Section A7.1.2.2.2/01 Water/sediment degradation study

Annex Point IIIA-XII.2.1

	substance	
8.2.1	Initial concentration of reference substance	Not relevant, the study is a model calculation.
8.3	**Testing procedure**	
8.3.1	Water/Sediment	Not relevant, the study is a model calculation.
8.3.2	Test system	Not relevant, the study is a model calculation.
8.3.3	Test conditions	Not relevant, the study is a model calculation.
8.3.4	Method of preparation of test solution	Not relevant, the study is a model calculation.
8.3.5	Application of test item	Not relevant, the study is a model calculation.
8.3.6	Duration of test	Not relevant, the study is a model calculation.
8.3.7	Sampling	Not relevant, the study is a model calculation.
8.3.8	Intermediates/ degradation products	Not relevant, the study is a model calculation.
8.3.9	Analytical methods	Not relevant, the study is a model calculation.
8.3.10	Statistics	Morlock (2006a (study no. 20051415/01-CUWS), 2006b (study no. 20051415/02-CUWS)) investigated the degradation of Permethrin and metabolites in two water-sediment systems. The respective studies were used to derive rates of degradation for the total system and two radiolabels (^{14}C-Vinyl- or ^{14}C-Phenoxyphenyl-Permethrin) with the model software KinGUI version 1.1[2]. Modelling was done using all data, no weighting and M0 (total amount at time 0) were not fixed for the parent. M0 of the metabolites were fixed to 0. Flows from parent to metabolites as well as from parent or metabolite, resp., to sink were considered for the simultaneous fittings. The proposed degradation pathway is shown in **Figure A7.1.2.2.2-01**. Dissipation half-lives (DT_{50}) were calculated by Morlock (2006a,b) on the basis of a one-compartmental approach. The kinetic data were recalculated based on the experimental results from Morlock (2006a,b) for the total system. The pertinent criteria of FOCUS kinetics working group were used to assess the goodness of fit.
		9 RESULTS
9.1	**Recovery**	Not relevant, the study is a model calculation.
9.2	**Degradation of**	

2 KinGUI vers. 1.1: User Interface for Kinetic Evaluations, Bayer CropScience (2006)

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Section A7.1.2.2.2/01 Water/sediment degradation study

Annex Point IIIA-XII.2.1

test substance

9.2.1 Test item — Not relevant, the study is a model calculation.

9.2.2 Metabolites — Not relevant, the study is a model calculation.

9.2.3 Degradation rate — For Permethrin, the obtained results indicate that SFO was the model that clearly fits best for both water-sediment systems. In all cases the kinetic evaluation using SFO resulted in better curve fittings and lower Chi² values. For FOMC the probabilities of the t-test indicated that the parameters α and β are not significantly different from zero. FOMC did also not provide better Chi² (χ2) values than the SFO model.

For one system/parent/metabolite combination (creek) the simultaneous fittings led to acceptable results. The resulting curve fittings and residual plots for parent and metabolite were visually acceptable considering the inherent scatter of degradation data.

Persistence and modelling endpoints for Permethrin and metabolites 3-Phenoxybenzyl alcohol and 3-Phenoxybenzoic acid in water sediment systems are summarised in **Table A7.1.2.2.2-1**

10 APPLICANT'S SUMMARY AND CONCLUSION

10.1 Materials and methods

Modelling and persistence endpoints for Permethrin and metabolites 3-Phenoxybenzyl alcohol and 3-Phenoxybenzoic acid in the water-sediment systems investigated by Morlock, G. (2006a,b) were re-calculated according to the FOCUS kinetics guidance (FOCUS, 2006).

Modelling was done using all data, no weighting and M0 (total amount at time 0) were not fixed for the parent. M0 of the metabolites were fixed to 0. Flows from parent to metabolites as well as from parent or metabolite, resp., to sink were considered for the simultaneous fittings.

10.2 Results and discussion

Endpoints for Permethrin:

The obtained results indicate that SFO was the model that clearly fits best for both water-sediment systems – pond and creek. In all cases the kinetic evaluation using SFO resulted in better curve fittings and lower Chi² values. However, in all cases the Chi² (χ2) values were slightly above the trigger of 15 which was considered to be acceptable due to the other parameters showing acceptable curve fittings.

For FOMC the probabilities of the t-test indicated that the parameters α and β are not significantly different from zero. FOMC did also not provide better Chi² (χ2) values for these water-sediment systems than the SFO model.

Endpoints for 3-Phenoxybenzyl alcohol and 3-Phenoxybenzoic acid

For the pond system no reliable $degDT_{50}$ could be derived for 3-Phenoxybenzyl alcohol. Due to the unreliable curve fitting for the precursor the $degDT_{50}$ of 3-Phenoxybenzoic acid is considered to have only indicative character. However, it confirms the findings for 3-Phenoxybenzoic acid found in the creek system.

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Section A7.1.2.2.2/01 Water/sediment degradation study

Annex Point IIIA-XII.2.1

For one system/parent/metabolite combination (creek) the simultaneous fittings led to acceptable results. The resulting curve fittings and residual plots for parent and metabolites were visually acceptable. However, the Chi² ($\chi 2$) value for the metabolite significantly exceeds the trigger of 15 which was considered to be acceptable due to the scatter of degradation data. Due to the transient occurrence of 3-Phenoxybenzyl alcohol this metabolite was not considered for creek.

X

10.3 Conclusion	Modelling and persistence endpoints for Permethrin and metabolites 3-Phenoxybenzyl alcohol and 3-Phenoxybenzoic acid in water-sediment systems were satisfactorily re-calculated according to the FOCUS kinetics guidance (FOCUS, 2006). The study is well conducted and reported and can be considered valid.
10.3.1 Reliability	1
10.3.2 Deficiencies	none

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Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	14-09-2011
Materials and Methods	Applicant's version is acceptable
Results and discussion	Adopt applicant's version with the following amendments:

Sub-heading 4.2.3 (Table A7.1.2.2.2-1)

DT_{50} values for Permethrin and its metabolites are evaluated from the cited study (Stangelj 2011). This, in turn, uses data from two earlier studies (Morlock 2006 a and b) which were carried out at 20 °C. The EC Technical Guidance Document (section 2.3.6.1) requires DT_{50} values to be reported at 12 °C. The following formula is provided to convert values from 20 °C to 12 °C:

$$DT_{50}(12\,^{\circ}C) = DT_{50}(20) \cdot e^{(0.08\,(20-12))}$$

Section A7.1.2.2.2/01 Water/sediment degradation study

Annex Point IIIA-XII.2.1

This allows for the figures in Table A7.1.2.2.2-1 to be supplemented as follows:

Whole-system degradation DT_{50} values

Substance	Kinetic model	$degDT_{50}$ @ 20°C	$degDT_{50}$ @ 12°C
Pond			
[Phenyl-U-14C]Permethrin			
Permethrin *	SFO	24.6	46.7
Permethrin	FOMC	24	45.5
Permethrin	SFO	22.4	42.5
3-Phenoxybenzyl alcohol *	SFO	2.7	5.1
3-Phenoxybenzoic acid *	SFO	33.4	63.3
[Vinyl-2-14C]Permethrin			
Permethrin *	SFO	14.3	27.1
Permethrin	FOMC	14.1	26.7
Creek			
[Phenyl-U-14C]Permethrin			
Permethrin*	SFO	24.6	46.7
Permethrin	FOMC	24	45.5
Permethrin	SFO	24.7	46.8
3-Phenoxybenzoic acid *	SFO	31.8	60.3
[Vinyl-2-14C]Permethrin			
Permethrin *	SFO	24.3	46.1
Permethrin	FOMC	23.7	44.9

Section A7.1.2.2.2/01 Water/sediment degradation study

Annex Point IIIA-XII.2.1

* proposed modelling and persistence endpoints

Sub-heading 5.1

It is noted that the ratio of isomers used in the original degradation study was approximately 75:25 trans:cis. This is in keeping with the representative product supported by the applicant. The metabolite DCVA formed at very significant levels, reaching a maximum of 84.1% AR in the pond system at day 62 and a maximum of 84.3% AR in the creek system at day 62. However reliable DT50 values could not be obtained for DCVA since it only showed a small decline by study end in both systems (to 756.3% AR for pond and 70.6% AR for creek).

Sub-heading 5.2

The applicant suggests omitting 3-Phenoxybenzyl alcohol from the kinetic fitting of the creek system as suitable fits can be achieved simply by modelling the system Permethrin to 3-Phenoxybenzoic acid. We are in agreement with this. When 3-Phenoxybenzyl alcohol is included in the fitting it is found not to significantly affect the derivation of endpoints for Permethrin and 3-Phenoxybenzoic acid.

Conclusion	Adopt applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	n/a
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Section A7.1.2.2.2/01 Water/sediment degradation study

Annex Point IIIA-XII.2.1

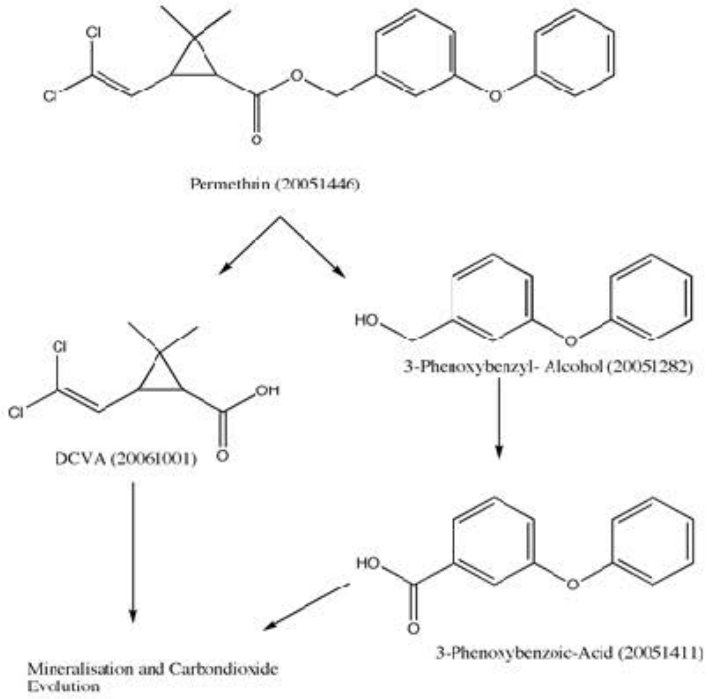

Figure A7.1.2.2.2-1. Proposed degradation pathway of Permethrin in the water-sediment systems.

Section A7.1.2.2.2/01 Water/sediment degradation study

Annex Point IIIA-XII.2.1

Table A7.1.2.2.2-1: Modelling and persistence endpoints for Permethrin and metabolites based on data obtained in two water-sediment studies (pond and creek) and considering FOCUS kinetics

Substance	Kinetic model	$degDT_{50}$ [d]	$degDT_{90}$ [d]	Formation fraction [a]	Plots visually acceptable	Chi^2 [b]	t-test [c]	EF [d]
Pond								
[Phenyl-U-^{14}C]Permethrin								
Permethrin	SFO	**24.6**	**81.6**	n.a.	yes	16.2	< 0.05	0.9200
Permethrin	FOMC	**24.0**	**83.2**	n.a.	yes	17.4	α: < 0.44 β: < 0.45	0.9174
Permethrin[e,f]	SFO	**22.4**	**74.5**	n.a.	yes	16.5	< 0.05	0.8465
3-Phenoxybenzyl alcohol (A1)	SFO	**2.7**	**8.9**	1.0	no	139.2	0.23	
3-Phenoxybenzoic acid (A2)	SFO	**33.4**	**111.0**	from A1: 0.927	yes	22.8	< 0.05	
[Vinyl-2-^{14}C]Permethrin								
Permethrin[e,f]	SFO	**14.3**	**47.6**	n.a.	yes	16.3	< 0.05	0.9441
Permethrin	FOMC	**14.1**	**48.5**	n.a.	yes	17.3	α: 0.455 β: 0.456	0.9434
Creek								
[Phenyl-U-^{14}C]Permethrin								
Permethrin	SFO	**24.6**	**81.8**	n.a.	yes	15.9	< 0.05	0.9312
Permethrin	FOMC	**24.0**	**83.1**	n.a.	yes	16.9	α: 0.438 β: 0.441	0.9292
Permethrin[e,f]	SFO	**24.7**	**82.1**	n.a.	yes	15.9	< 0.05	0.9205
3-Phenoxybenzoic acid[e,f]	SFO	**31.8**	**105.7**	0.867	yes	33.6	< 0.05	
[Vinyl-2-^{14}C]Permethrin								
Permethrin[e,f]	SFO	**24.3**	**80.8**	n.a.	yes	16.4	< 0.05	0.9346
Permethrin	FOMC	**23.7**	**82.2**	n.a.	yes	17.6	α: 0.441 β: 0.443	0.9318

n.a. not applicable

a Formation fraction from parent unless otherwise stated.

b Error value at which the Chi^2-test is passed should be below 15%. The Chi^2-test considers the deviations between observed and calculated values relative to the uncertainty of the measurements.

c A model parameter (e.g. k, α, β) is considered significantly different from zero if the probability corresponding to the calculated t-value is smaller than 0.05, i.e. considering a 5 percent significance level.

d corresponding to model efficiency ("EF", see FOCUS kinetics guidance, EC Document Reference Sanco/10058/2005 version 2.0)

e proposed modelling endpoints

f proposed persistence endpoints (used as triggers for additional work)

SFO Single First Order model

FOMC First Order Multi Compartment model

Section IIIA 7.1.3 Annex Point IIA, VII.7.7	Adsorption/Desorption screening test	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000), an adsorption/desorption screening test is required where the preliminary risk assessment indicates that this is necessary. A comprehensive investigation involving batch equilibrium testing on the adsorption/desorption of Permethrin in three soils was carried out and is described in the study "Studies on the Adsorption-Desorption of Permethrin" Joseph, Rachel (2004b) (IIIA, 7.2.3.1). Since this test is a higher-tier test with a higher level of information, the conduct of an HPLC screening test is not considered to be required as there is no relevant additional scientific information to be gained there from.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	02 June 2009	
Evaluation of applicant's justification	Applicant's justification is acceptable but the following points should be noted. The TNsG on data requirements state in relation to dossier section IIIA 7.1.3 that a screening test is always required (conducted according to EC method C.18 or OECD 106) in which adsorption at a single concentration is determined in five different soil types. The study provided in section IIIA 7.2.3.1 is a screening test in which adsorption at a single concentration was determined in three soils. Information on the mobility of permethrin in water-sediment systems can be obtained from the studies presented under AIII 7.1.2.2.2, which showed strong adsorption of permethrin to sediment. Additional information on sediment sorption behaviour is not required.	
Conclusion	An adsorption/desorption screening test on sediment is not required. An adsorption/desorption screening test using three soils is available in section IIIA 7.2.3.1.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

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Section IIIA 7.1.4.1 **Annex Point XII.2.1**	**Field study on accumulation in the sediment.**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	According to the 'Data requirements for biocidal product types, Version 4.3.2' (October, 2000), water-sediment simulation tests are required if the biocide is directly emitted to water or if the solids water equilibrium partition coefficient (K_p) of the substance being investigated is > 2000. The use pattern of the product is localised, of low volume and does not involve direct application to water. The K_p of Permethrin, derived from the equation $K_p = F_{oc} \times K_{oc}$, was calculated to be 993 and is thus below the threshold value set in the guidance. It is therefore proposed that a study is not required to address this point.	
Undertaking of intended data submission [ ]		

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Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

02 June 2009

Evaluation of applicant's justification

The applicant appears to have confused field testing of accumulation in sediment with water-sediment simulation testing. The rationale presented by the applicant is an argument that water-sediment simulation testing is not required. However the applicant has already presented two water-sediment studies under *AIII 7.1.2.2.2*, both of which were conducted according to OECD Guideline 308.

The applicant has not addressed the issue of whether or not a field study on accumulation in sediment is required. The decision on the need for this type of study should in the first instance be based on an analysis of the levels of non-extractable residues and mineralisation rates found in the water-sediment studies. Maximum levels of CO_2 formation and non-extractable residues found in the two studies presented under *AIII 7.1.2.2.2* (Morlock, G., 2006a and 2006b) are shown in the table below.

System	Creek		Pond	
Study	Morlock, G. (2006a)		Morlock, G. (2006b)	
Radiolabel	Phenoxyphenyl	Vinyl	Phenoxyphenyl	Vinyl
Max NER (% AR)	47.3 (day 120)	14.1 (day 120)	55.0 (day 86)	19.1 (day 86)
Max CO_2 (% AR)	45.4 (day 120)	14.1 (day 120)	30.1 (day 120)	8.4 (day 120)

NER = non-extractable residues

It can be seen from the table that the mineralisation rate of permethrin (25:75 *cis*:*trans*) by the end of the incubation period (120 days) exceeded 5% for both

Section IIIA 7.1.4.1 Annex Point XII.2.1	Field study on accumulation in the sediment.
	radiolabelled versions in each study. The maximum combined amount of non-extractable residues, summed over both radiolabels, was 61.4% AR for the creek-derived system and 74.1% AR for the pond-derived system. Although the maximum combined amount of non-extractable residues in the pond-derived system exceeded 70%, the RMS evaluator considers that a field study on accumulation in sediment would be of little value. If necessary, the potential for accumulation in sediment could be estimated using an appropriate calculation method or existing data from monitoring programmes could be used (e.g. programmes put in place to meet the requirements of the Water Framework Directive).
Conclusion	The applicant's justification is not acceptable because it fails to address the point. However, based on the alternative justification provided by the RMS evaluator, a field study on accumulation in sediment is not required.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.2.1/01 Annex Point IIIA-XII.1.1 — Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

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		1 REFERENCE
10.4	1.1 Reference	Traub, M. (2011): Aerobic degradation and metabolism of radiolabelled Permethrin in one soil at 20°C in the dark. Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany Unpublished report No. S10-00378 (5th May 2011)
10.5	1.2 Data protection	Yes
1.2.1	Data owner	Tagros Chemicals India Ltd.
1.2.2	Companies with letter of access	Not applicable.
1.2.3	Criteria for data protection	Data submitted to the MS after May 2000 on existing a.s. for the purpose of its entry Annex I/IA of Directive 98/8/EC.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	Guideline study	OECD 307 (2002) SETAC – Europe "Procedures for assessing the environmental fate and ecotoxicology of pesticides" (1995)
2.2	GLP	Yes
2.3	Deviations	None
		3 MATERIALS AND METHODS
3.1	Test material 1	[Phenyl-U-^{14}C]Permethrin (CFQ40816)
3.1.1	Lot/Batch number	Not stated
3.1.2	Specification	As given below
3.1.3	Purity	Radiochemical purity: 99.9%
3.1.4	Stability	Not applicable, purity was checked before application
3.1.5	Further relevant properties	Permethrin radiolabelled at Phenyl-U-carbon position Specific activity: 2.07 GBq/mmol, 56 mCi/mmol
3.2	Test material 2	[Vinyl-2-^{14}C]Permethrin (CFQ40815)
3.2.1	Lot/Batch number	Not stated
3.2.2	Specification	As given below
3.2.3	Purity	Radiochemical purity: 99.9%
3.2.4	Stability	Not applicable, purity was checked before application
3.2.5	Further relevant properties	Permethrin radiolabelled at Vinyl-2-carbon position Specific activity: 1.67 GBq/mmol, 45 mCi/mmol
10.6	3.3 Non labelled test item	Permethrin technical (purity: 93.34%) was used for biomass analysis

Section A7.2.1/01 Annex Point IIIA-XII.1.1

Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

10.7	3.4 Degradation products	Degradation products resulting from degradation and transformation of the parent compound tested
	3.4.1 Method of analysis for degradation products	Reversed phase TLC system and normal phase TLC system (confirmatory method)
	3.5 Reference substance	The following reference compounds were used: 3-phenoxybenzoic acid (Lot No. 6116X) Cypermethric acid (Lot No. CMA/382//B/10) 3-phenoxybenzyl alcohol (Lot No. 190284)
	3.5.1 Initial concentration of reference substance	Not reported
	3.6 Testing procedure	
	3.6.1 Soil	One German standard soil LUFA 2.2 (loamy sand) was used in this study. The soils in the flasks were wetted to 45% MWHC. The flasks were pre-incubated under oxic conditions at 20 ± 2°C in the dark. The moisture was checked regularly, and was adjusted with deionised water to about 45% MWHC if necessary. The soil characteristics are summarised in **Table A7.2.1-1**.
	3.6.2 Test system	The glass flasks (300 mL) contained about 50 g soil (d.w.). Flasks for biomass control (250 mL) contained 100 g soil (d.w.). Flasks were closed by traps. The organic volatiles in the flasks were trapped by Tenax as adsorbent and the carbon dioxide in the flasks was trapped by sodium hydroxide reservoir. The samples were incubated at 20 ± 2°C under oxic conditions, protected from light. The moisture of the soils was checked once a week and, if required, was adjusted with deionised water to about 45% of its MWHC. The traps for organic volatiles and carbon dioxide were changed at least every 4 weeks. Glass wool was used to close the end of Tenax trap to hold the adsorber materials in place.
	3.6.3 Temperature/light conditions	20 ± 2°C/dark
	3.6.4 Method of preparation of test solution	Test item was applied in a concentration of 9.9 µCi test item 1 ([Phenyl-U-^{14}C]Permethrin) or 7.8 µCi test item 2 ([Vinyl-2-^{14}C]Permethrin) in 200 µL acetone/water (50/50, v/v) to each flask containing 50 g soil.
	3.6.5 Application of test item	The test item solution was added drop by drop to the soil and subsequently mixed by shaking the flasks. The application rate was chosen based on typical soil concentrations resulting from the exposure assessment for biocidal use (e.g. 1.375 mg/kg dry soil) or typical field application rate (e.g. 1.375 kg Permethrin/ha). The resulting application rate corresponds to 68.75 µg Permethrin per 50 g dry soil. Every sample contained 9.9 µCi test item 1 or 7.8 µCi test item 2 per 50 g dry soil. This amount corresponds to 1.39 mg/kg for test item 1 and 1.36 mg/kg dry soil for test item 2. The biomass vessels were treated with the same unlabelled amount. The

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Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

untreated biomass samples received the same amount of solvent of the application solution without test item.

3.6.6 Duration of the test — 122 days

3.6.7 Number of replicates — 42 flasks for each soil system:

22 flasks treated with the test item (20 used for analysis and 2 as a reserve);

10 untreated flasks for determination of the biomass;

10 flasks treated with unlabelled test item for determination of the biomass.

3.6.8 Sampling — Two threated flasks were taken for analysis at the following sampling dates: 0, 3, 7, 11, 15, 21, 30, 58, 93 and 122 days after treatment. At every sampling date, a non-radiolabelled solution of Permethrin and reference items was added to optimise the extraction efficiency.

The biomass was determined at the start, after 29 days and after 119 days.

3.6.9 Analytical methods — The organic volatile traps (Tenax) were extracted with acetone and the amount of radioactivity was determined by Liquid Scintillation Counting (LSC). The radioactivity in the carbon dioxide traps was determined by LSC of an aliquot of the alkaline trapping reagent. Confirmation of the analyte was performed in representative samples through precipitation with barium hydroxide. Traps for carbon dioxide were monitored for radioactivity at every sampling interval.

The test item was extracted from the soil with 80 mL acetonitrile/water (80/20, v/v). The pH was lowered to a value below 5.0 with 1 mL acetic acid. The suspension was shaken overnight to evolve carbon dioxide and to extract the soil. The dispersed soil was transferred to a 200 mL glass centrifuge tube. The extract was separated from the sediment by centrifugation at 2600 rpm for 10 minutes. The extraction was repeated at least two times. The radioactivity after each extraction step and in the combined extract was determined by LSC of an aliquot. Afterwards the soil was extracted twice with 80 mL of pure acetone and the radioactivity was determined by LSC of an aliquot. After the final extraction, the soil was dried prior to combustion. The total amount of non-extractable radioactivity residues in soil after extraction was determined by combustion and LSC (3 × 0.5 g).

The thin layer chromatography (TLC) was used to characterize the extractable radioactivity.

The amount of radioactivity which could still be detected (LOD) was below 25 dpm and the lowest amount quantified by TLC (LOQ) was set to 50 dpm.

3.6.10 Statistics — Single first order fittings with associated r^2 analysis.

4 RESULTS

4.1 Recovery — The mean recovery ranged from 93 to 105% AR for [Phenyl-U-^{14}C]Permethrin and from 90 to 106% AR for [Vinyl-2-^{14}C]Permethrin during the whole study duration.

The distribution of the radioactivity and mean recoveries at each

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Section A7.2.1/01 Annex Point IIIA-XII.1.1	Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)	
	sampling point can be found in **Table A7.2.1-2** and **A7.2.1-3** for [Phenyl-U-^{14}C]Permethrin and [Vinyl-2-^{14}C]Permethrin, respectively.	
4.2 Degradation of test substance		
4.2.1 Mineralisation	Total mineralisation to CO_2 was in a range of 1 to 38% AR for [Phenyl-U-^{14}C]Permethrin and of 5 to 52% AR for [Vinyl-2-^{14}C]Permethrin. No organic volatiles were revealed within the study. Details can be found in **Table A7.2.1-2** and **A7.2.1-3** for [Phenyl-U-^{14}C]Permethrin and [Vinyl-2-^{14}C]Permethrin, respectively.	
4.2.2 Test item	[Phenyl-U-^{14}C]Permethrin decreased from 99% AR directly after application to 6% 122 days after treatment and [Vinyl-2-^{14}C]Permethrin decreased from 100% AR directly after application to 6% AR 122 days after application, respectively. Details can be found in **Table A7.2.1-4** and **A7.2.1-5** for [Phenyl-U-^{14}C]Permethrin and [Vinyl-2-^{14}C]Permethrin, respectively.	
4.2.3 Metabolites	Considering test substance 1 – [Phenyl-U-^{14}C]Permethrin, four minor metabolites were formed, all below< 5% AR. None of the metabolites co-eluated with the reference substance item 3-Phenoxybenzoic acid or 3-Phenoxybenzyl alcohol. In samples with test substance 2 – [Vinyl-2-^{14}C]Permethrin, one metabolite (M1) was > 5% AR. This metabolite increased from 0% to a plateau of 9% AR up to 21 days after application and decreased to 1% AR at the end of the incubation period. Three minor metabolites (M2, M3 and M9) were formed during the study duration; none of them was formed > 5% AR. None of the metabolites co-eluated with the reference item Cypermethric acid. Details can be found in **Table A7.2.1-4** and **A7.2.1-5** for [Phenyl-U-^{14}C]Permethrin and [Vinyl-2-^{14}C]Permethrin, respectively.	X
4.2.4 Degradation rates	The rate of degradation of [Phenyl-U-^{14}C]Permethrin and [Vinyl-2-^{14}C]Permethrin with one metabolite M1 in soil LUFA 2.2 was analysed by non-linear regression of the original data (single values) by using first order kinetics, which provided an acceptable fit to the data with r^2 of > 0.7. The first order DT_{50} and DT_{90} values of [Phenyl-U-^{14}C]Permethrin were 12 and 40 days (r^2 = 0.9725), 11 and 36 days (r^2 = 0.9668) for [Vinyl-2-^{14}C]Permethrin and 12 and 39 days (r^2 = 0.8792) for metabolite M1 ([Vinyl-2-^{14}C]M1). For metabolites M2, M3 and M9 no disappearance times were calculated. Details of calculated degradation lives for and resulting statistics can be found in **Table A7.2.1-6**.	X
4.2.5 Microbial biomass	The microbial biomass of each soil (treated samples) before and at the end of incubation varied from 27.7 to 10.6 mg C/100 g dry soil in the LUFA 2.2 soil. In the untreated samples it ranged from 30.2 to 11.2, mg C/100 g dry soil at the beginning and at the end of incubation in the LUFA 2.2 soil, respectively. No significant differences in biomass of the treated samples were observed in comparison to the untreated samples during the entire period of the test.	X

Section A7.2.1/01 Annex Point IIIA-XII.1.1 — Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

OECD 307 (2002)

SETAC Guideline "Procedures for assessing the environmental fate and ecotoxicology of pesticides"

Deviations: None

The degradation rate and metabolism of [Phenyl-U-^{14}C]Permethrin and [Vinyl-2-^{14}C]Permethrin and their degradation products was investigated in one soil (LUFA 2.2) under aerobic laboratory conditions in the dark at 20 ± 2°C for 122 days. Test item was applied with a concentration of 9.9 µCi test item 1 ([Phenyl-U-^{14}C]Permethrin) or 7.8 µCi test item 2 ([Vinyl-2-^{14}C]Permethrin) per 50 g soil in a flask. Liquid carbon dioxide and organic volatile traps were used.

The biomass vessels were treated with the same unlabelled amount. The untreated biomass samples received the same amount of solvent of the application solution without test item.

Soil samples were taken for analysis on day 0, 2, 7, 11, 15, 21, 30, 58 and 93 after treatment. The radioactivity was determined by TLC.

The disappearance times (DT_{50} and DT_{90}) for both test items and possible major metabolites were calculated.

5.2 Results and discussion

The mean recovery ranged from 93 to 105% AR for [Phenyl-U-^{14}C]Permethrin and from 90 to 106% AR for [Vinyl-2-^{14}C]Permethrin during the whole study duration. [Phenyl-U-^{14}C]Permethrin decreased from 99% AR directly after application to 6% 122 days after treatment and [Vinyl-2-^{14}C]Permethrin decreased from 100% AR directly after application to 6% AR 122 days after application, respectively.

Total mineralisation to CO_2 was in a range of 1 to 38% AR for [Phenyl-U-^{14}C]Permethrin and of 5 to 52% AR for [Vinyl-2-^{14}C]Permethrin. No organic volatiles were revealed within the study.

Considering test substance 1 four minor metabolites were formed, all below< 5% AR. In samples with test substance 2 one metabolite (M1) was > 5% AR. Three minor metabolites (M2, M3 and M9) were formed during the study duration; none of them was formed > 5% AR. None of the metabolites co-eluated with any of the reference item.

The rate of degradation of [Phenyl-U-^{14}C]Permethrin and [Vinyl-2-^{14}C]Permethrin with one metabolite M1 in soil LUFA 2.2 was analysed by non-linear regression of the original data by using first order kinetics. The first order DT_{50} values of [Phenyl-U-^{14}C]Permethrin and [Vinyl-2-^{14}C]Permethrin were 12 and 11 days, respectively. For metabolite M1 the first order DT_{50} was 12 days. For metabolites M2, M3 and M9 no disappearance times were calculated.

No significant differences in biomass of the treated samples were observed in comparison to the untreated samples during the entire period of the test.

5.3 Conclusion

Following incubation for 122 days in one soil under aerobic conditions at 20 °C, Permethrin degraded relatively fast with a degradation half-life

Section A7.2.1/01 Annex Point IIIA-XII.1.1	Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)
	based on SFO kinetics 11 and 12 days for [Vinyl-2-^{14}C]Permethrin and [Phenyl-U-^{14}C]Permethrin, respectively. For metabolite M1 the first order DT_{50} was 12 days. For metabolites M3, M4 and M9 no disappearance times were calculated.
5.3.1 Reliability	1
5.3.2 Deficiencies	None

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 07-10-11

Materials and Methods Applicant's version is acceptable

Results and discussion Adopt applicant's version with the addition of the following comments:

Sub-heading 4.2.3

Four metabolites were detected in both pond and creek systems (designated M1, M2, M3 and M9) but none were identified during the study. The known degradation products of permethrin (3-phenoxybenzoic acid, 3-phenoxybenzyl alcohol and Cypermethric acid) were not detected. Therefore this study can only be used in the determination of the rate of degradation of permethrin and not its route of degradation.

Sub-heading 4.2.4

Degradation rates for Permerthrin have been evaluated according to the recommendations of EC document 9188/VI/97 rev 8 (2000). This document has been superseded by the 2006 FOCUS guidance document SANCO/10058/2005 version 2.0. In particular the use of Chi-squared rather than r-squared values is recommended when assessing the goodness of a fit. Therefore the applicant has repeated the kinetic analysis using the more recent guidance in another study. Please refer to the report by Stangelj, A. (2011) presented in section A7.2.1/04 for a complete analysis of degradation rates.

Sub-heading 4.2.5

The microbial biomass of the soil at the end of the study is quoted as 10.6 mg C/100 g dry soil for the treated soil and 11.2 mg C/100 g dry soil for the untreated soil. These figures when expressed as percentages of organic carbon (OC) correspond to 0.48% and 0.51% respectively. OECD guidance document 307 recommends the use of soils with a microbial biomass of at least 1%. There appears to have been a substantial decline in microbial activity after day 30 in treated and untreated soil (~60% decrease in microbial biomass). Untreated soil biomass was 27.4 mg C/100g soil at day 29 (1.25% of OC) while treated soil biomass was 28.8 mg C/100g soil at day 29 (1.31% of OC). Fig. 1 and Fig. 2 in the original study report show that there was a definite decrease in the degradation rate after day 30 which corresponds with the time period over which microbial viability declined. Therefore only the data from the first 30 days is considered reliable.

Conclusion The kinetic analysis performed in this study needs to be repeated in light of the comments made above regarding current guidance and microbial viability. Please refer to the report by Stangelj, A. (2011) presented in section A7.2.1/04 for this analysis. No data is presented here regarding the nature of the metabolites formed from the degradation of permethrin in soil.

Reliability 2

Acceptability Acceptable but this study only provides information on the rate of degradation and

	not the route.
Remarks	Only the data from the first 30 days is considered reliable due to concerns about the microbial viability of the soil thereafter.
	COMMENTS FROM
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Section A7.2.1/01 Annex Point IIIA-XII.1.1 Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

10.8

10.9

10.10 Table A7.2.1-1: Soil parameters for aerobic degradation and metabolism study of vinyl- or phenyl-radiolabelled Permethrin

Soil name	LUFA 2.2
Soil description	2.2
pH ($CaCl_2$)	5.27
Organic carbon [%]	2.20
Maximum water holding capacitiy [g/100g]]	46.8
Cation exchange capacity [mval/100 g]	12.3
Soil density [g/L]	1200
Soil type (USDA)	loamy sand
Particle size [%]	
<0.002 mm	8.6
0.002-0.05 mm	17.6
0.05-2.00 mm	73.8

Section A7.2.1/01 Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

Annex Point IIIA-XII.1.1

Table A7.2.1-2: Distribution of the radioactivity for in % AR of [Phenyl-U-^{14}C]Permethrin (mean values)

Sampling interval [day]	% AR				
	Radioactivity in extracts	Bound residues	Carbon dioxide	Organic volatiles	Recovery
0	99	0	0	0	99
3	94	6	1	0	101
7	64	24	5	0	93
11	55	38	10	0	103
15	47	37	15	0	98
21	38	39	21	0	98
30	25	46	23	0	93
58	12	53	34	0	99
93	8	60	38	0	105
122	9	51	36	0	95

Table A7.2.1-3: Distribution of the radioactivity for in % AR of [Vinyl-2-^{14}C]Permethrin (mean values)

Sampling interval [day]	% AR				
	Radioactivity in extracts	Bound residues	Carbon dioxide	Organic volatiles	Recovery
0	100	0	0	0	100
3	95	3	0	0	98
7	76	13	5	0	94
11	59	26	11	0	95
15	56	33	17	0	106
21	46	33	14	0	93
30	38	39	26	0	103
58	17	34	42	0	90
93	15	33	47	0	94
122	9	29	52	0	88*

* used for evaluation, because mean recovery is still close to 90% AR

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Annex Point IIIA-XII.1.1

Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

10.11

Table A7.2.1-4: Characterisation of the extractable radioactivity of [Phenyl-U-^{14}C]Permethrin in soil LUFA 2.2 (mean values)

Sampling interval [days]	% AR				
	Permethrin	M1	M2	M3	M9
0	99.0	0.0	0.0	0.0	0.0
3	86.8	3.7	0.0	0.0	3.5
7	56.0	0.9	2.1	0.0	4.2
11	49.8	1.9	1.8	0.0	1.5
15	39.9	1.8	1.3	0.0	4.5
21	30.6	1.7	1.2	0.0	4.5
30	21.6	0.5	0.0	0.0	2.4
58	9.7	0.4	0.4	0.0	1.5
93	6.3	0.2	0.2	0.4	0.8
122	6.3	0.3	0.4	1.3	0.0

Table A7.2.1-5: Characterisation of the extractable radioactivity of [Vinyl-2-^{14}C]Permethrin in soil LUFA 2.2 (mean values)

Sampling interval [days]	% AR				
	Permethrin	M1	M2	M3	M9
0	100.0	0.0	0.0	0.0	0.0
3	84.5	5.5	0.0	0.0	4.4
7	58.2	9.4	1.1	3.1	4.2
11	44.8	8.9	1.6	1.6	1.5
15	36.2	9.3	1.6	2.9	4.6
21	29.9	8.8	1.1	1.3	3.0
30	18.1	3.3	1.6	0.0	2.1
58	10.4	1.8	0.6	0.6	1.4
93	10.6	0.8	0.5	0.4	1.4
122	6.0	0.8	0.5	0.9	0.0

Section A7.2.1/01 Annex Point IIIA-XII.1.1 Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

10.12

Table A7.2.1-6: DT_{50} and DT_{90} values (1st order) of [Phenyl-U-^{14}C]Permethrin, [Vinyl-2-^{14}C]Permethrin and metabolite M1 in soil LUFA 2.2

Substance	[Phenyl-U-^{14}C]Permethrin	[Vinyl-2-^{14}C]Permethrin	[Vinyl-2-^{14}C]M1
[Vinyl-2-^{14}C]Permethrin			
DT_{50} [days]	12 (11 – 14)	11 (9 – 13)	12 (7 – 57)
DT_{90} [days]	40 (35 – 47)	36 (31 – 43)	39 (22 – 190)
R^2	0.9725	0.9666	0.8792

numbers in parentheses are the 95% confidence limits

Section A7.2.1/02 Annex Point IIIA-XII.1.1 Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

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		2 REFERENCE
10.13	1.1 Reference	Hellstern J., (2011a): Aerobic degradation and metabolism of vinyl-radiolabelled Permethrin in three soils at 20°C in the dark Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany Unpublished report No. S10-00379 (4th May 2011)
10.14	1.2 Data protection	Yes
1.2.1	Data owner	Tagros Chemicals India Ltd.
1.2.2	Companies with letter of access	Not applicable.
1.2.3	Criteria for data protection	Data submitted to the MS after May 2000 on existing a.s. for the purpose of its entry Annex I/IA of Directive 98/8/EC.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	Guideline study	OECD 307 (2002)
2.2	GLP	Yes
2.3	Deviations	None
		3 MATERIALS AND METHODS
3.1	Test material 1	[Vinyl-2-^{14}C]Permethrin (CFQ40815)
3.1.1	Lot/Batch number	Batch No.: B1
3.1.2	Specification	As given below
3.1.3	Purity	Radiochemical purity: 99.9%
3.1.4	Stability	Not applicable, purity was checked before application
3.1.5	Further relevant properties	Permethrin radiolabelled at Vinyl-2-carbon position Specific activity: 1.67 GBq/mmol, 45 mCi/mmol
10.15	3.2 Non labelled test item	Permethrin technical (purity: 93.34%) was used for biomass analysis
10.16	3.3 Degradation products	Degradation products resulting from degradation and transformation of the parent compound tested
3.3.1	Method of analysis for degradation products	Reversed phase TLC system and normal phase TLC system (confirmatory method)
10.17	3.4 Reference substance	The following reference compound was used: Cypermethric acid (Lot No.: CMA/382//B/10)

Section A7.2.1/02 Annex Point IIIA-XII.1.1	Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)
3.4.1 Initial concentration of reference substance	Not reported
10.18 3.5 Testing procedure	
3.5.1 Soil	Three German standard soils were used in this study: LUFA 2.1 (loamy sand), LUFA 2.3 (sandy loam) and LUFA 2.4 (loam). The soils were already sieved over a 2 mm sieve. After arrival the soils were adjusted to 45% MWHC by adding deionised water. The soils were pre-incubated under aerobic conditions for 3 days (20 ± 2°C in the dark). The soil characteristics are summarised in **Table A7.2.1-1**.
3.5.2 Test system	The glass flasks (300 mL) contained about 50 g soil (d.w.). Flasks for biomass control (250 mL) contained 100 g soil (d.w.). Each flask was closed by cotton wool. The samples were incubated at 20 ± 2°C under oxic conditions in the dark. The moisture of the soils was checked twice a week and, if required, was adjusted with deionised water to about 45% of its MWHC.
3.5.3 Temperature/light conditions	20 ± 2°C/dark
3.5.4 Method of preparation of test solution	Test item was applied with a concentration of 67.88 µg in 200 µL acetone/water (50/50, v/v) to each flask containing 50 g soil.
3.5.5 Application of test item	The test item solution was added drop by drop to the soil and subsequently mixed by shaking the flasks. The application rate was chosen based on typical soil concentrations resulting from the exposure assessment for biocidal use (e.g. 1.375 mg/kg dry soil) or typical field application rate (e.g. 1.375 kg Permethrin/ha). The resulting application rate corresponds to 68.75 µg Permethrin per 50 g dry soil. Every sample contained 0.29 MBq (7.8 µCi) per vessel corresponding to 67.88 µg [Vinyl-2-^{14}C]Permethrin per 50 g soil or 1.36 mg/kg dry soil. The biomass vessels were treated with the same unlabelled amount. The untreated biomass samples received the same amount of the pure solvent of the application solution without test item (acetonitrile/water 50/50, v/v).
3.5.6 Duration of the test	122 days
3.5.7 Number of replicates	42 flasks for each soil system: 22 flasks treated with the test item (16 used for analysis and 6 as a reserve); 10 untreated flasks for determination of the biomass; 10 flasks treated with unlabelled test item for determination of the biomass.
3.5.8 Sampling	Two treated flasks taken for analysis at day 0, 2, 7, 11, 15, 21, 30, 58 and 93 after treatment for soils LUFA 2.3 and LUFA 2.4. In case of soil LUFA 2.1, an additional sampling 122 days after treatment was performed. At every sampling date, a non-radiolabelled solution of

Section A7.2.1/02 Annex Point IIIA-XII.1.1	Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)	
	Permethrin and reference items was added to optimise the extraction efficiency. The biomass was determined at the start, after 30 days and at the end of the study.	
3.5.9 Analytical methods	The test item was extracted from the soil with 80 mL acetonitrile/water (80/20, v/v). The suspension was shaken for at least 2 hours. The dispersed soil was transferred to a 200 mL glass centrifuge tube. The extract was separated from the sediment by centrifugation at 2600 rpm for 10 minutes. The extraction was repeated at least two times. The radioactivity after each extraction step was determined by LSC. The samples from single extraction with an amount of ≥ 5% AR were combined and analysed again. The soil was extracted with 80 mL of pure acetone and the radioactivity was determined by Liquid Scintillation Counting (LSC) of an aliquot. From every sample an aliquot was diluted and mixed with scintillation cocktail. The thin layer chromatography (TLC) was used to characterize the extractable radioactivity. The radioactivity on the TLC plates was determined optically by a digital autoradiography. The amount of radioactivity which could still be detected (LOD) was below 25 dpm and the lowest amount quantified by TLC (LOQ) was set to 50 dpm.	
3.5.10 Statistics	Single first order fittings with associated r^2 analysis.	
	4 RESULTS	
10.19 4.1 Recovery	No mass balance established. The % accounted radioactivity recovered at time 0 as a mean of both replicates ranged from 102.3 to 106.6% AR (see also **Table A7.2.1-2**).	
4.2 Degradation of test substance		
4.2.1 Mineralisation	Not evaluated.	
4.2.2 Test item	[^{14}C]Permethrin degraded from initial values between 102 and 107% to values below 5% AR within 93 days in soil LUFA 2.1 and within 58 days in soil LUFA 2.3 and soil LUFA 2.4, respectively. Details can be found in **Tables A7.2.1-3** to **A7.2.1-5**.	
4.2.3 Metabolites	Up to six metabolites were found. Metabolite M1 revealed a maximum amount of 19% AR in soil LUFA 2.1 (day 7), 36% AR in soil LUFA 2.3 (day 11) and 24% AR in soil LUFA 2.4 (day 7). In soil LUFA 2.3 two further metabolites M3 and M4 were detected with maximum amounts of 9% (day 58) or 6% (day 21), respectively. In soil LUFA 2.4 a further metabolite (M9) was detected with 6% AR after 7 days. All other metabolites were below 5% AR in any soil at any time. None of the metabolites co-eluated with a reference item. Details can be found in **Tables A7.2.1-3** to **A7.2.1-5**.	X
4.2.4 Degradation rates	Degradation kinetics of [Vinyl-2-^{14}C]Permethrin and metabolite M1 were modelled using first order kinetics, which provided an acceptable fit to the data with r^2 of > 0.7. For [Vinyl-2-^{14}C]Permethrin DT_{50} values were 10 days for the LUFA 2.1 and 7 days for LUFA 2.3 and LUFA 2.4	X

Section A7.2.1/02 Annex Point IIIA-XII.1.1	Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)	
	soils, respectively For metabolite M1 the DT_{50} values were 29, 25 and 11 days for soils LUFA 2.1, LUFA 2.3 and LUFA 2.4, respectively. Details of calculated degradation lives for and resulting statistics can be found in **Table A7.2.1-6.** For the metabolites M3, M4 and M9 no disappearance times were calculated. For M3 the number of data points after the maximum (9%) was not sufficient to derive robust DT_{50} values. M4 and M9 showed only a transient occurrence, hence being minor metabolites.	
4.2.5 Microbial biomass	The microbial biomass of each soil (treated samples) before and at the end of incubation varied from 13.4 to 6.4, 19.0 to 13.4 and 51.8 to 30.8 mg C/100 g dry soil in the LUFA 2.1, LUFA 2.3 and LUFA 2.4 soils, respectively. In the untreated samples it varied from 13.4 to 6.7, 24.1 to 13.4 and 49.6 to 31.1 mg C/100 g dry soil at the beginning and at the end of incubation in the LUFA 2.1, LUFA 2.3 and LUFA 2.4 soils, respectively. No significant differences in biomass of the treated samples were observed in comparison to the untreated samples during the entire period of the test.	X
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	OECD 307 (2002) Deviations: None The degradation rate of [Vinyl-2-^{14}C]Permethrin and possible metabolites was investigated in three different soils under aerobic laboratory conditions in the dark at 20 ± 2°C. Soil samples were treated with 67.88 µg [Vinyl-2-^{14}C]Permethrin per vessel (50 g soil), equivalent to 1.375 mg Permethrin/kg dry soil and incubated in glass flasks covered with cotton wool for up to 122 days at 45% of their MWHC. Flasks with untreated soil samples and with samples treated with non-radiolabelled test item were also incubated in order to demonstrate that the biomass of the soil samples is not affected by the test item during the complete study. Soil samples were taken for analysis on day 0, 2, 7, 11, 15, 21, 30, 58 and 93 after treatment for soils LUFA 2.3 and LUFA 2.4 and additional at day 122 after treatment for soil LUFA 2.1. The radioactivity was determined by TLC. The disappearance times (DT_{50} and DT_{90}) for both test items and possible major metabolites were calculated.	
5.2 Results and discussion	The amount of extractable radioactivity decreased during the incubation time; 107 – 102% AR at the study start to between 5 – 13% AR at the end of the study. [^{14}C]Permethrin was degraded from initial values between 102-107% to values below 5% AR at the end of the study for soils LUFA 2.1, LUFA 2.3 and LUFA 2.4. Up to six metabolites were found. Metabolite M1 revealed a maximum amount of 19% AR in soil LUFA 2.1, 36% AR in soil LUFA 2.3 and 24% AR in soil LUFA 2.4. In soil LUFA 2.3 two further metabolites M3 and M4 were detected with maximum amounts of 9% or 6%, respectively. In soil LUFA 2.4 a further metabolite (M9) was detected	

Section A7.2.1/02 Annex Point IIIA-XII.1.1	Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)
	with 6% AR. All other metabolites were below 5% AR in any soil at any time. None of the metabolites co-eluated with any reference item. The disappearance time (DT_{50} and DT_{90}) of the test item and metabolite M1 was calculated. A first order kinetics was assumed for the determination of the rate constants. The DT_{50} values range between 7 to 10 days for Permethrin and 11 to 29 days for metabolite M1. For metabolites M3, M4 and M9 no disappearance times were calculated. No significant differences in biomass of the treated samples were observed in comparison to the untreated samples during the entire period of the test.
5.3 Conclusion	Following incubation for up to 122 days in three different soils under aerobic conditions at 20°C, Permethrin degraded relatively fast with a degradation half-life based on SFO kinetics ranging from 7 to 10 days. For metabolite M1 the first order DT_{50} ranged between 11 to 29 days. For metabolites M3, M4 and M9 no disappearance times were calculated.
5.3.1 Reliability	1
5.3.2 Deficiencies	None

Section A7.2.1/02 Annex Point IIIA-XII.1.1 — Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

17-10-11

Materials and Methods

Applicant's version is acceptable

Results and discussion

Adopt applicant's version with the addition of the following comments:

Sub-heading 4.2.3

A total of six metabolites were detected in the three soils studied but none were identified. The expected degradation product of vinyl-labelled permethrin (Cypermethric acid) was not detected. Therefore this study can only be used in the determination of the rate of degradation of permethrin and not its route of degradation.

Sub-heading 4.2.4

Degradation rates for Permerthrin and one metabolite have been evaluated according to the recommendations of EC document 9188/VI/97 rev 8 (2000). This has been superseded by the 2006 FOCUS guidance document SANCO/10058/2005 version 2.0 which requires the use of Chi-squared rather than r-squared values when assessing the goodness of a fit. Also the combined fitting of parent and metabolites is preferred. Therefore the applicant has repeated the kinetic analysis using the more recent guidance in another study. Please refer to the report by Stangelj, A. (2011) presented in section A7.2.1/04 for a complete analysis of degradation rates.

Sub-heading 4.2.5

For soil Lufa 2.1 microbial viability was greater than 1% of OC by day 30 but had fallen to 0.66% by day 119. Therefore only the data for the first 30 days are considered reliable for soil Lufa 2.1. For the other soils, even though there was a decrease in biomass, levels remained above 1% OC throughout the incubation and so all data points were considered acceptable in these cases.

Conclusion

The kinetic analysis performed in this study needs to be repeated in light of the comments made above regarding current guidance and microbial viability. Please refer to the report by Stangelj, A. (2011) presented in section A7.2.1/04 for this analysis. No data is presented here regarding the nature of the metabolites formed from the degradation of permethrin in soil.

Reliability

2

Section A7.2.1/02 Annex Point IIIA-XII.1.1	Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)
Acceptability	Acceptable but this study only provides information on the rate of degradation and not the route.
Remarks	In the case of soil Lufa 2.1 only the data from the first 30 days is considered reliable due to concerns about the microbial viability of the soil thereafter.
	COMMENTS FROM
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Section A7.2.1/01 Annex Point IIIA-XII.1.1 Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

10.20

10.21

10.22 Table A7.2.1-1: Soil parameters for aerobic degradation and metabolism study of vinyl-radiolabelled Permethrin

Soil name	LUFA 2.1	LUFA 2.3	LUFA 2.4
Soil description	2.1	2.3	2.4
Batch No.	F2.11510	F2.31410	F2.41410
pH ($CaCl_2$)	5.3	6.87	7.25
Organic carbon [%]	0.97	1.10	2.67
Maximum water holding capacitiy [g/100g]]	37.5	40.1	45.3
Cation exchange capacity [mval/100 g]	6.6	11.7	32.3
Soil density [g/L]	1368	1277	1232
Soil type (USDA)	loamy sand	sandy loam	loam
Particle size [%]			
<0.002 mm	2.9	8.9	26.2
0.002-0.05 mm	10.6	26.4	42.1
0.05-2.00 mm	86.5	64.7	31.7
Biomass (before application)	13.4	24.1	49.6

Table A7.2.1-2: Extractable radioactivity in soils LUFA 2.1, LUFA 2.3 and LUFA 2.4 (mean values)

Sampling interval [days]	Radioactivity in extracts [% AR]		
	LUFA 2.1	LUFA 2.3	LUFA 2.4
0	104.6	106.6	102.3
2	101.2	95.9	96.7
7	87.6	81.3	81.0
11	79.3	69.6	56.0
15	72.4	61.3	45.5
21	58.6	47.4	30.1
30	49.4	37.7	15.2
58	28.3	12.8	5.7
93	15.3	5.1	4.8
122	13.1	-	-

Section A7.2.1/01 Annex Point IIIA-XII.1.1 Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

10.23

Table A7.2.1-3: Characterisation of the extractable radioactivity in soil LUFA 2.1 (mean values)

Sampling interval [days]	% of the applied radioactivity [% AR]			
	Permethrin	M1	M3	M4
0	104.6	n.d.	n.d.	n.d.
2	93.3	5.8	n.d.	n.d.
7	62.8	19.0	1.1	n.d.
11	54.5	18.1	2.2	n.d.
15	38.1	18.4	6.4	3.3
21	20.5	18.4	8.0	5.6
30	15.7	16.8	7.3	3.5
58	6.9	7.0	9.3	1.6
93	4.6	1.3	5.4	1.9
122	2.9	1.6	5.5	1.7

n.d. not detected

Table A7.2.1-4: Characterisation of the extractable radioactivity in soil LUFA 2.3 (mean values)

Sampling interval [days]	% of the applied radioactivity [% AR]	
	Permethrin	M1
0	106.7	n.d.
2	87.8	6.7
7	48.4	28.1
11	29.9	35.7
15	27.7	29.6
21	9.8	35.0
30	8.3	27.5
58	4.3	5.6
93	3.1	< 1

n.d. not detected

Table A7.2.1-5: Characterisation of the extractable radioactivity in soil LUFA 2.4 (mean values)

Sampling interval [days]	% of the applied radioactivity [% AR]		
	Permethrin	M1	M9
0	102.4	n.d.	n.d.
2	88.4	5.0	3.3
7	50.0	23.7	6.4
11	32.7	18.5	2.7
15	21.6	16.6	4.6
21	11.5	11.9	4.4
30	7.4	3.4	3.2
58	3.8	< 1	1.4
93	3.1	< 1	< 1

n.d. not detected

Table A7.2.1-6: DT_{50} and DT_{90} values (1st order) of [Vinyl-2-^{14}C]Permethrin and metabolite M1

Soil	LUFA 2.1	LUFA 2.3	LUFA 2.4
[Vinyl-2-^{14}C]-Permethrin			
DT_{50} [days]	10 (9 – 12)	7 (6 – 7)	7 (6 – 7)
DT_{90} [days]	34 (31 – 39)	22 (20 – 24)	22 (21 – 24)
R^2	0.983	0.989	0.994
Metabolite M1			
DT_{50} [days]	29 (22 – 45)	25 (17 – 45)	11 (9 – 15)
DT_{90} [days]	97 (72 – 150)	83 (58 – 150)	37 (30 – 49)
R^2	0.929	0.891	0.961

n.d. not detected

numbers in parentheses are the 95% confidence limits

Section A7.2.1/03 Annex Point IIIA-XII.1.1 Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

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		3 REFERENCE
10.24	1.1 Reference	Hellstern J., (2011b): Aerobic degradation and metabolism of phenyl-radiolabelled Permethrin in three soils at 20°C in the dark Eurofins Agroscience Services GmbH, Niefern-Öschelbronn, Germany Unpublished report No. S10-00380 (3rd May 2011)
10.25	1.2 Data protection	Yes
1.2.1	Data owner	Tagros Chemicals India Ltd.
1.2.2	Companies with letter of access	Not applicable.
1.2.3	Criteria for data protection	Data submitted to the MS after May 2000 on existing a.s. for the purpose of its entry Annex I/IA of Directive 98/8/EC.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	Guideline study	OECD 307 (2002)
2.2	GLP	Yes
2.3	Deviations	None
		3 MATERIALS AND METHODS
3.1	Test material 1	[Phenyl-U-^{14}C]Permethrin (CFQ40816)
3.1.1	Lot/Batch number	Batch No.: B1
3.1.2	Specification	As given below
3.1.3	Purity	Radiochemical purity: 99.9%
3.1.4	Stability	Not applicable, purity was checked before application
3.1.5	Further relevant properties	Permethrin radiolabelled at Phenyl-U-carbon position Specific activity: 2.07 GBq/mmol, 56 mCi/mmol
10.26	3.2 Non labelled test item	Permethrin technical (purity: 93.34%) was used for biomass analysis
10.27	3.3 Degradation products	Degradation products resulting from degradation and transformation of the parent compound tested
3.3.1	Method of analysis for degradation products	Reversed phase TLC system and normal phase TLC system (confirmatory method)
10.28	3.4 Reference substance	The following reference compounds were used: 3-phenoxybenzoic acid (Lot No. 6116X) 3-phenoxybenzyl alcohol (Lot No. 1396847)

Section A7.2.1/03 Annex Point IIIA-XII.1.1 — Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

3.4.1	Initial concentration of reference substance	Not reported
10.29	**3.5 Testing procedure**	
3.5.1	Soil	Three German standard soils were used in this study: LUFA 2.1 (loamy sand), LUFA 2.3 (sandy loam) and LUFA 2.4 (loam). The soils were already sieved over a 2 mm sieve. After arrival the soils were adjusted to 45% MWHC by adding deionised water. The soils were pre-incubated under aerobic conditions for 3 days (20 ± 2°C in the dark). The soil characteristics are summarised in **Table A7.2.1-1**.
3.5.2	Test system	The glass flasks (300 mL) contained about 50 g soil (d.w.). Flasks for biomass control (250 mL) contained 100 g soil (d.w.). Each flask was closed by cotton wool. The samples were incubated at 20 ± 2°C under oxic conditions in the dark. The moisture of the soils was checked twice a week and, if required, was adjusted with deionised water to about 45% of its MWHC.
3.5.3	Temperature/light conditions	20 ± 2°C/dark
3.5.4	Method of preparation of test solution	Test item was applied with a concentration of 76.41 µg in 200 µL acetone/water (50/50, v/v) to each flask containing 50 g soil.
3.5.5	Application of test item	The test item solution was added drop by drop to the soil and subsequently mixed by shaking the flasks. The application rate was chosen based on typical soil concentrations resulting from the exposure assessment for biocidal use (e.g. 1.375 mg/kg dry soil) or typical field application rate (e.g. 1.375 kg Permethrin/ha). The resulting application rate corresponds to 68.75 µg Permethrin per 50 g dry soil. Every sample contained 0.40 MBq (10.8 µCi) per vessel corresponding to 76.41 µg [Phenyl-U-^{14}C]-Permethrin per 50 g soil or 1.53 mg/kg dry soil. The biomass vessels were treated with the same unlabelled amount. The untreated biomass samples received the same amount of the pure solvent of the application solution without test item (acetonitrile/water 50/50, v/v).
3.5.6	Duration of the test	93 days
3.5.7	Number of replicates	42 flasks for each soil system: 22 flasks treated with the test item (16 used for analysis and 6 as a reserve); 10 untreated flasks for determination of the biomass; 10 flasks treated with unlabelled test item for determination of the biomass.
3.5.8	Sampling	Two treated flasks taken for analysis at day 0, 2, 7, 11, 15, 21, 30, 58 and 93 after treatment. At every sampling date, a non-radiolabelled solution of Permethrin and reference items was added to optimise the

Section A7.2.1/03 Annex Point IIIA-XII.1.1 — Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

extraction efficiency.

The biomass was determined at the start, after 30 days and at the end of the study.

3.5.9 Analytical methods

The test item was extracted from the soil with 80 mL acetonitrile/water (80/20, v/v). The suspension was shaken for at least 2 hours. The dispersed soil was transferred to a 200 mL glass centrifuge tube. The extract was separated from the sediment by centrifugation at 2600 rpm for 10 minutes. The extraction was repeated at least two times. The radioactivity after each extraction step was determined by LSC. The samples from single extraction with an amount of ≥ 5% AR were combined and analysed again. The soil was extracted with 80 mL of pure acetone and the radioactivity was determined by Liquid Scintillation Counting (LSC) of an aliquot. From every sample an aliquot was diluted and mixed with scintillation cocktail.

The thin layer chromatography (TLC) was used to characterize the extractable radioactivity. The radioactivity on the TLC plates was determined optically by a digital autoradiography.

The amount of radioactivity which could still be detected (LOD) was below 25 dpm and the lowest amount quantified by TLC (LOQ) was set to 50 dpm.

3.5.10 Statistics

Single first order fittings with associated r^2 analysis.

4 RESULTS

4.2 Recovery

No mass balance established.

The % accounted radioactivity recovered at time 0 as a mean of both replicates ranged from 97 to 102% AR (see also **Table A7.2.1-2**).

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4.2 Degradation of test substance

4.2.1 Mineralisation

Not evaluated.

4.2.2 Test item

[^{14}C]Permethrin degraded in 93 days from initially 97% to 5% AR in soil LUFA 2.1 and from values between 101 and 102% AR to values below 5% AR in a period of 58 days after application in soils LUFA 2.3 and LUFA 2.4.

Details can be found in **Table A7.2.1-3** to **A7.2.1-5**.

4.2.3 Metabolites

Up to six metabolites were found in all three soils which were always below 5% AR, except one metabolite – M1 in two soils. Maximum amount of metabolite M1 was 11% AR in soil LUFA 2.3 (day 7) and 14% in soil LUFA 2.4 (day7). None of the metabolites co-eluated with a reference item. X

Details can be found in **Table A7.2.1-3** to **A7.2.1-5**.

4.2.4 Degradation rates

Degradation kinetics of [Phenyl-U-^{14}C]Permethrin and metabolite M1 were modelled using first order kinetics, which provided an acceptable fit to the data with r^2 of > 0.7. For [Phenyl-U-^{14}C]Permethrin DT_{50} values were 11 days for the LUFA 2.1, 7 days for LUFA 2.3 and 6 days for LUFA 2.4 soils, respectively For metabolite M1 the DT_{50} values were 11 and 10 days for soils LUFA 2.3 and LUFA 2.4, respectively. X

Details of calculated degradation lives for and resulting statistics can be

Section A7.2.1/03 Annex Point IIIA-XII.1.1	Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)	
	found in **Table A7.2.1-6.**	
4.2.5 Microbial biomass	The microbial biomass of each soil (treated samples) before and at the end of incubation varied from 13.4 to 6.4, 19.0 to 13.4 and 51.8 to 30.8 mg C/100 g dry soil in the LUFA 2.1, LUFA 2.3 and LUFA 2.4 soils, respectively. In the untreated samples varied rom 13.4 to 6.7, 24.1 to 13.4 and 49.6 to 31.1 mg C/100 g dry soil at the beginning and at the end of incubation in the LUFA 2.1, LUFA 2.3 and LUFA 2.4 soils, respectively. No significant differences in biomass of the treated samples were observed in comparison to the untreated samples during the entire period of the test.	X
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	OECD 307 (2002) Deviations: None The degradation rate of [Phenyl-U-^{14}C]Permethrin and possible metabolites was investigated in three different soils under aerobic laboratory conditions in the dark at 20 ± 2°C. Soil samples were treated with 76.41 µg [Phenyl-U-^{14}C]Permethrin per vessel (50 g soil), equivalent to 1.53 mg Permethrin/kg dry soil and incubated in glass flasks covered with cotton wool for up to 93 days at 45% of their MWHC. Flasks with untreated soil samples and with samples treated with non-radiolabelled test item were also incubated in order to check the biomass. Soil samples were taken for analysis on day 0, 2, 7, 11, 15, 21, 30, 58 and 93 after treatment. The radioactivity was determined by TLC. The disappearance times (DT_{50} and DT_{90}) for both test items and possible major metabolites were calculated.	
5.2 Results and discussion	The amount of extractable radioactivity decreased during the incubation time; 97-102% AR at the study start to between 4.5 – 6.9% AR at the end of the study. [^{14}C]-Permethrin in the soil decreased during the incubation time from 97% AR directly after application to 7% AR in soil LUFA 2.1 at the end of the study. In soil LUFA 2.3 the amount of extractable radioactivity decreased from 102% to 5% AR and in soil LUFA 2.4 the extractable radioactivity decreased from 101% to 6% AR during the study. [^{14}C]Permethrin was degraded from initial values between 97 – 102% AR at the study start to values ≤ 5% AR at the end of the study for soils LUFA 2.1, LUFA 2.3 and LUFA 2.4. No significant differences in biomass of the treated samples were observed in comparison to the untreated samples during the entire period of the test. The disappearance time (DT_{50} and DT_{90}) of the test item and metabolite M1 was calculated. A first order kinetics was assumed for the determination of the rate constants. The DT_{50} values range between 6 to 11 days for Permethrin. For metabolite M1 the DT_{50} values were 11 and 10 days in soils LUFA 2.3 and LUFA 2.4, respectively.	

Section A7.2.1/03 Annex Point IIIA-XII.1.1	Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)
5.3 Conclusion	Following incubation for 93 days in three different soils under aerobic conditions at 20°C, Permethrin degraded relatively fast with a degradation half-life based on SFO kinetics ranging from 6 to 11 days. For metabolite M1 the DT_{50} values were 11 and 10 days in soils LUFA 2.3 and LUFA 2.4, respectively.
5.3.1 Reliability	1
5.3.2 Deficiencies	None

Section A7.2.1/03 Annex Point IIIA-XII.1.1 — Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

19-10-11

Materials and Methods

Applicant's version is acceptable

Results and discussion

Adopt applicant's version with the addition of the following comments:

Sub-heading 4.2.3

A total of six metabolites were detected in the three soils studied but none were identified. The expected degradation product of phenyl-labelled permethrin (3-phenoxybenzoic acid and 3-phenoxybenzyl alcohol) were not detected. Therefore this study can only be used in the determination of the rate of degradation of permethrin and not its route of degradation.

Sub-heading 4.2.4

Degradation rates for Permerthrin and one metabolite have been evaluated according to the recommendations of EC document 9188/VI/97 rev 8 (2000). This has been superseded by the 2006 FOCUS guidance document SANCO/10058/2005 version 2.0 which requires the use of Chi-squared rather than r-squared values when assessing the goodness of a fit. Also the combined fitting of parent and metabolites is preferred. Therefore the applicant has repeated the kinetic analysis using the more recent guidance in another study. Please refer to the report by Stangelj, A. (2011) presented in section A7.2.1/04 for a complete analysis of degradation rates.

Sub-heading 4.2.5

For soil Lufa 2.1 microbial viability was greater than 1% of OC by day 30 but had fallen to 0.66% by day 119. Therefore only the data for the first 30 days are considered reliable for soil Lufa 2.1. For the other soils, even though there was a decrease in biomass, levels remained above 1% OC throughout the incubation and so all data points were considered acceptable in these cases.

Conclusion

The kinetic analysis performed in this study needs to be repeated in light of the comments made above regarding current guidance and microbial viability. Please refer to the report by Stangelj, A. (2011) presented in section A7.2.1/04 for this analysis. No data is presented here regarding the nature of the metabolites formed from the degradation of permethrin in soil.

Section A7.2.1/03 Annex Point IIIA-XII.1.1	Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)
Reliability	2
Acceptability	Acceptable but this study only provides information on the rate of degradation and not the route.
Remarks	In the case of soil Lufa 2.1 only the data from the first 30 days is considered reliable due to concerns about the microbial viability of the soil thereafter.
	COMMENTS FROM
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Section A7.2.1/03 Annex Point IIIA-XII.1.1 Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

10.30

10.31 Table A7.2.1-1 Soil parameters for aerobic degradation and metabolism study of phenyl-radiolabelled Permethrin

Soil name	LUFA 2.1	LUFA 2.3	LUFA 2.4
Soil description	2.1	2.3	2.4
Batch No.	F2.11510	F2.31410	F2.41410
pH ($CaCl_2$)	5.3	6.87	7.25
Organic carbon [%]	0.97	1.10	2.67
Maximum water holding capacitiy [g/100g]]	37.5	40.1	45.3
Cation exchange capacity [mval/100 g]	6.6	11.7	32.3
Soil density [g/L]	1368	1277	1232
Soil type (USDA)	loamy sand	sandy loam	loam
Particle size [%]			
<0.002 mm	2.9	8.9	26.2
0.002-0.05 mm	10.6	26.4	42.1
0.05-2.00 mm	86.5	64.7	31.7
Biomass (before application)	13.4	24.1	49.6

Table A7.2.1-2: Extractable radioactivity in soils LUFA 2.1, LUFA 2.3 and LUFA 2.4

Sampling interval [days]	Radioactivity in extracts [% AR]		
	LUFA 2.1	LUFA 2.3	LUFA 2.4
0	97.0	102.0	101.0
2	86.1	90.4	86.5
7	73.9	66.6	65.7
11	53.1	47.5	46.8
15	43.4	30.8	34.3
21	30.4	18.3	21.2
30	19.7	12.3	13.5
58	9.6	6.5	7.2
93	6.9	4.7	5.5