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		separated by centrifugation (2456 g). The humin fraction was washed with further sodium hydroxide solution (0.5 M, 2 x 25 mL) and the washings were added to the original supernatant. The supernatant was acidified to ca pH 1 with hydrochloric acid (5 M) and the precipitate that formed was washed with hydrochloric acid (0.1 M, 25 mL). The supernatant and wash were combined (fulvic acid fraction) and the washed precipitate (humic acid fraction) was reconstituted in sodium hydroxide (0.5 M, 100 mL). Radioactivity in the fulvic acid and reconstituted humic acid fractions was determined by LSC. Radioactivity in the humin fraction was determined by combustion followed by LSC
3.7.5	Metabolite identification	Non-radiolabelled test substance and potential degradation products were co-chromatographed by high performance liquid chromatography (HPLC) with reference standards. The identities of metabolites were confirmed by thin layer chromatography (TLC) of selected samples using authentic reference standards.
3.7.6	Trapping solutions	Carbon dioxide was confirmed by precipitation of radioactivity from sodium hydroxide solution traps following the addition of barium chloride solution.
3.8	**Analytical methods**	HPLC-UV methods were used for the radiochemical purity and sample analysis (column: YMC-ODS-A, 25 cm x 4.6 mm, 5 µm using a 0.1% phosphoric acid/acetonitrile linear binary gradient system, flow rate 1 mL/min with UV detection at 220 nm). Extracts were co-chromatographed with the appropriate non-radiolabelled standards. Single dimension TLC solvent systems were used for the determination of radiochemical purity and sample analysis (Whatman K6F silica gel 60 Å plates with heaxane:dichloromethane 1:1 v/v or toluene:hexane:acetic acid 15:3:2 v/v/v solvent). Extracts were co-chromatographed with appropriate non-radiolabelled standards. Following chromatography, radiolabelled compounds were detected by preparation of a radioluminogram of the TLC plate using a Fuji BAS 1500 Bio-image analyser and non-radiolabelled compounds were visualised by irradiation with UV light at 254 nm. Chromatograms were evaluated from the radioluminograms using the associated software (Tina version 2.09g).
3.9	**Determination of radioactivity**	The exact concentrations of radioactivity in the [^{14}C]-cypermethrin treatment solutions at the time of application was determined by dispensing triplicate aliquots (50 µL pre- and post-dose) into volumetric flasks (10 mL) and making up to volume with acetonitrile. Aliquots (3 x 100 µL) of these solutions were assayed by liquid scintillation counting (LSC). The values obtained were used to calculate the quantity of [^{14}C]-cypermethrin applied to each unit. The radioactivity in samples and traps was determined by measuring the total sample weight and assaying duplicate aliquots by LSC. Weighed portions of these samples were added directly to scintillant (5 mL) prior to LSC quantification. Following extraction, air-dried sediment samples were ground and sub-sampled (ca 0.2 g) in triplicate prior to combustion in oxygen using a Harvey Biological Sample Oxidiser, model OX-300 or 500. The combusted products were absorbed in Permafluor® E+ and Carbo-Sorb® E (2:1 v/v) and the radioactivity absorbed was determined by LSC.

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Carbon-14 standards were combusted at the beginning and at regular intervals throughout the day to check the carry-over between samples and to determine the efficiency of the combustion process. Combustion and trapping efficiencies were 99 ± 4% and therefore no correction factors were applied to the reported data.

Radioactivity was measured using a Packard Tricarb model 1900TR liquid scintillation counter with facilities for computing quench corrected disintegrations per minute (dpm).

3.10 Determination of DT50, DT75 and DT90

The percent of applied radioactivity present as test substance was plotted against the incubation time in days. A curve was constructed through the data points using non-linear regression analysis to give a line of best fit.

The equation used for the surface water and total system was a single-phase exponential model (i.e. first order kinetics):

$$y = C_o \times e^{-kt}$$

Where y is the percent of test substance at time t days and C_0 is the computed initial concentration and k is the rate constant (slope). DT-50, DT-75 and DT-90 values were calculated from the equation of the lines. They were calculated as the value of t which gave a value of y equal to 50% (DT-50), 25% (DT-75) and 10% (DT-90) of the calculated initial concentration (intercept on the y-axis).

A different equation was used for the sediment as the level of cypermethrin accumulated before it decreased:

$$y = (b \times e^{-k_1 t}) - (a \times e^{-k_a t})$$

Where y is the percent of cypermethrin at time t days and a and b are constants. DT-50, DT-75 and DT-90 values were calculated as the time taken to decrease from the maximum computed concentration to half, 25% and to 10% of the maximum computed concentration, respectively.

4 RESULTS

4.1 Recovery and Distribution of radioactivity

Site A water-sediment system

The mean recoveries of radioactivity for incubation groups A (phenoxy label) and C (cyclopropyl label) at each sampling interval were ≥ 90%.

Mean levels of applied radioactivity in the surface water decreased from 66 and 69% at day 0 to <1 and 33% at 100 days following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl]cypermethrin, respectively. Mean levels of applied radioactivity extracted from the sediment into acetonitrile: water (1:1, v/v) increased to maximum values of 62% at 3 days and 68% at 1 day following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl]cypermethrin, respectively.

In the Site A system, mean levels of applied radioactivity unextracted from sediment increased from <1% of applied radioactivity, at day 0, to maximum values of 21 and 10% at 45 days following application of [^{14}C-phenoxy]cypermethrin and 100 days following application of [^{14}C-cyclopropyl]cypermethrin, respectively.

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Volatile radioactivity recovered in traps increased to mean values of 65 and 25% of applied radioactivity at 100 days, following application of [^{14}C-phenoxy]cypermethrin and [^{14}C-cyclopropyl]cypermethrin, respectively.

See Table A7_1_2_2_2-5

Swiss Lake water-sediment system

The mean recoveries of radioactivity for incubation groups B (phenoxy label) and D (cyclopropyl label) at each sampling interval were ≥ 90%. There was one exception of 89% at 100 days for dose group D.

The mean levels of applied radioactivity in the surface water decreased from 44 and 60% at day 0 to 1 and 19% at 100 days following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl]cypermethrin, respectively. Mean levels of applied radioactivity extracted from the sediment into acetonitrile: water (1:1, v/v) increased to maximum values of 54% at 3 days and 64% at 1 day following application of [^{14}C-phenoxy]cypermethrin and [^{14}C-cyclopropyl]cypermethrin, respectively.

In the Swiss Lake system, mean levels of applied radioactivity unextracted from sediment increased from <1% at day 0, to maximum values of 18 and 19% at 100 days following application of [^{14}C-phenoxy]cypermethrin and [^{14}C-cyclopropyl] cypermethrin, respectively.

Volatile radioactivity recovered in traps increased to mean values of 69 and 30% of applied radioactivity at 100 days, following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl]cypermethrin, respectively.

See Table A7_1_2_2_2-6

4.2 Bound residue analysis

Bound residue extraction of unextracted radioactivity showed that in the Site A system (both labels) and the Swiss Lake system (phenoxy label), radioactivity was evenly distributed across each fraction. In the Swiss Lake system (cyclopropyl label), most of the radioactivity was associated with the fulvic acid fraction.

See Table A7_1_2_2_2-7

4.3 Degradation of test substance

Site A water-sediment system

Levels of cypermethrin in the complete Site A system decreased from 96% and 91% at 0 days, to 8% and 7% at 100 days following application of [^{14}C-phenoxy] cypermethrin and from 96% and 95% at 0 days, to 2% and 3% at 100 days following application of [^{14}C-cyclopropyl] cypermethrin.

In the sediment, levels of cypermethrin increased to a maximum of 60% at 3 days and 66% at 1 day, decreasing to 7% and 2% at 100 days following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl] cypermethrin respectively.

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Swiss Lake water-sediment system

Levels of cypermethrin in the complete Swiss Lake system decreased from 94% and 92% at 0 days, to 3% and 9% at 100 days following application of [^{14}C-phenoxy] cypermethrin and from 97% and 96% at 0 days, to 6% and 3% at 100 days following application of [^{14}C-cyclopropyl] cypermethrin.

In the sediment, levels of cypermethrin increased to a maximum of 63% at 0 days and 68% at 1 day, decreasing to 2% and 3% at 100 days following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl] cypermethrin respectively.

4.4 Degradation products

Site A water-sediment system

3-Penoxybenzoic acid was present up to 21% in surface water and 11% in sediment at 10 days after application with [^{14}C-phenoxy] cypermethrin. Following application of [^{14}C-cyclopropyl] cypermethrin, (1RS)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (TDCVC) was present up to 38% in surface water at 10 days and 20% in sediment at 58 days. Also present was (1RS)-*cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (CDCVC) up to 15% in both surface water at 29 days and sediment at 58 days.

Following application of [^{14}C-cyclopropyl] cypermethrin, one unidentified metabolite (Unknown 1) reached 9% of applied radioactivity in one unit (C9) at 58 days and also reached 6 and 10% in both 100 day units (C11 and C12).

See Table A7_1_2_2_2-8 and Table A7_1_2_2_2-10

Swiss Lake water-sediment system

3-Penoxybenzoic acid was present up to 12% in surface water at 29 days and 6% in sediment at 3 days after application with [^{14}C-phenoxy] cypermethrin. Following application of [^{14}C-cyclopropyl] cypermethrin, (1RS)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (TDCVC) was present up to 44% in surface water at 3 days and 16% in sediment at 58 days. Also present was (1RS)-*cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (CDCVC) up to 22% in surface water at 29 days and 9% in sediment at 58 days.

Unknown 1 again appeared following application of [^{14}C-cyclopropyl] cypermethrin, reaching 9% in one unit at 58 days (D10) and also reaching 14% and 11% in both 100 day units (D11 and D12).

A further unidentified metabolite (Unknown 3) reached 6% in one unit after 3 days (D3). This metabolite then decreased over the remaining incubation period to <1% applied radioactivity. Similarly, another unknown metabolite (Unknown A) reached 6% at day 10 in one unit (B6), with levels then decreasing to <1% applied radioactivity.

See Table A7_1_2_2_2-9 and A7_1_2_2_2-11

Section A7.1.2.2.2 Water/sediment degradation study

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4.5 Degradation rates

Using first order kinetics, degradation rates were calculated for both cis and trans cypermethrin in both water-sediment systems and also for the identified metabolites 3-phenoxybenzoic acid, TDCVC and CDCVC.

See Table A7_1_2_2_2-12

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The rate of degradation of cypermethrin was been studied in two water-sediment systems over a period of 100 days according to OECD guideline 308. The application rate was 4.3 µg per unit (water surface area of 15.9 cm^2). This was calculated as the equivalent of ten times the maximum drift calculation of 3 µg/L (i.e. 30 µg/L) for plant protection applications of cypermethrin.

Samples of the 2 mm sieved sediment (3 cm depth in a 4.5 cm internal diameter vessel) and 0.2 mm sieved water (9 cm above sediment) were dispensed into individual glass vessels through which moistened air was drawn. The units were maintained in the dark at 20 ± 2°C for 25 days to enable equilibrium to be established. After treatment with radiolabelled test substance, the air drawn over the surface of the units was passed through a series of traps (ethanediol, 2% paraffin in xylene and two 2M sodium hydroxide solution traps) to collect evolved radiolabelled material.

Dosing was carried out by dropwise application of the radiolabelled test substance (4.3 µg, 21.22 kBq for phenoxy label; 4.3 µg, 20.34 kBq for cyclopropyl label), in acetonitrile (92 µL or 90 µL for the phenoxy and cyclopropyl labels respectively) to the surface water of each water-sediment system. The water-sediment units were incubated in the dark at 20 ± 2°C.

The water was separated from the sediment by aspiration and the two phases were separately analysed. Non-radiolabelled test substance and potential degradation products were co-chromatographed by high performance liquid chromatography (HPLC) with reference standards. The identities of metabolites were confirmed by thin layer chromatography (TLC) of selected samples using authentic reference standards. Carbon dioxide was confirmed by precipitation of radioactivity from sodium hydroxide solution traps following the addition of barium chloride solution.

5.2 Results and discussion

5.2.1 Recovery

Mean recoveries of radioactivity for both the [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl]cypermethrin were ≥90% at each sampling interval, with one exception of 89% at 100days for dose group D (cyclopropyl label, Swiss Lake).

5.2.2 Distribution

In the Site A system, levels of applied radioactivity in the surface water decreased from 66 and 69% at day 0 to <1 and 33% at 100 days following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl]cypermethrin, respectively. Mean levels of applied radioactivity extracted from the sediment increased to maximum values of 62% at 3 days and 68% at 1 day following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl]cypermethrin, respectively.

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		Applied radioactivity unextracted from sediment increased from <1% of applied radioactivity, at day 0, to maximum values of 21 and 10% at 45 days following application of [^{14}C-phenoxy] cypermethrin and 100 days following application of [^{14}C-cyclopropyl]cypermethrin, respectively. Bound residue analysis showed radioactivity was distributed evenly across each fraction. In the Swiss Lake system, levels of applied radioactivity in the surface water decreased from 44 and 60% at day 0 to 1 and 19% at 100 days following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl] cypermethrin, respectively. Mean levels of applied radioactivity extracted from the sediment increased to maximum values of 54% at 3 days and 64% at 1 day following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl] cypermethrin, respectively. Applied radioactivity unextracted from sediment increased from <1% at day 0, to maximum values of 18 and 19% at 100 days following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl] cypermethrin, respectively. Bound residue analysis showed radioactivity was distributed evenly across each fraction.
5.2.3	Mineralization	In the Site A system, volatile radioactivity recovered in traps increased to mean values of 65 and 25% of applied radioactivity at 100 days, following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl]cypermethrin, respectively. In the Swiss Lake system, volatile radioactivity increased to mean values of 69 and 30% of applied radioactivity at 100 days, following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl] cypermethrin, respectively.
5.2.4	Identification of radioactivity	In the Site A system, levels of cypermethrin decreased 96% and 91% at 0 days, to 8% and 7% at 100 days following application of [^{14}C-phenoxy] cypermethrin and from 96% and 95% at 0 days, to 2% and 3% at 100 days following application of [^{14}C-cyclopropyl] cypermethrin. In the sediment, levels of cypermethrin increased to a maximum of 60% at 3 days and 66% at 1 day, decreasing to 7% and 2% at 100 days following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl] cypermethrin respectively. In the Swiss Lake system, levels of cypermethrin decreased from 94% and 92% at 0 days, to 3% and 9% at 100 days following application of [^{14}C-phenoxy] cypermethrin and from 97% and 96% at 0 days, to 6% and 3% at 100 days following application of [^{14}C-cyclopropyl] cypermethrin. In the sediment, levels of cypermethrin increased to a maximum of 63% at 0 days and 68% at 1 day, decreasing to 2% and 3% at 100 days following application of [^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropyl] cypermethrin respectively. The significant metabolites were 3-phenoxybenzoic acid (following application of the phenoxy label) and TDCVC and CCVC (following application of the cyclopropyl label). In the Site A system, 3-Penoxybenzoic acid was present up to 21% in surface water and 11% in sediment, TDCVC was present up to 38% in surface water and 20% in sediment and CDCVC up to 15% in both surface water and sediment. In the Swiss lake system, 3-phenoxybenzoic acid was present up to 12% in

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		surface water and 6% in sediment, TDCVC was present up to 44% in surface water and 16% in sediment and CDCVC up to 22% in surface water and 9% in sediment One unidentified metabolite (Unknown 1) was present in units from both systems, reaching a maximum of 10% and 14% in 100 day units. Despite extensive development of sample cleanup, it was not possible to identify this metabolite within the study. This metabolite is thought to be related to the TDCVC.	
5.2.5	Rate of degradation	Degradation of cypermethrin was very rapid in both systems with DT50 values between 3.5 and 9.8 days in sediment. Dissipation from the water phase was more rapid with DT50 values of 0.5 days in both systems.	
5.3	**Conclusion**	Degradation of cypermethrin was very rapid in both water-sediment systems was very rapid (DT50 values between 3.5 and 9.8 days in sediment and 0.5 days in the water phase). The significant metabolites were 3-phenoxybenzoic acid (from the phenoxy label), TDCVC and CDCVC (from the cyclopropyl label). A further unknown metabolite (Unknown 1) was identified at levels >10% in the units dosed with the cyclopropyl label. In both systems there were no other single unidentified metabolites which individually comprised 5% of applied radioactivity at any timepoint.	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2007
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is adopted.
Conclusion	The sentence of the first paragraph should be modified as follow: "Degradation of cypermethrin *cis:trans*/40:60 was very rapid in both water-sediment system(DT50 values between 3.5 and 9.8 days in sediment and 0.5 days in the water phase)."
Reliability	1
Acceptability	Acceptable
Remarks	Unknown metabolite at levels>10%.
	COMMENTS FROM ...

Section A7.1.2.2.2 Water/sediment degradation study

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Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table A7_1_2_2_2-1: Radiolabelled test substance

Isomer name	Lot No.	Specific radioactivity	Radiochemical purity
[^{14}C-phenoxy]cypermethrin-cis	04BLY115b	2.2 mCi (55.4 mCi/mmol)	100%
[^{14}C-phenoxy]cypermethrin-*trans*	04BLY115b	3.7 mCi (55.4 mCi/mmol)	100%
[^{14}C-cyclopropyl]cypermethrin-*cis*	04BLY115b	0.9 mCi (53 mCi/mmol)	100%
[^{14}C-cyclopropyl]cypermethrin-*trans*	04BLY115b	1.7 mCi (53 mCi/mmol)	100%

Table A7_1_2_2_2-2: Sediment properties

Water-sediment system name	pH (H_20)	OM %	Sand (UK) %	Silt (UK) %	Clay (UK) %	CEC mEq/100g	Biomass µg C/g soil[1]
Site A*	7.4	8.3	30	63	7	21.0	769
Swiss Lake	6.1	1.6	79	20	1	5.68	144

*Site A = Calwich Abbey Lake
[1] On arrival at Covance
CEC = cation exchange capacity
OM = organic matter

Table A7_1_2_2_2-3: Water properties

Water-sediment system name*	pH (H_20) on collection	Total organic carbon (mg/L)	Hardness (mg $CaCO_3$/L)	Suspended solids (mg/L)
Site A	8.22	2.1	234	4.0
Swiss Lake	5.85	10.2	34	9

*Site A = Calwich Abbey Lake

Table A7_1_2_2_2-4: Incubation groups

Incubation group	Radiolabel	System name	Number of units*
A	Phenyl	Site A	16
B	Phenyl	Swiss Lake	16
C	Cyclopropyl	Site A	16
D	Cyclopropyl	Swiss Lake	16

*Exclude control units used for biomass determination

Table A7_1_2_2_2-5: Mean % Recovery of applied radioactivity from the site A water-sediment system

Label	Sampling Interval (days)	Surface Water[1]	Sediment Extract	Unextracted from Sediment	Total Volatiles	$C0_2$ aq[2]	Mass Balance
Phenoxy	0	66.0	28.3	0.8	NA	NA	95.0
Phenoxy	1	28.3	60.1	4.2	1.2	NA	93.7
Phenoxy	3	14.5	61.8	6.7	4.8	5.2	92.8
Phenoxy	10	21.0	53.9	8.2	7.2	NA	90.3
Phenoxy	29	1.7	22.6	14.9	48.7	2.0	89.8
Phenoxy	45	0.5	13.2	20.5	59.9	NA	94.0
Phenoxy	100	0.5	11.6	18.8	65.3	NA	96.1
Cyclopropyl	0	69.1	27.9	0.7	NA	NA	97.6
Cyclopropyl	1	28.8	67.7	2.4	0.1	NA	98.9
Cyclopropyl	3	31.7	65.3	2.9	0.3	NA	100.1
Cyclopropyl	10	51.5	42.9	3.3	0.8	NA	98.4
Cyclopropyl	29	46.3	40.5	6.3	5.2	NA	98.2
Cyclopropyl	58	37.3	41.3	6.1	11.2	NA	95.9
Cyclopropyl	100	33.2	29.5	10.1	25.1	NA	97.7

[1]Includes acetonitrile pipette wash
[2] aqueous carbon dioxide
NA=Not Applicable

Table A7_1_2_2_2-6: Mean % Recovery of applied radioactivity from the Swiss Lake water-sediment system

Unit	Sampling Interval (days)	Surface Water[1]	Sediment Extract	Unextracted from Sediment	Total Volatiles	$C0_2$ aq[2]	Mass Balance
Phenoxy	0	43.5	51.2	ND	NA	NA	94.7
Phenoxy	1	19.9	51.0	9.5	5.9	8.2	94.4
Phenoxy	3	11.8	53.7	12.2	12.6	5.6	95.7
Phenoxy	10	9.4	26.6	18.0	31.4	9.7	95.0
Phenoxy	29	14.8	17.1	14.4	43.1	3.4	92.6
Phenoxy	45	4.1	15.9	13.9	57.7	0.0	91.5
Phenoxy	100	1.0	8.1	18.0	68.8	NA	95.8
Cyclopropyl	0	60.1	37.8	ND	NA	NA	97.9
Cyclopropyl	1	34.3	63.8	0.4	0.1	NA	98.5
Cyclopropyl	3	50.4	46.9	1.3	0.6	NA	99.1
Cyclopropyl	10	60.6	33.8	2.7	1.0	NA	98.1
Cyclopropyl	29	58.2	31.1	3.5	5.0	NA	97.8
Cyclopropyl	58	43.6	29.1	9.5	10.0	NA	92.2
Cyclopropyl	100	18.5	22.5	18.6	29.7	NA	89.2

[1]Includes acetonitrile pipette wash
[2] aqueous carbon dioxide
NA=Not Applicable

Table A7_1_2_2_2-7: Bound residue analysis (expressed as % AR)

System	Label	Time (days)	Unextracted radioactivity	Bound reside extract	Fulvic acid	Humic acid	Humin
Site A	Phenoxy	45	20.7	11.7	5.7	5.5	8.4
Swiss Lake	Phenoxy	10	20.0	15.0	6.3	8.0	4.9
Site A	Cyclopropyl	29	6.7	4.3	3.2	1.1	2.1
Swiss Lake	Cyclopropyl	100	18.8	14.6	11.0	4.0	3.3

Table A7_1_2_2_2-8: %Recovery of AR - Site A system, [14C-phenoxy] cypermethrin application

Unit	(days)	Sample Type	TPC	CPC	Total Cypermethrin	3PBOH	3PBA	3PBAD	Unknown A	Total Other Unknown	Unresolved Background	Total
A1	0	Water	37.6	23.5	61.2	ND	ND	ND	ND	0.5	0.1	61.7
		Sediment	20.4	14.2	34.6	ND	ND	ND	ND	ND	0.1	34.7
		Total	58.1	37.7	95.8	ND	ND	ND	ND	0.5	0.1	96.4
A2	0	Water	42.1	27.0	69.1	ND	ND	ND	ND	0.5	0.2	69.8
		Sediment	12.7	9.1	21.9	ND	ND	ND	ND	ND	0.0	21.9
		Total	54.8	36.2	90.9	ND	ND	ND	ND	0.5	0.2	91.7
A13	1	Water	5.8	7.1	12.9	ND	11.6	ND	0.1	0.5	0.1	25.2
		Sediment	30.7	28.7	59.4	ND	ND	ND	0.8	ND	0.1	60.3
		Total	36.6	35.7	72.3	ND	11.6	ND	0.9	0.5	0.2	85.5
A14	1	Water	8.1	8.6	16.6	ND	9.4	ND	0.5	0.5	0,1	27.2
		Sediment	27.7	28.6	56.3	ND	1,5	ND	1.8	ND	0.2	59.8
		Total	35.8	37.1	72.9	ND	10.9	ND	2.3	0.5	0.4	87.0
A3	3	Water	3.0	2.9	5.9	ND	6.2	ND	ND	ND	0.1	12.2
		Sediment	27.8	32.2	60.0	ND	1.5	ND	1.4	ND	0.1	63.0
		Total	30.7	35.1	65.9	ND	7.7	ND	1.4	ND	0.2	75.2
A4	3	Water	1.5	2.2	3.6	ND	10.2	ND	0.1	0.5	0.1	14.5
		Sediment	21.7	31.2	52.9	ND	3.7	ND	2.1	1.6	0.2	60.5
		Total	23.2	33.4	56.5	ND	13.9	ND	2.2	2.1	0.3	75.0
A5	10	Water	0.2	1.1	1.3	ND	21.2	ND	ND	1.0	0.1	23.6
		Sediment	19.9	30.5	50.4	ND	11.4	ND	1.7	0.8	0.1	64.3
		Total	20.1	31.5	51.7	ND	32.6	ND	1.7	1.8	0.2	87.9
A6	10	Water	0.1	0.6	0.8	ND	14.2	ND	ND	0.9	0.2	16.1
		Sediment	10.8	25.2	36.0	ND	4.0	ND	3.3	ND	0.1	43.5
		Total	10.9	25.9	36.8	ND	18.2	ND	3.3	0.9	0.3	59.6
A7	29	Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Sediment	1.2	8.6	9.7	ND	3.7	ND	0.8	0.5	0.2	14.9
		Total	1.2	8.6	9.7	ND	3.7	ND	0.8	0.5	0.2	14.9
A8	29	Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Sediment	10.0	14.2	24.2	ND	3.6	ND	1.0	1.0	0.5	30.3
		Total	10.0	14.2	24.2	ND	3.6	ND	1.0	1.0	0.5	30.3
A9	45	Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Sediment	1.9	7.8	9.7	ND	2.3	ND	0.6	0.2	0.1	12.8
		Total	1.9	7.8	9.7	ND	2.3	ND	0.6	0.2	0.1	12.8
A10	45	Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Sediment	1.6	6.5	8.0	ND	3.6	ND	1.1	0.5	0.3	13.5
		Total	1.6	6.5	8.0	ND	3.6	ND	1.1	0.5	0.3	13.5
A11	100	Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Sediment	1.1	6.4	7.5	ND	2.1	ND	0.5	1.5	0.2	11.9
		Total	1.1	6.4	7.5	ND	2.1	ND	0.5	1.5	0.2	11.9
A12	100	Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Sediment	1.8	5.5	7.3	ND	1.9	ND	0.6	1.3	0.1	11.2
		Total	1.8	5.5	7.3	ND	1.9	ND	0.6	1.3	0.1	11.2

Table A7_1_2_2_2-9: %Recovery of AR – Swiss Lake system, [14C-phenoxy] cypermethrin application

Unit	Days	Sample Type	TPC	CPC	Total Cypermethrin	3PBOH	3PBA	3PBAD	Unknown A	Total other Unknown	Unresolved Background	Total
B1	0	Water	19.2	11.9	31.2	ND	ND	ND	ND	0.7	0.3	32.2
		Sediment	37.2	25.6	62.8	ND	0.4	ND	ND	ND	0.0	63.2
		Total	56.4	37.5	94.0	ND	0.4	ND	ND	0.7	0.3	95.4
B2	0	Water	32.5	20.9	53.4	ND	ND	ND	ND	0.9	0.5	54.8
		Sediment	23.4	15.1	38.5	ND	ND	ND	ND	0.6	0.1	39.2
		Total	55.9	36.0	91.9	ND	ND	ND	ND	1.5	0.5	94.0
B13	1	Water	5.2	7.4	12.6	ND	2.1	ND	0.7	1.6	0.1	17.0
		Sediment	20.6	28.9	49.6	ND	0.2	ND	2.5	0.1	0.1	52.4
		Total	25.8	36.3	62.1	ND	2.3	ND	3.2	1.6	0.2	69.4
B14	1	Water	5.7	6.2	12.0	ND	1.8	ND	0.8	3.2	0.1	17.8
		Sediment	19.5	27.7	47.2	ND	0.1	ND	0.9	0.8	0.4	49.5
		Total	25.3	33.9	59.2		1.9	ND	1.7	4.0	0.5	67.3
B3	3	Water	2.2	4.5	6.6	ND	4.5	ND	0.2	0.8	0.1	12.2
		Sediment	18.7	30.5	49.2	ND	0.2	ND	2.1	1.9	0.2	53.6
		Total	20.9	35.0	55.8	ND	4.6	ND	2.3	2.7	0.3	65.8
B4	3	Water	1.1	3.9	4.9	ND	0.6	ND	0.4	1.5	0.1	7.5
		Sediment	15.7	28.1	43.8	ND	6.3	ND	1.1	2.0	0.4	53.7
		Total	16.8	32.0	48.8	ND	6.9	ND	1.6	3.5	0.5	61.2
B5	10	Water	0.6	1.4	2.0	ND	1.7	ND	0.3	2.1	0.4	6.4
		Sediment	8.7	20.0	28.7	ND	ND	ND	2.8	0.0	0.2	31.7
		Total	9.3	21.4	30.7	ND	1.7	ND	3.1	2.1	0.6	38.1
B6	10	Water	0.0	1.6	1.7	ND	2.5	ND	0.8	3.5	0.2	8.6
		Sediment	3.2	12.9	16.1	ND	0.1	ND	4.9	0.2	0.2	21.5
		Total	3.3	14.5	17.7	ND	2.7	ND	5.7	3.6	0.4	30.1
B7	29	Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Sediment	1.4	7.3	8.6	NA	1.4	NA	1.4	2.9	0.3	14.6
		Total	1.4	7.3	8.6	NA	1.4	NA	1.4	2.9	0.3	14.6
B8	29	Water	ND	0.6	0.6	ND	11.9	ND	ND	7.2[1]	0.4	20.1
		Sediment	1.3	7.8	9.0	ND	4.3	ND	0.8	5.0[2]	0.4	19.5
		Total	1.3	8.4	9.6	ND	16.2	ND	0.8	12.2	0.8	39.6
B9	45	Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Sediment	4.9	11.5	16.4	ND	1.7	ND	1.0	3.1	0.4	22.7
		Total	4.9	11.5	16.4		1.7		1.0	3.1	0.4	22.7
B10	45	Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Sediment	1.6	4.8	6.3	ND	0.6	ND	0.8	1.4	0.0	9.1
		Total	1.6	4.8	6.3		0.6		0.8	1.4	0.0	9.1
B11	100	Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Sediment	0.7	2.0	2.7	ND	0.4	ND	0.8	1.0	0.0	4.9
		Total	0.7	2.0	2.7	ND	0.4	ND	0.8	1.0	0.0	4.9
B12	100	Water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Sediment	3.2	6.0	9.1	ND	0.4	ND	1.1	0.6	0.2	11.3
		Total	3.2	6.0	9.1	ND	0.4	ND	1.1	0.6	0.2	11.3

Table A7_1_2_2_2-10: %Recovery of AR - Site A system, [14C-cyclopropyl] cypermethrin application

Unit	days	Sample Type	TCC	CCC	Total Cypermethrin	TDCVC	CDCVC	Unk 1	Unk 2	Unk 3	Total Other Unknown	Unresolved Background	Total
C1	0	Water	38.5	24.4	62.9	0.1	0.1	ND	ND	ND	0.1	0.3	63.4
		Sediment	19.9	12.9	32.8	ND	ND	ND	ND	ND	0.1	0.3	33.2
		Total	58.3	37.4	95.7	0.1	0.1	ND	ND	ND	0.2	0.5	96.6
C2	0	Water	44.3	28.6	72.9	ND	ND	ND	ND	ND	0.1	0.3	73.3
		Sediment	13.1	9.1	22.2	0.2	ND	ND	ND	ND	ND	0.2	22.5
		Total	57.4	37.7	95.1	0.2	ND	ND	ND	ND	0.1	0.4	95.8
C13	1	Water	7.0	5.4	12.4	11.3	0.9	ND	0.1	ND	ND	0.1	24.8
		Sediment	34.8	30.7	65.5	0.8	ND	ND	1.7	0.4	ND	0.4	68.7
		Total	41.8	36.0	77.8	12.1	0.9	ND	1.8	0.4	ND	0.5	93.5
C14	1	Water	8.2	7.3	15.4	12.9	0.9	ND	ND	0.2	ND	0.1	29.5
		Sediment	31.8	30.0	61.8	3.4	0.1	ND	0.8	0.4	ND	0.1	66.6
		Total	40.0	37.2	77.2	16.3	1.0	ND	0.8	0.6	ND	0.3	96.1
C3	3	Water	0.7	1.5	2.2	28.6	3.4	ND	ND	ND	ND	0.3	34.4
		Sediment	22.6	33.4	56.1	3.3	ND	ND	2.4	1.7	ND	0.3	63.6
		Total	23.3	34.9	58.2	31.9	3.4	ND	2.4	1.7	ND	0.5	98.0
C4	3	Water	1.2	1.3	2.6	22.2	3.0	ND	ND	ND	ND	0.2	28.0
		Sediment	26.8	32.6	59.4	2.5	0.2	ND	2.9	1.6	ND	0.3	67.0
		Total	28.1	33.9	62.0	24.8	3.2	ND	2.9	1.6	ND	0.6	95.0
C5	10	Water	0.1	0.5	0.6	35.1	11.4	ND	ND	ND	ND	0.1	47.2
		Sediment	11.0	19.8	30.8	8.2	3.0	ND	0.8	2.7	ND	0.5	45.9
		Total	11.1	20.3	31.4	43.2	14.5	ND	0.8	2.7	ND	0.6	93.1
C6	10	Water	0.2	2.2	2.4	38.3	10.8	0.8	ND	ND	0.2	0.2	52.7
		Sediment	5.5	17.9	23.3	8.8	2.7	ND	1.8	2.6	ND	0.6	39.8
		Total	5.6	20.1	25.7	47.2	13.5	0.8	1.8	2.6	0.2	0.8	92.5
C7	29	Water	ND	ND	ND	28.4	9.8	3.4	ND	ND	ND	0.3	41.9
		Sediment	4.2	13.7	17.9	15.9	7.5	0.5	0.3	1.1	ND	0.5	43.7
		Total	4.2	13.7	17.9	44.2	17.3	3.9	0.3	1.1	ND	0.8	85.6
C8	29	Water	ND	ND	ND	31.8	14.5	2.6	ND	ND	ND	0.9	49.9
		Sediment	2.3	9.2	11.5	15.6	7.6	ND	0.2	1.6	0.4	0.4	37.3
		Total	2.3	9.2	11.5	47.4	22.1	2.6	0.2	1.6	0.4	1.3	87.2
C9	58	Water	ND	ND	ND	18.1	7.1	4.7	ND	ND	0.3	0.2	30.5
		Sediment	3.8	2.5	6.3	19.1	15.2	4.6	ND	ND	2.0	0.4	47.6
		Total	3.8	2.5	6.3	37.2	22.3	9.4	ND	ND	2.3	0.6	78.1
C10	58	Water	ND	ND	ND	25.9	12.6	3.9	ND	ND	0.4	0.3	43.1
		Sediment	0.8	4.8	5.7	19.9	8.9	ND	ND	ND	ND	0.4	34.9
		Total	0.8	4.8	5.7	45.9	21.4	3.9	ND	ND	0.4	0.8	78.0
C11	100	Water	ND	ND	ND	19.6	9.9	2.9	ND	ND	1.3	0.2	33.9
		Sediment	0.6	1.8	2.4	13.2	9.5	2.7	0.1	0.2	1.3	0.2	29.5
		Total	0.6	1.8	2.4	32.7	19.4	5.5	0.1	0.2	2.6	0.4	63.4
C12	100	Water	ND	ND	ND	16.5	7.4	6.4	ND	ND	0.7	0.0	31.0
		Sediment	1.0	1.6	2.6	14.3	8.0	3.2	0.4	0.2	0.7	0.2	29.4
		Total	1.0	1.6	2.6	30.8	15.4	9.6	0.4	0.2	1.4	0.2	60.4

Table A7_1_2_2_2-11: %Recovery of AR–Swiss Lake system, [14C-cyclopropyl] cypermethrin application

Unit	(days)	Sample Type	TCC	CCC	Total Cypermethrin	TDCVC	CDCVC	Unk 1	Unk 2	Unk 3	Total Other Unknown	Unresolved Background	Total
D1	0	Water	41.0	26.9	67.9	ND	ND	ND	ND	ND	0.1	0.3	68.3
		Sediment	17.5	11.5	29.0	0.1	ND	ND	ND	ND	ND	0.3	29.4
		Total	58.6	38.3	96.9	0.1	ND	ND	ND	ND	0.1	0.6	97.7
D2	0	Water	29.5	20.8	50.4	0.2	ND	ND	ND	ND	0.3	0.4	51.3
		Sediment	27.6	18.1	45.7	0.1	0.1	ND	ND	ND	ND	0.3	46.2
		Total	57.1	39.0	96.1	0.4	0.1	ND	ND	ND	0.3	0.6	97.5
D13	1	Water	4.4	4.8	9.1	14.2	0.9	ND	0.4	ND	0.2	0.0	24.8
		Sediment	35.4	32.7	68.1	0.9	ND	ND	1.4	1.1	ND	0.4	71.9
		Total	39.8	37.4	77.2	15.1	0.9	ND	1.8	1.1	0.2	0.5	96.7
D14	1	Water	5.8	7.1	12.9	25.5	2.4	ND	0.7	ND	0.1	0.2	41.8
		Sediment	23.4	28.9	52.3	1.1	ND	ND	1.2	0.7	ND	0.3	55.6
		Total	29.2	35.9	65.1	26.7	2.4	ND	1.9	0.7	0.1	0.5	97.4
D3	3	Water	0.8	2.8	3.6	44.0	7.9	0.6	0.3	ND	ND	0.4	56.8
		Sediment	9.0	26.6	35.6	3.4	0.3	ND	1.8	5.7	0.3	0.3	47.4
		Total	9.8	29.4	39.2	47.4	8.2	0.6	2.1	5.7	0.3	0.6	104.2
D4	3	Water	1.6	3.2	4.7	34.4	3.7	0.2	0.1	0.1	ND	0.2	43.4
		Sediment	15.2	25.6	40.8	2.0	0.2	ND	0.8	2.3	0.2	0.1	46.4
		Total	16.8	28.7	45.5	36.3	3.9	0.2	1.0	2.4	0.2	0.3	89.8
D5	10	Water	0.3	1.3	1.6	41.2	13.5	1.5	ND	ND	1.5	0.5	59.7
		Sediment	6.8	16.1	22.9	5.2	1.6	0.3	0.7	3.2	0.5	0.6	35.1
		Total	7.1	17.3	24.4	46.4	15.2	1.8	0.7	3.2	2.0	1.1	94.8
D6	10	Water	0.2	1.8	2.0	41.5	12.9	1.4	ND	ND	1.7	0.9	60.5
		Sediment	5.2	15.8	21.0	5.9	1.5	ND	1.0	2.3	0.5	0.3	32.5
		Total	5.4	17.7	23.0	47.4	14.5	1.4	1.0	2.3	2.2	1.3	93.0
D7	29	Water	ND	ND	ND	35.6	22.4	2.5	ND	ND	1.3	0.4	62.2
		Sediment	2.9	5.9	8.8	10.8	5.7	ND	0.5	ND	ND	0.3	26.1
		Total	2.9	5.9	8.8	46.4	28.1	2.5	0.5	ND	1.3	0.7	88.3
D8	29	Water	0.2	0.2	0.4	29.8	16.6	2.6	ND	ND	3.1	0.8	53.3
		Sediment	9.3	14.9	24.2	7.3	3.9	ND	ND	ND	ND	0.6	36.1
		Total	9.5	15.1	24.7	37.1	20.5	2.6	ND	ND	3.1	1.4	89.4
D9	58	Water	ND	ND	ND	29.6	15.1	1.8	ND	ND	2.2	0.2	49.0
		Sediment	2.7	4.4	7.1	16.0	7.6	0.5	0.3	0.4	ND	0.2	32.0
		Total	2.7	4.4	7.1	45.7	22.7	2.2	0.3	0.4	2.2	0.4	81.0
D10	58	Water	ND	ND	ND	19.4	8.4	5.6	ND	ND	3.5	0.2	37.1
		Sediment	ND	2.0	2.0	11.1	8.7	3.5	0.5	ND	ND	0.4	26.2
		Total	ND	2.0	2.0	30.5	17.1	9.2	0.5	ND	3.5	0.6	63.3
D11	100	Water	ND	ND	ND	1.1	0.1	9.5	ND	ND	1.8	0.3	12.8
		Sediment	3.0	2.8	5.8	3.0	1.9	4.2	0.3	ND	ND	0.0	15.2
		Total	3.0	2.8	5.8	4.1	2.0	13.7	0.3	ND	1.8	0.3	28.0
D12	100	Water	ND	ND	ND	9.3	4.0	6.5	ND	ND	2.2	0.4	22.4
		Sediment	0.6	2.9	3.4	11.8	7.2	4.2	0.9	ND	1.9	0.4	29.8
		Total	0.6	2.9	3.4	21.1	11.2	10.7	0.9	ND	4.1	0.8	52.2

Table A7_1_2_2_2-12: Rates of degradation

Water-sediment System	Compound	Label	Degradation rate (days)			R squared
			DT_{50}	DT_{75}	DT_{90}	
Site A	Total cypermethrin	Both	8.6	17.3	28.7	0.959
Site A	Total cypermethrin	Both	5.6	21.0	43.8	0.976
Site A	*cis* cypermethrin	Both	16.9	33.8	56.1	0.972
Site A	*trans* cypermethrin	Both	2.9	5.7	9.5	0.973
Site A	Total cypermethrin	Phenoxy	9.8	19.7	32.7	0.949
Site A	Total cypermethrin	Phenoxy	8.7	23.7	43.4	0.974
Site A	Total cypermethrin	Cyclopropyl	6.1	12.2	20.3	0.970
Site A	Total cypermethrin	Cyclopropyl	4.7	13.7	44.3	0.996
Swiss Lake	Total cypermethrin	Both	4.2	8.3	13.8	0.930
Swiss Lake	Total cypermethrin	Both	2.7	10.7	40.1	0.973
Swiss Lake	*cis* cypermethrin	Both	12.5	25.0	41.5	0.962
Swiss Lake	*trans* cypermethrin	Both	1.5	2.9	4.8	0.939
Swiss Lake	*trans* cypermethrin	Both	1.1	4.6	11.1	0.977
Swiss Lake	Total cypermethrin	Phenoxy	5.0	9.9	16.5	0.914
Swiss Lake	Total cypermethrin	Phenoxy	5.1	16.9	32.6	0.977
Swiss Lake	Total cypermethrin	Cyclopropyl	3.5	7.0	11.6	0.951
Swiss Lake	Total cypermethrin	Cyclopropyl	2.5	8.5	35.8	0.990
Site A	TDCVC	Cyclopropyl	144.3[1]	301.0[1]	468.7[1]	0.974
Site A	CDCVC	Cyclopropyl	187.5[1]	402.0[1]	593.4[1]	0.972
Site A	3-Phenoxybenzoic acid	Phenoxy	12.9	28.3	29.9	0.879
Swiss Lake	TDCVC	Cyclopropyl	79.9	165.2[1]	261.5[1]	0.946
Swiss Lake	CDCVC	Cyclopropyl	62.0	136.7[1]	158.8[1]	0.947
Water Phase						
Site A	Total cypermethrin	Both	0.5	0.9	1.5	0.989
Swiss Lake	Total cypermethrin	Both	0.5	0.9	1.5	0.965
Sediment Phase						
Site A[†]	Total cypermethrin	Both	14.3	NR	47.3	0.955
Swiss Lake[†]	Total cypermethrin	Both	10.9	NR	36.1	0.823

[1]extrapolated values [†] Accumulation and dissipation

Section A7.2.2.1 Aerobic degradation in soil

Annex Point IIIA XII.1.1, XII.1.4

Rate and route of degradation, including identification of metabolites and degradation products.

			Official use only
		REFERENCE	
1.1	**Reference**	Brice, A., Cooke, C. (2005); [^{14}C]-Cypermethrin cis:trans 40:60: Aerobic soil degradation and metabolism; Covance Laboratories Ltd., report no. 1669/012-D2149, 21 March 2006 (unpublished). Dates of work: 11 February 2005 – 17 August 2005	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. OECD Guideline 307 (April 2002)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		MATERIALS AND METHODS	
3.1	**Test material**	**[^{14}C phenoxy]cypermethrin-*cis*** Batch 04BLY115b, Specific radioactivity: 2.2 mCi (55.4 mCi/mmol) Radiochemical purity: 100 % **[^{14}C phenoxy]cypermethrin-*trans*** Batch 04BLY115b, Specific radioactivity: 3.7 mCi (55.4 mCi/mmol) Radiochemical purity: 100 % **[^{14}C cyclopropyl]cypermethrin-*cis*** Batch 04BLY115b, Specific radioactivity: 0.9 mCi (53 mCi/mmol) Radiochemical purity: 100 % **^{14}C cyclopropyl]cypermethrin-*trans*** Batch 04BLY115b, Specific radioactivity: 1.7 mCi (53 mCi/mmol) Radiochemical purity: 100 %	
3.2	**Reference substance**	No	

Section A7.2.2.1 Aerobic degradation in soil

Annex Point IIIA XII.1.1, XII.1.4 **Rate and route of degradation, including identification of metabolites and degradation products.**

3.3 Testing procedure

3.3.1 Soil types

Four UK soils, PT102, PT103, SK920191 and SK15556090 were supplied by Land Research Associates, or Agronomy Enterprise. All soils were passed through a 2 mm sieve with the minimum of air drying. Prior to use, the soils were stored in an incubator routinely maintained at 4 ± 2°C, in loosely tied plastic bags in accordance with the ISO international standard ISO 10381-6:1993(E). The soil characteristics are given in Table A7_2_2_1-1

3.3.2 Test system

Samples of the sieved soil (50 g) were dispensed into individual glass vessels through which moistened air was drawn. The units were maintained in the dark at 20 ± 2°C or 10 ± 2°C for four days to enable equilibrium to be established. The air drawn over the surface of the units was passed through a series of traps (sodium hydroxide, ethanediol and paraffin in xylene) to collect evolved radiolabelled material.

The mean percent water holding capacity (between the values at pF 2 and pF 2.5) was calculated for each soil.

Soil name	Mean percent water holding capacity (pF 2 and pF 2.5)
PT102	21.65
PT103	12.5
SK920191	32.9
SK15556090	35.3

During the incubation period, each unit was maintained at the relevant mean percent water holding capacity.

3.3.3 Test conditions

Five incubation groups were set up, four at 20±2°C and one additional group (soil PT102) at 10±2°C as shown in Table A7_2_2_1-2

Moistened air was drawn through the units and, after test substance application, through a series of traps. The first trap was empty and acted as a security trap, the second and third contained ethanediol and 2% liquid paraffin in xylene to trap polar and non-polar volatiles respectively, and the final two contained 2 M sodium hydroxide solution to trap liberated carbon dioxide.

Soil units were maintained under experimental conditions for four days at 20 ± 2°C or at 10 ± 2°C, in the dark prior to test substance application. During the pre-incubation phase the security trap was filled with water to check for air-flow through the unit. The water was emptied and trapping reagents were added to the other traps prior to test substance application.

3.3.4 Application of test solution

The application rate was 15 µg/50 g dry weight equivalent of soil. This was based on the application rate for agricultural use of 0.15 kg/ha (calculated using a depth of 5 cm and assuming a bulk density of 1.0 g/cm^3).

Section A7.2.2.1 Aerobic degradation in soil

Annex Point IIIA XII.1.1, XII.1.4 **Rate and route of degradation, including identification of metabolites and degradation products.**

		Dosing was carried out by dropwise application of the radiolabelled test substance (ca 15 µg, 73.3 kBq for the phenoxy label and ca 15 µg, 71.3 kBq for the cyclopropyl label), in acetonitrile (90 µL for the phenoxy label and 92 µL for the cyclopropyl label) to the soil samples. The soil was mixed thoroughly before incubation.
3.3.5	Artificial light source	In the dark at 20 ± 2°C or 10 ± 2°C.
3.3.6	Sampling procedure	Microbial biomass was determined at day 60 and at the end of the incubation period using units dosed with acetonitrile only. For incubation groups A, B, D and E, soil samples were taken immediately after application and at 3, 7, 14, 30, 45 and 90 days after application. For incubation groups C and F, soil samples were taken immediately after application and at 3 (group C only), 7 (group C only), 14, 30, 58, 90 (group F - cyclopropyl label only) and 120 days after application.
3.3.7	Extraction method	Soil samples were subjected to the following extraction sequence: a) Primary extraction. Extraction by shaking four times with acetonitrile:water (1:1 v/v, 100 mL). b) Reflux extraction (selected samples only). Extraction for approximately 5 hours in acetonitrile:water:hydrochloric acid (50:50:1 v/v/v, 250 mL). c) Bound residue fractionation (selected samples only). The residues resulting from the primary and reflux extractions were base extracted to further separate them into fulvic acid, humic acid and humin fractions.
3.3.8	Analytical methods	The soil extracts were co-chromatographed by high performance liquid chromatography (HPLC) with reference standards. The identities of metabolites were confirmed by thin layer chromatography (TLC) of selected samples using authentic reference standards. See Table A7_2_2_1-3
3.3.9	Statistics	The degradation rate of cypermethrin was determined in each soil using a single phase first-order model. The equation used was single phase (first order kinetics): $y = C_o \times e^{-kt}$ where y is the percent of test substance at time t days, C_0 is the computed initial concentration and k is the rate constant (slope). DT-50, DT-75 and DT-90 values were calculated from the equation of the lines

Section A7.2.2.1 Aerobic degradation in soil

Annex Point IIIA XII.1.1, XII.1.4 **Rate and route of degradation, including identification of metabolites and degradation products.**

RESULTS

4.1 Recovery and distribution of radioactivity

The overall recovery of applied radioactivity in all the samples was ≥96% with the exception of two values, 79 (unit A22, dose group A, 90 days) and 86% (Unit F6, dose group F, phenoxy label, 120 days).

4.2 Degradation products

[^{14}C-phenoxy]cypermethrin

Primary extracts contained 95 to 100% of applied radioactivity immediately after dosing, decreasing to 8 to 39% at the terminal timepoint (90 days for dose groups A, D and E; 120 days for dose groups C and F).

The reflux extract from unit A2 (soil PT102, phenoxy label) contained an additional 10% of applied radioactivity at 90 days. Remaining radioactivity was in the fulvic acid (6%), humic acid (12%) and humin (9%) fractions.

Volatile radioactivity (carbon dioxide) increased to 29 to 54% of applied radioactivity at the terminal timepoint. $^{14}CO_2$ confirmatory analysis was performed on the pooled sodium hydroxide traps from each terminal incubation unit, by barium chloride precipitation. No radioactivity was detected in any of the supernatant traps, confirming the presence of carbon dioxide only.

Following application with [^{14}C-phenoxy]cypermethrin, one significant metabolite, 3-phenoxybenzoic acid was present in each soil. The maximum level of 3-phenoxybenzoic acid was 10.2% total applied radioactivity:

[^{14}C-cyclopropyl]cypermethrin

Primary extracts contained 94 to 100% of applied radioactivity immediately after dosing, decreasing to 6 to 35% at the terminal timepoint (90 days for dose groups B, D and E; 120 days for dose groups C and F).

The reflux extract from unit B21 (soil PT102, cyclopropyl label) contained an additional 4% of applied radioactivity at 90 days. Remaining radioactivity was in the fulvic acid (3%), humic acid (4%) and humin (3%) fractions.

Volatile radioactivity (carbon dioxide) increased to 49 to 78% of applied radioactivity at the terminal timepoint. $^{14}CO_2$ confirmatory analysis was performed on the pooled sodium hydroxide traps from each terminal incubation unit, by barium chloride precipitation. No radioactivity was detected in any of the supernatant traps, confirming the presence of carbon dioxide only.

Following application with [^{14}C-cyclopropyl]cypermethrin there were two significant metabolites, (1*RS*)-*cis*-3-(2, 2-dichlorovinyl)- 2,2-dimethylcyclopropanecarboxylic acid (CDCVC) and (1*RS*)-*trans*-3-(2, 2-dichlorovinyl)- 2,2-dimethylcyclopropanecarboxylic acid (TDCVC)

Section A7.2.2.1 **Aerobic degradation in soil**

Annex Point IIIA XII.1.1, XII.1.4 **Rate and route of degradation, including identification of metabolites and degradation products.**

present in each soil. The maximum levels of CDCVC and TDCVC were 3.9 and 13.6% total applied radioactivity, respectively

See Table A7_2_2_1-4

4.3 Degradation rate

The DT-50 values for the degradation of cypermethrin in the four soils were within the range 6 to 24 days following incubation at 20 ± 2°C. In soil PT 102, incubated at 10 ± 2°C, the DT-50 value for the degradation of cypermethrin was 52 days.

See Table A7_2_2_1-5 to A7_2_2_1-7

APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The route and rate of degradation of cypermethrin was studied in one soil type (soil PT102) at 20 ± 2°C. The rate of degradation of cypermethrin was also studied in this soil at 10 ± 2°C, and in three other soil types at 20 ± 2°C. The application rate was 15 µg/50 g dry weight equivalent of soil. This was based on the agricultural application rate of 0.15 kg/ha (calculated using a depth of 5 cm and assuming a bulk density of 1.0 g/cm^3).

Samples of the sieved soil (50 g) were dispensed into individual glass vessels through which moistened air was drawn. The units were maintained in the dark at 20 ± 2°C or 10 ± 2°C for four days to enable equilibrium to be established. The air drawn over the surface of the units was passed through a series of traps (sodium hydroxide, ethanediol and paraffin in xylene) to collect evolved radiolabelled material.

Dosing was carried out by dropwise application of the radiolabelled test substance (*ca* 15 µg, 73.3 kBq for the phenoxy label and *ca* 15 µg, 71.3 kBq for the cyclopropyl label), in acetonitrile (90 µL for the phenoxy label and 92 µL for the cyclopropyl label) to the soil samples. The soil was mixed thoroughly before incubation in the dark at 20 ± 2°C or 10 ± 2°C.

The soil extracts were co-chromatographed by high performance liquid chromatography (HPLC) with reference standards. The identities of metabolites were confirmed by thin layer chromatography (TLC) of selected samples using authentic reference standards.

The degradation rate of cypermethrin was determined in each soil using a single phase first-order model.

5.2 Results and discussion

Following application with [^{14}C-phenoxy]cypermethrin, primary extracts contained 95 to 100% of applied radioactivity immediately after dosing, decreasing to 8 to 39% at the terminal timepoint. Volatile radioactivity (carbon dioxide) increased to 29 to 54% of applied radioactivity at the terminal timepoint. $^{14}CO_2$ confirmatory analysis was performed on the pooled sodium hydroxide traps from each terminal incubation unit, by barium chloride precipitation. No radioactivity was detected in any of the supernatant traps, confirming the presence of carbon dioxide only.

Section A7.2.2.1 Aerobic degradation in soil

Annex Point IIIA XII.1.1, XII.1.4 **Rate and route of degradation, including identification of metabolites and degradation products.**

		Following application with [^{14}C-phenoxy]cypermethrin, one significant metabolite, 3-phenoxybenzoic acid was present in each soil with a maximum level of 10.2% total applied radioactivity. Primary extracts contained 94 to 100% of applied radioactivity immediately after dosing, decreasing to 6 to 35% at the terminal timepoint. Volatile radioactivity (carbon dioxide) increased to 49 to 78% of applied radioactivity at the terminal timepoint. $^{14}CO_2$ confirmatory analysis was performed on the pooled sodium hydroxide traps from each terminal incubation unit, by barium chloride precipitation. No radioactivity was detected in any of the supernatant traps, confirming the presence of carbon dioxide only. Following application with [^{14}C-cyclopropyl]cypermethrin there were two significant metabolites, (1RS)-cis-3-(2, 2-dichlorovinyl)- 2,2-dimethylcyclopropanecarboxylic acid (CDCVC) and (1RS)-trans-3-(2, 2-dichlorovinyl)- 2,2-dimethylcyclopropanecarboxylic acid (TDCVC) present in each soil. The maximum levels of CDCVC and TDCVC were 3.9 and 13.6% total applied radioactivity, respectively.	X
5.2.1	DT50	The DT-50 values for the degradation of cypermethrin in the four soils were within the range 6 to 24 days following incubation at 20 ± 2°C. In soil PT 102, incubated at 10 ± 2°C, the DT-50 value for the degradation of cypermethrin was 52 days.	
5.3	**Conclusion**	Cypermethrin was metabolised to three significant metabolites in soil, 3-phenoxybenzoic acid, CDCVC and TDCVC. Their maximum levels were 2.4 to 10.2, 0.6 to 3.9 and 3.8 to 13.6% of applied radioactivity, respectively. Further metabolism of cypermethrin and/or these metabolites lead to bound residues and mineralisation to carbon dioxide. The DT-50 values for the degradation of cypermethrin in the four soils were within the range 6 to 24 days following incubation at 20 ± 2°C. In soil PT 102, incubated at 10 ± 2°C, the DT-50 value for the degradation of cypermethrin was 52 days.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*May 2007*
Materials and Methods	Applicants version is acceptable.
Results and discussion	5.2 The third paragraph doesn't specify that the radiolabeled substance used was [^{14}C-cyclopropyl]cypermethrin. Therefore, this paragraphe should begin with:

Section A7.2.2.1 Aerobic degradation in soil

Annex Point IIIA XII.1.1, XII.1.4

Rate and route of degradation, including identification of metabolites and degradation products.

	Following application with[^{14}C-cyclopropyl]cypermethrin, primary extracts contained 94 to 100% of applied radioactivity immediately after dosing,...
	It is not mentionned that some radioactivity was found in the reflux extract from unit B21 (soil PT102, cyclopropyl label) that contained an additional 4% of applied radioactivity at 90 days. Remaining radioactivity was in the fulvic acid (3%), humic acid (4%) and humin (3%) fractions.
Conclusion	Applicant's version is adopted
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_2_2_1-1: Soil Characteristics

Soil name	pH (H_20)	OM %	Sand [1]%	Silt[1] %	Clay[1] %	Classification[1]	MWHC %	CEC mEq/100g	Biomass µg C/g soil
PT102	7.3	4.0	52	39	9	Sandy loam	63.2	19.1	440
PT103	5.3	1.9	74	14	12	Sandy loam	36.4	10.2	283
SK920191	7.5	7.2	30	37	33	Clay loam	66.7	36.6	747
SK15556090	6.8	7.9	20	60	20	Silty clay loam / clay loam	87.6	27.0	798

[1] UK Particle Size Distribution and Classification
CEC = cation exchange capacity, OM = organic matter, MWHC = maximum water holding capacity at pF 0

Table A7_2_2_1-2: Incubation groups

Incubation group	Soil type	Incubation Temperature (°C)	Radiolabel	Study type	Number of units[1,3]
A	PT 102	20±2	Phenoxy	Route of degradation	22
B	PT 102	20±2	Cyclopropyl	Route of degradation	22
C	PT 103	20±2	Phenoxy/ Cyclopropyl[2]	Rate of degradation	22
D	SK 920191	20±2	Phenoxy/ Cyclopropyl[2]	Rate of degradation	22
E	SK 15556090	20±2	Phenoxy/ Cyclopropyl[2]	Rate of degradation	22
F	PT102	10±2	Phenoxy/ Cyclopropyl[2]	Rate of degradation	16

[1]Excludes biomass units
[2]One replicate with the phenoxy label, one replicate with the cyclopropyl label
[3]Includes six extra units for possible additional timepoints

Table A7_2_2_1-3: Analytical methods

HPLC method 2 (radiochemical purity and sample analysis)			
Column:	YMC-ODS-A, 25 cm x 4.6 mm, 5 µm		
Gradient:	A linear binary gradient system was used consisting of		
	Solvent A:	0.1% Phosphoric acid	
	Solvent B:	Acetonitrile	
	Time (minutes)	% A	% B
	0	50	50
	5	40	60
	20	16	84
	30	16	84
	35	0	100
	40	50	50
Flow rate:	1 mL/min		
Detection:	UV absorbance of the eluent was monitored at 220 nm using an ABI Model 759A absorbance detector or an Agilent G1313A absorbance detector. Radioactivity in the eluent was monitored using a β-ram flow through radioactivity monitor equipped with a liquid mixing (500 µL) cell with Flowlogic 1:1 (1 mL/min) as liquid scintillant.		
HPLC method 3 (sample analysis)			
Column:	YMC-ODS-A, 25 cm x 4.6 mm, 5 µm		
Gradient:	A linear binary gradient system was used consisting of		
	Solvent A:	0.1% Phosphoric acid	
	Solvent B:	Acetonitrile	
	Time (minutes)	% A	% B
	0	80	20
	120	35	65
	140	16	84
	150	16	84
	160	0	100
	170	80	20
	180	80	20
Flow rate:	1 mL/min		
Detection:	UV absorbance of the eluent was monitored at 220 nm using an Agilent G1313A absorbance detector. Radioactivity in the eluent was monitored using a β-ram flow through radioactivity monitor equipped with a liquid mixing (500 µL) cell with Flowlogic 1:1 (1 mL/min) as liquid scintillant.		
HPLC method 4 (sample analysis)			
Column:	YMC-ODS-A, 25 cm x 4.6 mm, 5 µm		
Gradient:	A linear binary gradient system was used consisting of		
	Solvent A:	0.1% Phosphoric acid	
	Solvent B:	Acetonitrile	
	Time (minutes)	% A	% B
	0	95	5
	5	95	5
	40	0	100
Flow rate:	1 mL/min		
Detection:	UV absorbance of the eluent was monitored at 220 nm using an Agilent G1313A absorbance detector. Radioactivity in the eluent was monitored using a β-ram flow through radioactivity monitor equipped with a liquid mixing (500 µL) cell with Flowlogic 1:1 (1 mL/min) as liquid scintillant.		
TLC System 1 (radiochemical purity and sample analysis)			
Plates:	Whatman K6F Silica Gel 60 Å		
Solvent:	Hexane : dichloromethane (1:1 v/v)		
TLC System 2 (sample analysis)			
Plates:	Whatman K6F Silica Gel 60 Å		
Solvent:	Toluene: dichloromethane : hexane (15:3:2 v/v/v)		

Table A7_2_2_1-4: Percentage recovery and distribution of applied radioactivity recovered from soil

Soil	[14C] Label	Days	Soil Extract	Un-extracted	CO2	Cypermethrin cis	Cypermethrin trans	Cypermethrin total	3PBA	TDCVC	CDCVC
PT102 20±2°C	Phenoxy	0	98.5	0.7	NA	39.7	58.6	98.3	ND	NA	NA
		3	81.5	9.5	6.3	33.0	37.8	70.8	7.4	NA	NA
		7	67.1	17.7	12.3	28.2	26.5	54.8	6.4	NA	NA
		14	53.4	24.6	19.2	25.9	19.2	45.1	3.7	NA	NA
		30	26.6	34.6	34.7	12.0	5.9	18.0	1.7	NA	NA
		45	21.0	34.6	40.0	10.7	4.8	15.5	1.2	NA	NA
		90	12.8	36.8	38.84 8.6[1]	6.1	2.9	9.0	0.5	NA	NA
	Cyclo-propyl	0	99.4	1.0	NA	40.3	58.9	99.2	NA	ND	ND
		3	92.4	3.5	3.4	36.2	42.3	78.4	NA	8.4	1.4
		7	84.0	5.6	9.6	31.9	31.1	63.0	NA	11.9	2.3
		14	60.3	10.9	26.7	23.6	17.6	41.2	NA	7.7	2.0
		30	40.4	13.1	44.5	17.8	8.7	26.5	NA	4.5	1.6
		45	27.6	13.7	55.9	12.5	5.4	17.9	NA	2.0	0.9
		90	15.5	13.8	70.3	7.7	3.4	11.1	NA	0.7	0.5
PT103 20±2°C	Phenoxy	0	97.9	0.3	NA	40.5	57.1	97.6	0.0	NA	NA
		3	88.7	4.7	3.9	36.2	48.7	84.8	1.1	NA	NA
		7	84.8	7.6	6.1	36.8	42.0	78.8	2.4	NA	NA
		14	65.3	15.7	17.0	29.5	28.9	58.4	1.9	NA	NA
		30	43.2	23.5	30.2	20.6	13.7	34.3	1.6	NA	NA
		58	31.5	24.5	39.5	13.4	6.8	20.2	1.2	NA	NA
		120	38.9	20.4	36.7	18.8	10.4	29.2	1.3	NA	NA
	Cyclo-propyl	0	97.6	0.3	NA	39.6	57.6	97.2	NA	0.0	0.0
		3	96.3	1.6	1.3	38.4	51.7	90.1	NA	3.2	0.3
		7	88.1	4.3	5.7	34.4	41.9	76.2	NA	3.8	0.6
		14	76.8	7.5	14.3	30.3	30.6	60.9	NA	3.7	0.6
		30	53.9	12.9	31.6	21.7	15.1	36.8	NA	2.9	0.6
		58	34.0	15.5	48.4	13.0	6.5	19.5	NA	1.0	0.5
		120	25.3	16.2	56.3	8.4	3.8	12.2	NA	0.8	0.5
SK 920191 20±2°C	Phenoxy	0	94.7	4.2	NA	38.7	55.9	94.6	ND	NA	NA
		3	78.9	11.9	7.0	32.0	34.1	66.1	10.0	NA	NA
		7	51.5	24.0	20.9	20.8	14.6	35.4	10.2	NA	NA
		14	29.3	32.1	34.4	12.6	8.1	20.8	5.4	NA	NA
		30	15.0	36.8	45.1	6.7	4.0	10.7	1.4	NA	NA
		45	9.6	35.8	50.3	3.0	2.2	5.2	0.5	NA	NA
		90	7.7	36.4	53.7	3.1	1.8	4.9	0.2	NA	NA
	Cyclo-propyl	0	96.5	2.7	NA	38.4	57.7	96.1	NA	ND	ND
		3	81.3	10.0	6.3	31.3	32.3	63.6	NA	9.7	2.8
		7	69.0	12.9	17.0	25.6	18.9	44.5	NA	13.6	3.9
		14	39.8	17.6	40.4	15.5	8.6	24.0	NA	7.2	3.5
		30	17.8	17.4	62.3	7.6	4.0	11.6	NA	2.1	1.3
		45	12.4	17.2	68.9	5.5	3.7	9.1	NA	0.4	0.4
		90	6.4	15.8	77.8	3.4	2.3	5.7	NA	0.2	0.1
SK155 56090 20±2°C	Phenoxy	0	95.7	3.7	NA3 9.4	39.45 5.9	55.99 5.3	95.3ND	NDNA	NA	NA39.4
		3	70.1	16.3	10.33 2.1	32.12 9.5	29.56 1.7	61.75. 5	5.5NA	NA	NA32.1
		7	50.9	26.0	21.62 5.4	25.41 7.2	17.24 2.6	42.63. 6	3.6NA	NA	NA25.4
		14	31.7	33.3	32.11 7.6	17.61 0.2	10.22 7.7	27.72. 1	2.1NA	NA	NA17.6
		30	17.7	34.9	43.99 .7	9.73. 8	3.813 .4	13.41. 1	1.1NA	NA	NA9.7
		45	11.5	34.3	51.26 .1	6.12. 1	2.18. 1	8.10.6	0.6NA	NA	NA6.1
		90	9.2	33.3	54.24 .4	4.41. 7	1.76. 0	6.00.5	0.5NA	NA	NA4.4
	Cyclo-propyl	0	94.3	4.4	NA3 8.9	38.95 4.8	54.89 3.7	93.7NA	NAND	NDND	ND38.9
		3	81.5	8.7	8.432 .8	32.83 5.1	35.16 7.9	67.9NA	NA7.5	7.51.4	1.432.8
		7	66.3	11.6	20.82 8.9	28.92 3.2	23.25 2.1	52.1NA	NA7.5	7.51.8	1.828.9
		14	38.4	16.7	44.11 8.5	18.51 0.4	10.42 8.9	28.9NA	NA3.6	3.61.2	1.218.5
		30	17.6	17.0	64.29 .3	9.33. 8	3.813 .1	13.1NA	NA0.7	0.70.5	0.59.3
		45	13.5	16.2	69.36 .7	6.72. 7	2.79. 4	9.4NA	NA0.5	0.50.2	0.26.7
		90	8.5	14.9	75.24 .9	4.92. 4	2.47. 3	7.3NA	NAND	NDND	ND4.9

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ND=Not detected; NA=Not applicable; 3PBA=3-Penoxybenzoic acid; [1] One sample due to mass balance too low in the second sample.

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TDCVC=(1RS)-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid
CDCVC=(1RS)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid

Table A7_2_2_1-4 (cont..)

Soil	[14C] Label	Days	Soil Extract	Un-extracted	CO2	cis	trans	total	3PBA	TDCVC	CDCVC
PT102 10±2°C	Phenoxy	0	97.7	0.7	NA	39.2	58.5	97.7	ND	NA	NA
		14	82.3	9.8	6.2	34.1	37.6	71.7	7.3	NA	NA
		30	65.5	17.9	14.2	29.3	26.0	55.3	4.0	NA	NA
		58	54.1	23.0	19.8	25.4	19.4	44.8	3.1	NA	NA
		120	24.9	26.7	34.6	11.6	6.6	18.2	1.3	NA	NA
	Cyclo-propyl	0	99.5	0.7	NA	40.8	58.6	99.4	NA	ND	ND
		14	89.2	5.1	5.5	34.7	36.1	70.8	NA	10.5	1.9
		30	86.9	5.4	8.6	32.9	33.7	66.6	NA	12.3	2.3
		58	64.2	10.2	23.4	25.6	16.7	42.3	NA	9.9	2.7
		90	45.4	12.9	40.1	18.2	10.1	28.3	NA	5.3	2.1
		90	53.7	11.2	34.5	20.6	12.3	33.0	NA	8.5	2.8
		120	35.3	14.1	49.3	13.8	7.4	21.2	NA	4.1	1.9

Table A7_2_2_1-5: Degradation rates for total cypermethrin

Soil	Label	Model	Degradation rate of cypermethrin (days)			
			DT-50	DT-75	DT-90	R^2
PT102	Both	One phase	13.9	27.8	46.2	0.947
PT102	Phenoxy	One phase	13.0	26.1	43.3	0.947
PT102	Cyclopropyl	One phase	14.8	29.7	49.3	0.956
PT103	Both	One phase	24.2	48.3	80.3	0.934
SK920191	Both	One phase	6.4	12.9	21.4	0.974
SK15556090	Both	One phase	8.4	16.7	27.7	0.968
PT102[1]	Both	One phase	51.9	103.7	172.2[2]	0.975

[1] Incubation at 10 ± 2°C [2] Extrapolated value

Table A7_2_2_1-6: Degradation rates for cis and trans-cypermethrin

Soil	Label	Compound	Model	Degradation rate (days)			
				DT-50	DT-75	DT-90	R^2
PT102	Both	*cis-cypermethrin*	One phase	25.0	49.9	82.9	0.948
PT102	Phenoxy	*cis-cypermethrin*	One phase	22.7	NA	75.4	0.950
PT102	Cyclopropyl	*cis-cypermethrin*	One phase	27.4	NA	90.9	0.957
PT103	Both	*cis-cypermethrin*	One phase	46.6	NA	154.9[2]	0.864
SK920191	Both	*cis-cypermethrin*	One phase	11.0	NA	36.4	0.974
SK15556090	Both	*cis-cypermethrin*	One phase	15.0	NA	49.7	0.975
PT102[1]	Both	*cis-cypermethrin*	One phase	81.5	NA	270.6[2]	0.976
PT102	Both	*trans*-cypermethrin	One phase	8.3	16.6	27.5	0.965
PT102	Phenoxy	*trans*-cypermethrin	One phase	7.8	NA	26.0	0.957
PT102	Cyclopropyl	*trans*-cypermethrin	One phase	8.7	NA	29.0	0.976
PT103	Both	*trans*-cypermethrin	One phase	15.8	NA	52.4	0.974
SK920191	Both	*trans*-cypermethrin	One phase	3.9	NA	19.0	0.997
SK15556090	Both	*trans*-cypermethrin	One phase	5.0	NA	16.7	0.977
PT102[1]	Both	*trans*-cypermethrin	One phase	34.9	NA	116.0[2]	0.966

[1] Incubation at 10 ± 2°C [2] Extrapolated values NA = Not Applicable

Table A7_2_2_1-7: Degradation rates for cypermethrin metabolites

Soil	Label	Compound	Model	Degradation rate (days)		
				DT-50	DT-90	R^2
PT102	Phenoxy	3-Phenoxybenzoic acid	One phase	13.2	41.7	0.987
PT103	Phenoxy	3-Phenoxybenzoic acid	One phase	119.8	391.8[2]	0.839
SK920191	Phenoxy	3-Phenoxybenzoic acid	One phase	9.9	26.7	0.998
SK15556090	Phenoxy	3-Phenoxybenzoic acid	One phase	9.7	31.9	0.969
PT102[1]	Phenoxy	3-Phenoxybenzoic acid	One phase	35.0	116.2	0.954
PT102	Cyclopropyl	TDCVC	One phase	17.6	51.6	0.970
PT103	Cyclopropyl	TDCVC	One phase	35.8	113.1	0.967
SK920191	Cyclopropyl	TDCVC	One phase	10.3	24.0	0.984
SK15556090	Cyclopropyl	TDCVC	One phase	8.9	22.9	0.997
PT102[1]	Cyclopropyl	TDCVC	One phase	65.6	182.5[2]	0.998
PT102	Cyclopropyl	CDCVC	One phase	34.3	114.0	0.975
PT103	Cyclopropyl	CDCVC	One phase	357.2[2]	1186.7[2]	0.754
SK920191	Cyclopropyl	CDCVC	One phase	14.6	48.4	0.966
SK15556090	Cyclopropyl	CDCVC	One phase	12.2	40.5	1.000
PT102[1]	Cyclopropyl	CDCVC	One phase	133.9[2]	444.7[2]	0.519

[1] Incubation at 10 ± 2°C [2] Extrapolated values

Section A7.2.2.4 (01) Other soil degradation studies

Annex Point IIA XII.1.1

Soil Photolysis study

Official use only

REFERENCE

1.1	**Reference**	Swales, S. (2003); (^{14}C)-cypermethrin: Photodegradation on a soil surface; Covance Laboratories Ltd., Report N° 40/44-D2149 (CYP/M71), 28 April 2003 (unpublished). Dates of experimental work: 16 August 2002 – 19 March 2003
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes EC Directive 95/36/EC (July 1995), SETAC Procedures for assessing the Environmental Fate and Ecotoxicity of Pesticides, section 2 (March 1995)
2.2	**GLP**	Yes
2.3	**Deviations**	No

MATERIALS AND METHODS

3.1	**Test material**	[^{14}C phenoxy] cis-cypermethrin Batch 01BLY095C Specific activity: 50 mCi/mmole (4.444 MBq/mg) Radiochemical purity: > 98 % [^{14}C phenoxy] trans-cypermethrin Batch 01BLY095B Specific activity: 50 mCi/mmole (4.444 MBq/mg) Radiochemical purity: > 98 % [^{14}C cyclopropane] cis-cypermethrin Batch 01BLY095 Specific activity: 57 mCi/mmole (5.066 MBq/mg) Radiochemical purity: > 98 %

Section A7.2.2.4 (01) Other soil degradation studies

Annex Point IIA XII.1.1 **Soil Photolysis study**

[^{14}C cyclopopane] trans-cypermethrin
Batch 01BLY095A
Specific activity: 57 mCi/mmole (5.066 MBq/mg)
Radiochemical purity: > 98 %

The 2 phenoxy labels of cypermethrin were mixed together such that the cis/trans ratio in the formulation was 40/60. The 2 cyclopropane labels were similarly mixed to form a second dosing solution.

3.2 Soil types

Agricultural (grassland) silt loam soil (see table A7_2_2_4_01-1). No pesticides had been used on this soil for 12 years.

3.3 Testing procedure

3.3.1 Test system

The photodegradation of each of the two dosing solutions was investigated on thin layers of a silty clay loam soil exposed to simulated sunlight, filtered to remove wavelengths below 290 nm. The soil layers were formed by spreading a slurry of the soil (*ca* 4g) in water on metal trays (4 cm in diameter) and allowing the soil to dry at *ca* 35°C. The temperature of the test soil was maintained at 20 ± 3°C. Additional samples were kept in a temperature controlled dark incubation room.

All test vessels were connected with traps to absorb volatile compounds. Carbon dioxide free air was drawn through the chambers and through a series of five traps. The first trap was an empty security trap, the second contained ethanediol, the third 2% paraffin in xylene and the fourth and fifth sodium hydroxide (2 M) solution.

3.3.2 Application of test solution

The test article was applied in acetonitrile solution to the surface of the soils at a rate approximately equivalent to 25 g/ha (the application rate for cypermethrin used in agriculture), based on the surface area of the soil dish.

Eighteen units were prepared in total for each test material, eight to be irradiated, eight as dark controls and a further two for analysis immediately after test article application.

3.3.3 Artificial light source

Soil samples were exposed to artificial light in an irradiation cabinet (Atlas Suntest CPS+ accelerated exposure machine – Alpas technology) equipped with a xenon lamp for 15 days (continuous irradiation) . The wavelength of the light source ranged form 300 to 800 nm and the wavelengths below 290 nm were eliminated by a special UV filter system. The light intensity of the xenon lamp was 306.9-335.7 W/m^2.

3.3.4 Sampling procedure

For the (^{14}C phenoxy) cypermethrin (cis:trans/40:60) test material, samples were removed for analysis immediately after test article application, and at a further eight sampling intervals after 0.08, 0.2, 0.3, 1, 3, 4, 7 and 15 days of continuous irradiation. These times are approximately equivalent to 0.2, 0.32, 0.6, 2, 5.6, 7.6, 13 and 30 days of

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Annex Point IIA XII.1.1 **Soil Photolysis study**

Harrogate summer sunlight; values were also calculated in terms of the standardised Florida summer sunlight.

For the (^{14}C cyclopropane) cypermethrin (cis:trans/40:60) test material, samples were removed for analysis immediately after test article application, and at a further eight sampling intervals after 1, 2, 5, 7, 9, 12 and 15 (duplicate units) days of continuous irradiation. These times are approximately equivalent to 2, 4, 9, 13, 17, 23, 29 and 30 days of summer sunlight.

Dark control samples were removed for analysis at the same time as the irradiated units.

3.3.5 Extraction method

Soils samples were extracted once with acetonitrile/water (1/1). The extracts were concentrated and quantified by LSC. Bound residues and trapping solutions were quantified by LSC. All samples were analysed by HPLC and selected samples were analysed by TLC for confirmation.

RESULTS

4.1 Recovery and distribution of radioactivity

Overall recoveries were in the range 95% to 106% Applied radioactivity (AR) for irradiated and dark control samples. The percentage of applied radioactivity extracted from soil decreased with time from 97% to 78% for the (^{14}C cyclopropane) cypermethrin irradiated samples and from 98% to 69% for the (^{14}C phenoxy) cypermethrin irradiated samples, over the 15-day incubation period. For the dark control samples, the percentage of applied radioactivity extracted from soil decreased from 98% to 91% and 96% to 91% for the (^{14}C cyclopropane) and (^{14}C phenoxy) cypermethrin respectively.

There was a concomitant increase in the unextracted soil residues that comprised 13 and 22% of applied radioactivity in irradiated samples after 15 days for the (^{14}C cyclopropane) and (^{14}C phenoxy) cypermethrin respectively. Unextracted soil residues in the dark control samples increased to 11% of applied radioactivity for the two labels. In the irradiated samples, 5 and 6% of applied radioactivity was present in the sodium hydroxide traps for the (^{14}C cyclopropane) and (^{14}C phenoxy) cypermethrin respectively. Sodium hydroxide traps for the dark control samples contained lower levels of applied radioactivity at 0.2% for the (^{14}C cyclopropane) label and 2.5% for the (^{14}C phenoxy) label.

The radioactivity recovered after bound residue extraction of the unextracted radioactivity was distributed between the fulvic acid, humic acid and humin. For the (^{14}C cyclopropane) cypermethrin the majority of the applied radioactivity was found in the fulvic acid fraction for both irradiated and dark control samples (6-8%) with lower levels present in the humic acid (2%) and humin (2-3%). For the (^{14}C phenoxy) cypermethrin the majority of the applied radioactivity was found in the humic acid (7%) and humin fractions (7%) for the irradiated sample, with lower levels in the fulvic acids (5%).

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Annex Point IIA XII.1.1 **Soil Photolysis study**

For the dark controls, the humin fraction contained the largest proportion of applied radioactivity (4%) compared with 2% for the fulvic and humic acids.

See Table A7_2_2_4_01-2.

4.2 Degradation products

The major photolysis degradation product was the carboxamide derivative of cypermethrin along with smaller amounts of 3-phenoxybenzoic acid, DCVC acid and carbon dioxide. It appears that the carboxamide derivative is formed due to photolysis, while the DCVC acid and the 3-phenoxybenzoic acid metabolites are formed by hydrolysis – this hypothesis fits with the fact that the irradiated samples show lower levels of the DCVC acid and 3-phenoxybenzoic acid than the dark control samples, and correspondingly higher levels of the carboxamide derivative, presumably due to a drying effect on the surface of the soils. Although not conclusively shown in the study it may be possible for cypermethrin to directly react to produce DCVC acid and 3-phenoxybenzoic acid.

See tables A7_2_2_4_01-4 to A7_2_2_4_01-7.

4.3 Degradation rate

The rate of degradation in the irradiated units was two-phase with an initial rapid phase, followed by a slower phase. Half-life values were calculated using first-order kinetics, and DT-50 and DT-90 values were calculated using two phase degradation curves. First order half-lives were calculated as 34.2 days (light), 39.8 days (dark) for the (^{14}C phenoxy) cypermethrin and 38.2 days (light) and 58.8 days (dark) for the (^{14}C cyclopropane) cypermethrin respectively. All figures are quoted as equivalent to Florida summer sunlight days. Using a two-phase decay curve, with improved correlation, the DT50 values were 29.6 days and 43.9 days (light samples) and the DT90 values were 201 and 230 days (light samples) for the (^{14}C phenoxy) cypermethrin and the (^{14}C cyclopropane) cypermethrin, respectively.

It was proposed that the initial rapid degradation, followed by a slower phase, may indicate that only a proportion of the applied cypermethrin was on the soil surface and could undergo photolysis. For this reason a half-life was calculated using the first order rate constant from the initial rapid portion of the two phase degradation curve. This method of calculation resulted in half-life values of 3 days for the (^{14}C - phenoxy) cypermethrin and 2.5 days for the (^{14}C - cyclopropane) cypermethrin.

See tables A7_2_2_4_01-8 to A7_2_2_4_01-9.

Section A7.2.2.4 (01) **Other soil degradation studies**

Annex Point IIA XII.1.1 **Soil Photolysis study**

APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The photodegradation of (^{14}C phenoxy) cypermethrin and (^{14}C cyclopropane) cypermethrin, cis:trans ratio 40:60 was investigated on thin layers of a silty clay loam soil exposed to simulated sunlight, filtered to remove wavelengths below 290 nm.

The soils layers were formed by spreading a slurry of the soil on metal trays and allowing the soil to dry at 35°C. [^{14}C phenoxy] cypermethrin (40/60) and [^{14}C cyclopropane] cypermethrin (40/60) were applied to the surface of the soil at the rate of 25 g/ha, based on the surface area of the soil dish.

The temperature of the test soil was maintained at 20 ± 3°C. Additional samples were kept in a temperature controlled dark incubation room. All test vessels were connected with traps to absorb volatile compounds.

The extracts were concentrated and quantified by LSC. Bound residues and trapping solutions were quantified by LSC. All samples were analysed by HPLC and selected samples were analysed by TLC for confirmation.

5.2 Results and discussion

In irradiated soil, the major degradation product is the carboxamide derivative of cypermethrin (19% AR after 7-9 days continuous irradiation) along with smaller amounts of 3-phenoxybenzoic acid (6% AR) and DCVC acid ((2,2-dichlorovinyl)- 2,2-dimethylcyclopropanecarboxylic acid) (3% AR). Bound residue reached 12.8-21.9 % AR at day 15, mineralisation reached 5.4-6.2 % AR at day 15.

In dark samples, the major degradation products are 3-phenoxybenzoic acid (13% AR) and DCVC (24 %AR); carboxamide is formed at a lower level (5% AR).

Bound residue reached 10.6-10.7% AR at day 15, mineralisation reached 0.2-2.5 % AR at day 15.

5.2.1 DT50

Using a two phase decay curve the DT50 values were 29.6 days and 43.9 days (light samples) and the DT90 values were 201 and 230 days (light samples) for the (^{14}C phenoxy) cypermethrin and the (^{14}C cyclopropane) cypermethrin, respectively.

A two-phase decay was used as there was an initial rapid degradation, followed by a slower phase, which may indicate that only a proportion of the applied cypermethrin was on the soil surface and could undergo photolysis. For this reason a half-life was calculated using the first order rate constant from the initial rapid portion of the two-phase degradation curve. This method of calculation resulted in half-life values of 3 days for the (^{14}C phenoxy) cypermethrin and 2.5 days for the (^{14}C

Section A7.2.2.4 (01) **Other soil degradation studies**

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		cyclopropane) cypermethrin.
5.3	**Conclusion**	Data on distribution of radioactivity and DT50 for cis- and trans isomers indicate that soil photolysis is a minor route of degradation of the active substance.
5.3.1	Reliability	1
5.3.2	Deficiencies	No
		Study evaluated and accepted under Directive 91/414/EC

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2007
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Aplicant's version is adopted.
Conclusion	Aplicant's version is adopted.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*

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Soil Photolysis study

Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_2_2_4_01-1: Soil characteristics for soil photolysis study

Characteristic	Soil
Soil name	SK15556090
Location	Derbyshire, UK
PH (H_2O)	5.7
Sand (%)	18
Silt (%)	63
Clay (%)	19
Texture[1]	Silty clay loam
Organic matter (%)	7.8
Organic carbon (%)	4.5
Cation exchange capacity (meq/100 g)	22.2
Water holding capacity at pF2.5 (1/3 bar) (%)	35.3

[1] UK classification.

Table A7_2_2_4_01-2: Distribution of radioactivity for (^{14}C cyclopropane) cypermethrin

Timepoint	Soil Extract		Residue		Volatile traps						Mass balance	
					Organic traps		NaOH 1		NaOH 2			
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
0 Day	96.6	97.5	4.2	4.1	NA	NA	NA	NA	NA	NA	100.8	101.6
1 Day	92.6	97.6	8.1	4.8	0.0	0.0	0.4	0.0	0.1	0.0	101.2	102.4
2 Day	91.4	100.3	9.5	4.3	0.0	0.0	1.0	0.0	0.2	0.0	102.1	104.6
5 Day	87.0	99.1	10.8	4.5	0.0	0.0	2.4	0.0	0.0	0.0	100.2	103.6
7 Day	86.0	95.4	10.0	6.1	0.0	0.0	3.1	0.0	0.0	0.0	99.1	101.5
9 Day	82.9	99.1	12.3	7.1	0.0	0.0	3.7	0.0	0.0	0.0	98.9	106.2
12 Day	83.0	95.7	13.3	6.9	0.0	0.0	4.4	0.1	0.0	0.0	100.7	102.7
15 Day	78.2	91.7	12.8	9.0	0.0	0.0	5.1	0.2	0.3	0.0	96.4	100.9
15 Day	85.9	91.0	11.1	10.7	0.0	0.0	5.1	0.2	0.3	0.0	102.4	101.9

NA- Not applicable
Timepoints are the actual time between dosing and removal of the units from the incubation system

Table A7_2_2_4_01-3: Distribution of radioactivity for (^{14}C phenoxy) cypermethrin

Timepoint	Soil Extract		Residue		Volatile traps						Mass balance	
					Organic traps		NaOH 1		NaOH 2			
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
0 Day	97.5	96.2	3.4	4.8	NA	NA	NA	NA	NA	NA	100.9	101.0
0.08 Day	92.9	93.2	5.6	4.4	0.0	0.0	0.0	0.0	0.0	0.0	98.5	97.6
0.2 Day	93.7	93.2	7.1	7.1	0.0	0.0	0.0	0.0	0.0	0.0	100.8	100.3
0.3 Day	93.2	95.0	7.0	4.5	0.0	0.0	0.0	0.0	0.0	0.0	100.2	99.5
1 Day	91.5	93.0	8.0	4.1	0.0	0.0	0.2	0.0	0.0	0.0	99.7	97.1
3 Day	86.4	93.7	9.9	5.3	0.0	0.0	0.7	0.0	0.0	0.0	97.0	99.0
4 Day	84.3	93.1	13.2	5.6	0.0	0.0	1.1	0.1	0.0	0.0	98.6	98.8
7 Day	80.2	91.9	13.0	10.3	0.0	0.0	1.8	0.5	0.0	0.0	95.0	102.7
15 Day	69.2	91.2	21.9	10.6	0.0	0.0	6.0	2.5	0.2	0.0	97.3	104.3

NA- Not applicable
Timepoints are the actual time between dosing and removal of the units from the incubation system

Table A7_2_2_4_01-4: Percentage of applied radioactivity as parent/degradation products (^{14}C-cyclopropane) cypermethrin – irradiated samples

Timepoint (days)	Cypermethrin			Carboxamide[1]			DCVC Acid			Unknowns	Back ground	Total
	Cis	Trans	Cis+Trans	Cis	Trans	Cis+Trans	Cis	Trans	Cis+Trans			
0	39.7	56.0	95.7	ND	ND	ND	0.5	0.4	0.8	ND	0.1	96.6
1	34.8	50.2	85.0	2.9	3.8	6.7	0.4	0.6	1.0	ND	0.0	92.6
2	31.4	45.9	77.3	4.1	6.0	10.2	0.8	1.4	2.2	1.4	0.3	91.4
5	29.4	41.4	70.8	6.9	8.3	15.2	ND	0.9	0.9	ND	0.1	87.0
7	28.2	39.3	67.4	6.5	10.3	16.9	0.5	1.2	1.6	ND	0.1	86.0
9	24.1	35.9	59.9	7.9	11.0	18.9	0.7	1.8	2.5	1.6	0.1	82.9
12	24.7	35.7	60.4	6.2	10.1	16.4	1.1	2.0	3.1	2.5	0.7	83.0
15	23.8	33.7	57.5	7.3	9.3	16.6	0.7	2.1	2.8	1.0	0.3	78.2
15	29.0	41.5	70.5	5.0	7.5	12.5	1.0	1.9	2.9	ND	0.1	85.9

[1]Carboxamide derivative of cypermethrin
ND - Not detected
Timepoints are the actual time between dosing and removal of the units from the incubation system

Table A7_2_2_4_01-5: Percentage of applied radioactivity as parent/degradation products (^{14}C-cyclopropane) cypermethrin – dark control samples

Timepoint (days)	Cypermethrin			Carboxamide[1]			DCVC Acid			Unknowns	Back ground	Total
	Cis	Trans	Cis+Trans	Cis	Trans	Cis+Trans	Cis	Trans	Cis+Trans			
0	39.9	57.4	97.3	ND	ND	ND	ND	ND	ND	ND	0.2	97.5
1	37.2	57.7	94.9	0.8	1.7	2.5	ND	ND	ND	ND	0.2	97.6
2	38.4	57.2	95.6	1.9	1.9	3.8	0.3	0.5	0.9	ND	0.1	100.3
5	39.2	53.4	92.6	1.0	2.4	3.3	0.6	2.6	3.2	ND	0.0	99.1
7	36.1	51.9	88.0	1.1	2.0	3.2	0.7	2.9	3.6	ND	0.7	95.4
9	37.7	50.9	88.6	1.9	2.6	4.6	1.0	4.9	5.9	ND	0.1	99.1
12	37.2	45.6	82.8	0.8	1.2	2.0	1.4	9.2	10.6	ND	0.2	95.7
15	34.9	32.0	66.9	1.9	2.0	3.9	3.0	17.9	20.9	ND	0.0	91.7
15	35.8	29.4	65.2	0.5	0.6	1.0	2.8	21.1	23.9	0.7	0.2	91.0

[1]Carboxamide derivative of cypermethrin
ND - Not detected
Timepoints are the actual time between dosing and removal of the units from the incubation system

Table A7_2_2_4_01-6: Percentage of applied radioactivity as parent/degradation products (^{14}C-phenoxy) cypermethrin – irradiated samples

Timepoint	Cypermethrin			Carboxamide[1]			PBA[2]	Unknowns	Background	Total
(days)	Cis	Trans	Cis+Trans	Cis	Trans	Cis+Trans				
0	38.0	58.9	96.8	ND	ND	ND	0.6	ND	0.1	97.5
0.08	36.3	54.6	91.0	0.7	ND	0.7	1.0	ND	0.3	92.9
0.2	36.3	54.8	91.1	0.7	0.5	1.2	1.3	ND	0.2	93.7
0.3	35.5	51.9	87.4	1.5	2.3	3.8	1.2	0.4	0.5	93.2
1	32.3	51.2	83.5	1.9	2.8	4.7	2.6	0.5	0.1	91.5
3	28.1	43.0	71.0	5.0	5.7	10.8	3.2	1.3	0.1	86.4
4	26.1	39.1	65.2	4.1	8.6	12.8	5.7	0.6	0.1	84.3
7	23.9	35.7	59.5	5.8	9.4	15.1	5.1	0.2	0.3	80.2
15	24.2	34.4	58.6	3.4	4.6	8.1	1.9	0.6	0.0	69.2

1. Carboxamide derivative of cypermethrin
2. 3-Phenoxybenzoic acid

ND - Not detected

Timepoints are the actual time between dosing and removal of the units from the incubation system

Table A7_2_2_4_01-7: Percentage of applied radioactivity as parent/degradation products (^{14}C-phenoxy) cypermethrin – dark control samples

Timepoint	Cypermethrin			Carboxamide[1]			PBA[2]	Unknowns	Background	Total
(days)	Cis	Trans	Cis+Trans	Cis	Trans	Cis+Trans				
0	39.4	56.1	95.4	ND	ND	0.0	0.7	ND	0.0	96.2
0.08	36.7	55.8	92.5	ND	ND	0.0	0.6	ND	0.1	93.2
0.2	39.0	53.9	92.9	ND	ND	0.0	ND	ND	0.3	93.2
0.3	36.7	56.5	93.2	ND	ND	0.0	1.4	0.2	0.2	95.0
1	34.3	55.3	89.7	1.0	1.3	2.2	1.0	ND	0.0	93.0
3	36.9	53.9	90.8	1.0	0.9	1.9	0.9	ND	0.0	93.7
4	33.8	51.3	85.1	1.2	2.5	3.8	4.3	ND	0.0	93.1
7	33.5	44.7	78.2	1.3	2.5	3.8	9.6	ND	0.2	91.9
15	40.7	33.2	73.9	ND	ND	0.0	12.7	ND	0.3	86.9

3. Carboxamide derivative of cypermethrin
4. 3-Phenoxybenzoic acid

ND - Not detected

Timepoints are the actual time between dosing and removal of the units from the incubation system

Table A7_2_2_4_01-8: DT50 of cypermethrin (*)

	[^{14}C phenoxy] cypermethrin		[^{14}C cyclopropane] cypermethrin	
	Irradiated samples	Dark control samples	Irradiated samples	Dark control samples
First order decay curve				
DT50	34.2	39.8	38.2	55.8
DT90	-	132	-	185
R^2	0.748	0.908	0.874	0.948
Two phase decay curve				
DT50	29.6	-	43.9	-
DT90	201	-	230	-
R^2	0.943	-	0.984	-

(*): DT50 were calculated assuming equivalent summer sunlight conditions at 30° N

Table A7_2_2_4_01--9: First order DT50 of cis and trans isomers of cypermethrin (*)

		[^{14}C phenoxy] cypermethrin		[^{14}C cyclopropane] cypermethrin	
		Cis-cypermethrin	Trans-cypermethrin	Cis-cypermethrin	Trans-cypermethrin
Irradiated	samples DT50 (days)	37.0	32.7	38.1	38.4
	R^2	0.707	0.769	0.852	0.888
Dark cond.	samples DT50 (days)	37.8	20.9	121	20.3
	R^2	0.527	0.961	0.588	0.819

(*): DT50 were calculated assuming equivalent summer sunlight conditions at 30° N

Section A7.2.2.4 (02) Other soil degradation studies

Annex Point IIA XII.1.1 **Anaerobic soil degradation study**

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REFERENCE

1.1	**Reference**	Brice, A., Cooke, C. (2006); [^{14}C]-Cypermethrin *cis*:*trans* 40:60: Anaerobic Soil Metabolism and Degradation; Covance Laboratories Limited, report no. 1669/013-D2149, 21 March 2006, (unpublished). Dates of experimental work: 15 February 2005 – 1 February 2006
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD Guideline 307 (April 2002)
2.2	**GLP**	Yes
2.3	**Deviations**	No

MATERIALS AND METHODS

3.1	**Test material**	**[^{14}C phenoxy]cypermethrin-*cis*** Batch 04BLY115b, Specific radioactivity: 2.2 mCi (55.4 mCi/mmol) Radiochemical purity: 100 % **[^{14}C phenoxy]cypermethrin-*trans*** Batch 04BLY115b, Specific radioactivity: 3.7 mCi (55.4 mCi/mmol) Radiochemical purity: 100 % **[^{14}C cyclopropyl]cypermethrin-*cis*** Batch 04BLY115b, Specific radioactivity: 0.9 mCi (53 mCi/mmol) Radiochemical purity: 100 % **^{14}C cyclopropyl]cypermethrin-*trans*** Batch 04BLY115b, Specific radioactivity: 1.7 mCi (53 mCi/mmol) Radiochemical purity: 100 %

Section A7.2.2.4 (02) Other soil degradation studies

Annex Point IIA XII.1.1 **Anaerobic soil degradation study**

3.2	**Soil types**	UK sandy loam (PT102) supplied by Agronomy Enterprise (see Table A7_2_2_4_02-1). Soil was passed through a 2 mm sieve with minimum air-drying and was stored according to ISO 10381-6:1993(E) prior to use. See Table A7_2_2_4_02-1
3.3	**Testing procedure**	
3.3.1	Test system	Samples of the 2 mm sieved soil (50 g) were dispensed into individual glass vessels through which moistened air was drawn. The units were maintained in the dark at 20 ± 2°C for 2 days to enable equilibrium to be established. The air drawn over the surface of the units was passed through a series of traps (sodium hydroxide, ethanediol and paraffin in xylene) to collect evolved radiolabelled material. Aerobic incubation was maintained for 10 days after dose application (equivalent to one half-life/DT-50). After this time, reverse osmosis (RO) water was added to the soil to form an overlying layer of 3 cm. The system was flushed with nitrogen and anaerobic conditions were continued for up to a further 182 days.
3.3.2	Application of test solution	An application rate of 15 µg/50 g soil was used which corresponds to a field application rate of 150 g/ha, assuming an incorporation depth of 5 cm and a soil density of 1.0 g/cm^3 (in support of the agricultural uses of cypermethrin). Solutions of [^{14}C-phenoxy]-cypermethrin or [^{14}C-cyclopropyl]-cypermethrin in acetonitrile were dispensed dropwise over the soil surface of 18 units in each incubation group. The soil was mixed thoroughly before incubation in the dark at 20 ± 2°C. See Table A7_2_2_4_02-2
3.3.3	Artificial light source	None. Units were maintained in the dark.
3.3.4	Sampling procedure	Duplicate samples were removed for analysis at 0, 10, 24, 42, 69, 130 and 192 days after application. A single additional unit from dose group A (unit A15) was removed 193 days after application (183 days post-flooding) to replace a 192 day unit which showed unexpectedly low levels of radioactivity.

Section A7.2.2.4 (02) **Other soil degradation studies**

Annex Point IIA XII.1.1 **Anaerobic soil degradation study**

3.3.5 Extraction method

The water was separated from the soil by aspiration and the two phases were separately analysed.

Water

Following the addition of concentrated hydrochloric acid (1.5 mL), the water samples were partitioned three times with dichloromethane and twice with ethyl acetate to give aqueous and organic phases. Organic phases were concentrated by rotary evaporation and then reduced to dryness under nitrogen and reconstituted in acetonitrile.

Soil

Primary extraction. Extraction by shaking four times with acetonitrile:water (1:1 v/v, 100 mL). The extracts were combined and a sub-sample was reduced in volume by rotary evaporation and reconstituted in acetonitrile for chromatographic analysis.

Reflux extraction (selected samples only). Extraction for approximately 5 hours in acetonitrile:water:hydrochloric acid (50:50:1 v/v/v, 250 mL).

Bound residue fractionation (selected samples only). The residues resulting from the primary and reflux extractions were base extracted to further separate them into fulvic acid, humic acid and humin fractions.

3.3.6 Analytical methods

Cypermethrin and metabolites were detected in the water and soil extracts by HPLC (YMC-PACK ODS A, 250 x 4.6 mm, 5 µm, flow rate 1ml/min) with UV detection (220 nm). Metabolites were confirmed by co-chromatography with authentic reference compounds by TLC (Whatman K6F Silica gel plates with hexane:dichloromethane or toluene:heaxance:acetic acid as solvent).

See Table A7_2_2_4_02-3.

RESULTS

4.1 Recovery and distribution of radioactivity

The overall recovery of applied radioactivity in all units was ≥ 94% with the exception of unit A3 (phenoxy label, 0 days post-flooding) and unit A15 (phenoxy label, 183 days post-flooding) where the recovery values were 89 and 88%, respectively.

[^{14}C-phenoxy]-cypermethrin (dose group A)

Radioactivity in the surface water increased from a mean value of 5% of applied radioactivity at 14 days post-flooding, to a mean value of 19% of applied radioactivity at 120 days post-flooding. The level of applied radioactivity in the surface water was maintained at 182 days post-flooding.

Radioactivity extracted from soil decreased from a mean value of 99% of applied radioactivity, initially, to a mean value of 22% at nominally 182 days post-flooding. Radioactivity unextracted from soil increased from

Section A7.2.2.4 (02) **Other soil degradation studies**

Annex Point IIA XII.1.1 **Anaerobic soil degradation study**

<1% of applied radioactivity, initially, to a maximum mean value of 25% at 182 days post-flooding.

The reflux extract from unit A15 contained an additional 8% of applied radioactivity at 183 days post-flooding. Remaining radioactivity was in the fulvic acid (4%), humic acid (7%) and humin (6%) fractions

Volatile radioactivity (carbon dioxide) increased from a mean value of 13% applied radioactivity at 0 days post-flooding to 31% at the terminal timepoint. Confirmatory $^{14}CO_2$ analysis was performed by barium chloride precipitation, using the pooled sodium hydroxide traps from unit A15 (a terminal incubation unit). Only a trace level of radioactivity (0.2 % applied radioactivity) was detected.

[^{14}C-cyclopropyl]-cypermethrin (dose group B)

Radioactivity in surface water increased from a mean value of 19% of applied radioactivity at 14 days post-flooding, to a maximum mean value of 43% of applied radioactivity at 182 days post-flooding.

Radioactivity extracted from soil decreased from a mean value of 100% of applied radioactivity, initially, to a minimum mean value of 24% at 182 days post-flooding. Radioactivity unextracted from soil increased from <1% of applied radioactivity, initially, to a maximum mean value of 9% at 120 days post-flooding.

The reflux extract from unit B8 contained an additional 3% of applied radioactivity at 32 days post-flooding. Remaining radioactivity was in the fulvic acid (2%), humic acid (2%) and humin (2%) fractions.

Volatile radioactivity (carbon dioxide) increased from a mean value of 16% applied radioactivity at 0 days post-flooding to 23% at 32 days post-flooding. Levels of volatile radioactivity were maintained at the terminal timepoint (182 days post-flooding). Confirmatory $^{14}CO_2$ analysis was performed by barium chloride precipitation , using the pooled sodium hydroxide traps from unit B14 (a terminal incubation unit). Only a trace level of radioactivity (0.1 % applied radioactivity) was detected

See Table A7_2_2_4_02-4 and Table A7_2_2_4_02-5

Section A7.2.2.4 (02) Other soil degradation studies

Annex Point IIA XII.1.1 **Anaerobic soil degradation study**

4.2 Degradation products

Throughout the duration of this study, no cypermethrin was detected in the surface water of either flooded soil system following application with [^{14}C]-cypermethrin.

Following application with [^{14}C-phenoxy]-cypermethrin, one significant metabolite, 3-phenoxybenzoic acid (3PBA) was present in the flooded soil system. The maximum level of this metabolite in the surface water, soil and total flooded system was 16.6, 18.5 and 35.1 % respectively (120 days post flooding). One very minor identified metabolite, 3-Phenoxybenzaldehyde (3PBAD), was present in the flooded soil system at mean levels no greater than 0.7% of applied radioactivity.

Following application with [^{14}C-cyclopropyl]-cypermethrin, two significant metabolites, (1*RS*)-*trans*-3-(2, 2-dichlorovinyl)- 2,2-dimethylcyclopropanecarboxylic acid (TDCVC) and (1*RS*)-*cis*-3-(2, 2-dichlorovinyl)- 2,2-dimethylcyclopropane carboxylic acid (CDCVC) were present in the flooded soil system. The maximum levels of these metabolites in the surface water, soil and total flooded system was 15.3, 7.6 and 22.8 % respectively for CDCVC (182 days post flooding) and 21.3, 15.4 and 31.2 % for TDCVC (120 days post flooding in surface water and total system, 0 days post flooding in soil).

See Tables A7_2_2_4_02-6 and A7_2_2_4_02-7

Units A15 (phenoxy label, 183 days post-flooding) and B8 (cyclopropyl label, 32 days post-flooding) were selected for harsher extraction by reflux analysis. Following the initial extraction, units A15 and B8 contained 25.6 and 9.4% unextracted radioactivity, respectively. From units A15 and B8, 8% and 3% total applied radioactivity was extracted by reflux analysis, respectively. The extract from unit A15 was then analysed by HPLC and no single component was found to be present in this extract at a level greater than 2% total applied radioactivity.

4.3 Degradation rate

See Table A7_2_2_4_02-8

APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The route and rate of cypermethrin was studies in one soil type under anaerobic conditions (incubation in the dark at 20 ±2°C) according to OECD guideline 307 using an application rate of 0.15 kg/ha. Anaerobic conditions were maintained for 182 days with duplicate samples removed for analysis 0, 10, 24, 42, 69, 130 and 192 days after application. The water and soil phases were separated and analysed separately by HPLC and TLC to determine lebvels of cypermethrin and its metabolites.

Section A7.2.2.4 (02) Other soil degradation studies

Annex Point IIA XII.1.1 **Anaerobic soil degradation study**

5.2 Results and discussion

Cypermethrin was metabolised to three extractable metabolites 3PBA, CDCVC, TDCVC and carbon dioxide in the total flooded soil system. Their maximum levels were 36.6, 25.8, 33.4 and 28.2% of applied radioactivity, respectively. Further metabolism of cypermethrin and/or these metabolites resulted in bound residue and mineralisation to carbon dioxide

Throughout the duration of this study, no cypermethrin was detected in the surface water of the flooded soil system following application with [^{14}C]-cypermethrin

In each case, cis-cypermethrine degraded slower than trans cypermethrin. See table A7_2_2_4_02-9

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5.2.1 DT50

The DT-50, DT-75 and DT-90 values for cypermethrin in the total flooded soil system were as follows:

Label	Model	Rate of degradation of cypermethrin (days)		
		DT-50	DT-75	DT-90
Phenoxy	One phase	46	92	153
Cyclopropyl	One phase	46	92	152

5.3 Conclusion

Due to the very low water solubility of cypermethrin, degradation of cypermethrin only occurred in the flooded soil. The relatively water soluble metabolites, compared with cypermethrin, then steadily migrated to the surface water.

5.3.1 Reliability

1

5.3.2 Deficiencies

No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2007
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is adopted
Conclusion	Applicant's version is adopted
Reliability	1

Section A7.2.2.4 (02) Other soil degradation studies

Annex Point IIA XII.1.1 **Anaerobic soil degradation study**

Acceptability	Acceptable
Remarks	/
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_2_2_4_02-1: Soil Characteristics

Soil name	pH (H_20)	OM %	Sand[1]%	Silt[1]%	Clay[1]%	Classification[1]	MWHC%	CEC mEq/100g	Biomass µg C/g soil
PT102	7.3	4.0	52	39	9	Sandy loam	63.2	19.1	440

[1] UK Particle Size Distribution and Classification
CEC = cation exchange capacity, OM = organic matter, MWHC = maximum water holding capacity at pF 0

Table A7_2_2_4_02-2: Application of test substance

Incubation group	Radiolabel	No. of units	Volume of dose formulation applied (µL)	Weight of test article applied (µg)	Radioactivity applied (kBq)
A	[^{14}C-phenoxy]	18	88	15.2	74.63
B	[^{14}C-cyclopropyl]	18	87	15.2	71.42

Table A7_2_2_4_02-3: Analytical methods

HPLC method 1			
Column:	YMC-ODS-A, 25 cm x 4.6 mm, 5 µm		
Gradient	Solvent A:	0.1% Phosphoric acid	
	Solvent B:	Acetonitrile	
	Time (minutes)	% A	% B
	0	50	50
	5	40	60
	20	16	84
	30	16	84
	35	0	100
	40	50	50
Flow rate:	1 mL/min		
Detection:	UV absorbance of the eluent was monitored at 220 nm using an ABI Model 759A absorbance detector or an Agilent G1313A absorbance detector. Radioactivity in the eluent was monitored using a β-ram flow through radioactivity monitor equipped with a liquid mixing (500 µL) cell with Flowlogic 1:1 (1 mL/min) as liquid scintillant.		
TLC System			
Plates:	Whatman K6F Silica Gel 60 Å		
Solvent:	Hexane : dichloromethane (1:1 v/v)		

Table A7_2_2_4_02-4: Mean recovery (%) of applied radioactivity

Timepoint[1]	Surface Water	Soil Extract	Residue	Unit Wash	Organic Traps[2]	2 M NaOH 1	2 M NaOH 2	Total
[14C-Phenoxy]-Cypermethrin (Group A)								
0-hour	NA	99.2	0.6	ND	NA	NA	NA	99.8
10-day (F + 0 days)	NA	58.7	21.8	ND	ND	13.1	0.1	93.6
24-day (F + 14 days)	4.6	53.6	20.6	ND	ND	19.1	ND	97.8
42-day (F + 32 days)	10.7	48.2	19.9	ND	ND	18.3	0.1	97.0
69-day (F + 59 days)	11.7	34.2	24.2	ND	ND	26.1	0.2	96.4
130-day (F + 120 days)	19.1	29.2	24.1	ND	ND	23.9	ND	96.2
192-day (F + 182 days)	17.7	21.5	25.1	ND	ND	27.2	0.1	91.4
[14C-Cyclopropyl]-Cypermethrin (Group B)								
0-hour	NA	99.9	0.7	ND	NA	NA	NA	100.6
10-day (F + 0 days)	NA	74.9	7.6	ND	ND	16.0	ND	98.5
24-day (F + 14 days)	19.1	55.7	7.5	ND	ND	15.4	ND	97.7
42-day (F + 32 days)	26.3	39.8	9.1	ND	ND	22.8	ND	97.9
69-day (F + 59 days)	32.9	34.2	8.7	ND	ND	22.0	ND	97.7
130-day (F + 120 days)	42.2	27.8	9.1	ND	ND	20.1	0.1	99.2
192-day (F + 182 days)	43.1	23.9	8.6	ND	ND	21.8	0.1	97.4

[1] F + X days = Number of days after the flooding of the soil; [2] Organic traps = ethanediol and 2% paraffin in xylene.
NA = Not applicable
ND = Not detected

Table A7_2_2_4_02-5: Reflux and bound residue analysis

Soil, Label	Incubation unit	Percent applied radioactivity (%)			
		Reflux extract	Fulvic Acid	Humic Acid	Humin
PT 102, phenoxy	A15	8.2	3.9	7.0	5.7
PT 102, cyclopropyl	B8	3.3	2.1	1.9	1.9

Table A7_2_2_4_02-6: Mean (%) of A.R. as parent compound and metabolites (Group A)

Timepoint[1]	Fraction	Cis cyper	Trans cyper	Total cyper	3PBA	3PBAD	Uknowns	unresolved	Total
[14C-Phenoxy]-Cypermethrin (Group A)									
0-hour	Surface water	NA	NA	NA	NA	NA	NA	NA	NA
	Soil	41.0	57.7	98.7	ND	ND	ND	0.5	99.2
	Total system	41.0	57.7	98.7	ND	ND	ND	0.5	99.2
10-day (F + 0 days)	Surface water	NA	NA	NA	NA	NA	NA	NA	NA
	Soil	26.8	22.2	49.0	5.0	ND	4.2	0.3	58.7
	Total system	26.8	22.2	49.0	5.0	ND	4.2	0.3	58.7
24-day (F + 14 days)	Surface water	ND	ND	ND	3.7	ND	0.1	ND	3.9
	Soil	23.6	15.1	38.7	11.2	ND	3.3	0.4	53.6
	Total system	23.6	15.1	38.7	14.9	ND	3.5	0.4	57.5
42-day (F + 32 days)	Surface water	ND	ND	ND	9.7	ND	0.2	0.1	9.9
	Soil	18.6	10.4	28.9	16.0	0.7	2.3	0.3	48.2
	Total system	18.6	10.4	28.9	25.7	0.7	2.4	0.3	58.1
69-day (F + 59 days)	Surface water	ND	ND	ND	9.4	ND	0.6	0.1	10.1
	Soil	13.0	5.5	18.4	13.7	0.1	1.8	0.2	34.2
	Total system	13.0	5.5	18.4	23.1	0.1	2.4	0.3	44.3
130-day (F + 120 days)	Surface water	ND	ND	ND	16.6	ND	1.0	0.1	17.6
	Soil	6.1	3.0	9.1	18.5	ND	1.4	0.2	29.2
	Total system	6.1	3.0	9.1	35.1	ND	2.4	0.2	46.8
192-day (F + 182 days)	Surface water	ND	ND	ND	16.0	ND	1.6	0.1	17.7
	Soil	3.5	1.7	5.3	15.1	ND	0.9	0.1	21.5
	Total system	3.5	1.7	5.3	31.1	ND	2.5	0.2	39.1

[1] F + X days = Number of days after the flooding of the soil.
NA = Not analysed ND = Not detected
3PBA = 3-Phenoxybenzoic acid, 3PBAD = 3-Phenoxybenzaldehyde,

Table A7_2_2_4_02-7: Mean (%) of A.R. as parent compound and metabolites (Group B)

Timepoint[1]	Fraction	Cis cyper	Trans cyper	Total cyper	CDCVC	TDCVC	Uknowns	Unresolved	Total
[14C-Cyclopropyl]-Cypermethrin (Group B)									
0-hour	Surface water	NA	NA	NA	NA	NA	NA	NA	NA
	Soil	40.9	58.2	99.1	ND	ND	0.4	0.4	99.9
	Total system	40.9	58.2	99.1	ND	ND	0.4	0.4	99.9
10-day (F + 0 days)	Surface water	NA	NA	NA	NA	NA	NA	NA	NA
	Soil	40.9	58.2	99.1	ND	ND	0.4	0.4	99.9
	Total system	27.2	20.4	47.6	3.5	15.4	7.9	0.6	74.9
24-day (F + 14 days)	Surface water	ND	ND	ND	3.7	11.9	3.2	ND	18.8
	Soil	24.6	16.0	40.6	3.2	8.5	3.3	0.2	55.7
	Total system	24.6	16.0	40.6	6.9	20.4	6.5	0.2	74.5
42-day (F + 32 days)	Surface water	ND	ND	ND	5.9	15.2	4.7	0.2	26.0
	Soil	16.6	8.8	25.4	4.1	6.9	3.2	0.2	39.8
	Total system	16.6	8.8	25.4	10.0	22.1	7.9	0.3	65.7
69-day (F + 59 days)	Surface water	ND	ND	ND	9.1	19.2	4.1	0.3	32.7
	Soil	12.3	6.5	18.8	5.6	7.7	2.0	0.2	34.2
	Total system	12.3	6.5	18.8	14.7	26.8	6.1	0.5	66.9
130-day (F + 120 days)	Surface water	ND	ND	ND	12.7	21.3	5.0	0.2	39.2
	Soil	6.4	2.5	9.0	7.2	9.9	1.7	ND	27.8
	Total system	6.4	2.5	9.0	19.9	31.2	6.7	0.2	67.0
192-day (F + 182 days)	Surface water	ND	ND	ND	15.3	19.1	5.2	0.1	39.7
	Soil	4.3	1.9	6.2	7.6	8.8	1.1	0.1	23.9
	Total system	4.3	1.9	6.2	22.8	27.9	6.4	0.2	63.5

[1] F + X days = Number of days after the flooding of the soil.
NA = Not analysed, ND = Not detected
CDCVC = (1*RS*)-*cis*-3-(2, 2-dichlorovinyl)- 2,2-dimethylcyclopropane carboxylic acid
TDCVC = (1*RS*)-*trans*-3-(2, 2-dichlorovinyl)- 2,2-dimethylcyclopropane carboxylic acid

Table A7_2_2_4_02-8: Degradation rates in total flooded soil system

Label	Model	Compound	DT-50	DT-75	DT-90	R^2
Phenoxy	One phase	*Total Cypermethrin*	46	92	153	0.972
Cyclopropyl	One phase	*Total Cypermethrin*	46	92	152	0.973

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Table A7_2_2_4_02-9: Degradation rate for *cis* and *trans*- cypermethrin

Label	Model	Compound	DT-50	DT-75	DT-90	R^2
Phenoxy	One phase	*Cis-cypermthrin*	58	115	191	0.977
Phenoxy	One phase	*Trans-cypermthrin*	31	63	104	0.976
Cyclopropyl	One phase	*Cis-cypermthrin*	55	111	184	0.972
Cyclopropyl	One phase	*Trans-cypermthrin*	34	68	113	0.973

Section A 7.1.3, 7.2.3.1 Adsorption / desorption screening test

Annex Point IIA7.7, , XII.1.2

Adsorption / desorption in four soils and one sediment

			Official use only
		1 REFERENCE	
1.1	**Reference**	Brice, A., Cooke, C. (2005); [14C]-Cypermethrin cis:trans 40:60: Adsorption/Desorption in soil; Covance Laboratories Ltd., report no. 1669/015-D2149, 22 March 2006, unpublished. Dates of experimental work: 2 March 2005 – 6 June 2005	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar SA	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD guideline 106 (January 2000)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material (radiolabelled)**	Radiolabelled Cypermethrin (one isomer per label) supplied by BlyChem Ltd : [^{14}C-phenoxy]cypermethrin-cis and [^{14}C-phenoxy]cypermethrin-trans.	
3.1.1	Lot/Batch number	Name:-[^{14}C-phenoxy]cypermethrin-*cis* Supplier batch number:-04BLY115b Specific radioactivity:-2.2 mCi (55.4 mCi/mmol) Radiochemical purity:-100% Name:-[^{14}C-phenoxy]cypermethrin-*trans* Supplier batch number:-04BLY115b Specific radioactivity:-3.7 mCi (55.4 mCi/mmol) Radiochemical purity:-100%	
3.1.2	Further relevant properties	Solubility in water: 0.004 mg/L (published value)	X
3.1.3	Method of analysis	HPLC (YMC ODS-A, 25 cm x 4.6 mm, 5 µm), flow rate 1 mL/min. UV absorbance of the eluent was monitored at 220 nm using either an ABI 759A absorbance detector or an Agilent G1313A absorbance detector. Radioactivity in the eluent was monitored using a β-ram flow through radioactivity monitor (LabLogic) equipped with a liquid mixing (500 µL) cell with Flowlogic 1:1 (1 mL/min) as liquid scintillant. TLC was used for determination of radiochemical purity and reference standard analysis (Whatman Silica Gel K6F plates, Dichloromethane : hexane (1:1 v/v) solvent). Following chromatography, radiolabelled compounds were detected by preparation of a radioluminogram of the TLC plate using a Fuji BAS 1500 Bio-image analyser and non-radiolabelled compounds were visualised at 254 nm under a UV lamp.	
3.2	**Degradation products**	Degradation products tested: No, due to low levels of radioactivity found in the supernatant.	
3.2.1	Method of analysis	Not applicable	

Section A 7.1.3, 7.2.3.1 Adsorption / desorption screening test

Annex Point IIA7.7, , XII.1.2		**Adsorption / desorption in four soils and one sediment**
	for degradation products	
3.3	**Reference substance**	No
3.4	**Soil types**	See Table A7.2.3.1-1
3.5	**Testing procedure**	
3.5.1	Test system	The four soil samples were air-dried, thoroughly mixed, 2 mm sieved and stored in the dark at room temperature prior to use. The sediment sample was passed through a 2 mm sieve and the associated water filtered through a 0.2 mm sieve. Prior to use the sediment was stored waterlogged (6-10 cm water layer) in the dark, in an incubator routinely maintained at 4 ± 2°C. Soils were dispensed into pre-weighed centrifuge tubes and were re-equilibrated by shaking with 0.01M calcium chloride solution (25 mL) overnight before the day of the experiment.
3.5.2	Test solution and Test conditions	The tests were initiated by addition of aliquots of an appropriate concentration of [^{14}C]-cypermethrin cis:trans 40:60 treatment solution (acetonitrile). The volume used was 20 µL, which gave the required final solution concentrations. All tests were performed in a temperature controlled room (20 ± 2°C) in the dark. Soils and solutions were mixed mechanically using a Stuart Scientific Rotator Drive STR4 end-over-end mixer at a speed that ensured efficient mixing of the soils and solution. The water solubility of cypermethrin (4 µg/L) was known prior to the start of this study. The highest concentration chosen for testing was 2 µg/L. The lowest concentration used was two orders of magnitude lower, 0.02 µg/L.
3.6	**Test performance**	--
3.6.1	Preliminary testing	According to OECD 106: Yes Initial 'adsorption to container', 'ratio of soil to aqueous phase', 'stability' and 'adsorption equilibrium time' tests were first conducted in the dark at 20 ± 2°C, under non-sterile conditions. Duplicate solutions of [^{14}C]-cypermethrin at concentrations of 0.02 or 2 µg/L were prepared in 0.01 M calcium chloride solution (25 mL) and four different tube types (Teflon, silanised glass, glass and PPCO). Solutions were prepared by addition of aliquots (20 µL) of the acetonitrile dose formulation. The samples were shaken for 24 hours and the radioactivity in the supernatants was determined by LSC. Due to low recoveries of radioactivity from the supernatant, acetonitrile unit washes and pipette rinses were performed. The wash/pipette rinse solutions were then quantified by LSC. When combining recovery data from the supernatant, unit wash and pipette rinse for each sample, Teflon tubes were found to be the most suitable for the concentration range being tested Adsorption to the surface of the Teflon tubes was also investigated in the

Section A 7.1.3, 7.2.3.1 Adsorption / desorption screening test

Annex Point IIA7.7, , XII.1.2

Adsorption / desorption in four soils and one sediment

presence of soil at a soil:solution ratio of 1:50 (w/v). Duplicate samples of soil SK566696 were dosed at each of three different concentrations of [^{14}C]-cypermethrin in acetonitrile (0.01 M calcium chloride solution concentrations, equivalent to 0.02, 0.2 and 2 µg/L). The samples were shaken for 24 hours, centrifuged (4700 rpm) and quantified by LSC. The supernatant was then transferred to a new container and an acetonitrile unit wash performed and quantified by LSC.

3.6.2 Ratio of soil to aqueous phase

The ratio of 1:50 w/v (0.5 g soil and 25 mL 0.01 M calcium chloride) was confirmed by performing a ratio test using duplicate samples of each of the five soils using the preconditioned Teflon tubes. Samples were dosed with a solution of [^{14}C]-cypermethrin in acetonitrile (2.55 µg/mL, 20 µL) to achieve an initial nominal concentration of 2.04 µg/L in the supernatant. The samples were mixed by shaking for 24 hours, centrifuged (4700 rpm) and the radioactivity in the weighed supernatants determined by taking duplicate weighed aliquots for LSC.

3.6.3 Adsorption equilibrium time determination

Eight test units were prepared for each soil at a soil:aqueous phase ratio of 1:50 w/v (0.5 g of soil to 25 mL 0.01M calcium chloride) and pre-conditioned by shaking overnight. A measured volume (20 µL) of a solution of [^{14}C]-cypermethrin (2.55 µg/mL) in acetonitrile was added to each tube to give an initial nominal solution concentration of 2.04 µg/L and a soil to aqueous phase ratio of 1:50 w/v. The samples were mixed for up to 48 hours. Duplicate test vessels for each soil were removed from the shaker after 3, 6, 24 and 48 hours and radioactivity in the weighed supernatant determined by taking duplicate weighed aliquots (5mL) for LSC.

The remaining supernatant was then transferred to a separate container, to which acetonitrile (ca 15 mL) was added. Acetonitrile:water 1:1 v/v was then added to the soil and the diluted supernatant and soil placed in freezer storage prior to stability testing.

3.6.4 Stability test

The adsorption supernatants from soils EL-7 and SK961089 only were analysed as supernatants from the remaining soils did not contain sufficient levels of radioactivity to enable an accurate assessment of test substance content.

The supernatants were reduced to dryness by rotary evaporation and reconstituted in acetonitrile:water (1:1, v/v,). Duplicate aliquots of the concentrated extract were then removed for LSC quantification.

Following LSC analysis, only the supernatant from soil SK961089 was found to contain a sufficient level of radioactivity to enable accurate determination of the test substance content. The adsorption supernatant from soil SK961089 was partitioned three times with dichloromethane and the organic extract reduced to dryness by rotary evaporation and reconstituted in acetonitrile.

Soil samples were extracted four times with acetonitrile: water (1:1 v/v) and the resulting extracts pooled and quantified by LSC. One extract for each soil type was then reduced to *ca* 1 mL in volume by rotary evaporation, acetonitrile:water (1:1 v/v 15 mL) was then added and the extract reduced to dryness by rotary evaporation . The concentrated

Section A 7.1.3, 7.2.3.1 Adsorption / desorption screening test

Annex Point IIA7.7, , XII.1.2 **Adsorption / desorption in four soils and one sediment**

extract was reconstituted in acetonitrile:water (1:1 v/v) and duplicate aliquots removed for LSC.

The amount of test substance in the supernatant and combined soil extracts was determined by HPLC

3.6.5 Ratio of soil to aqueous phase using sterile soil

The initial ratio of soil to aqueous phase test under non-sterile conditions demonstrated that only very low levels of radioactivity were present in the supernatant and that only trace levels of the radioactivity in the supernatant corresponded to cypermethrin.

In an attempt to reduce the degradation of cypermethrin, the 'ratio of soil to aqueous phase' and 'stability' tests were repeated in the dark at 20 ± 2°C under sterile conditions. The soils used for these tests were sterilised by gamma irradiation and the 0.01M calcium chloride solutions were sterilised by autoclaving, prior to use.

Because cypermethrin was found to be unstable for 24 hours the 'ratio of soil to aqueous phase' test was repeated over a reduced length of time. Duplicate samples were mixed by shaking for 3 hours, centrifuged and the radioactivity in the weighed supernatants was determined by taking duplicate weighed aliquots for LSC.

3.6.6 Desorption equilibrium time determination

Results from the ratio tests demonstrated that only very low levels of radioactivity were in the supernatant following shaking of the units at the optimum equilibrium time (3 hours). As it was not possible to accurately evaluate such low levels of radioactivity, the desorption phase of this study could not be conducted.

3.6.7 Definitive study, Freundlich sorption isotherms

Due to the relatively low water solubility of cypermethrin and its instability in the test units, it was not possible to accurately determine adsorption and desorption isotherms for this test substance.

In order to calculate the minimum distribution coefficient for adsorption (K_d) and the minimum organic carbon normalised adsorption coefficient (K_{oc}) values for cypermethrin in each soil, it was assumed that any radioactivity determined in each adsorption supernatant and soil/sediment sample was cypermethrin.

Because of the relatively low water solubility of cypermethrin and its instability in the test system, it was not possible to determine adsorption and desorption isotherms for this compound. Freundlich adsorption coefficient (K) values could not therefore be determined for cypermethrin within the design of this study.

4 RESULTS

4.1 Preliminary test

Analysis of the supernatant, unit washes and pipette rinses from the Teflon tubes provided the most reproducible recovery data with values ranging from 95 to 116% applied radioactivity.

4.2 Adsorption equilibrium time (non-sterile conditions)

The data from this 'adsorption equilibrium time' test suggested that for all the soils except the Matanuska sample, equilibrium had not been reached at 48 hours. For some samples in this test, particularly the Site C1 sediment at 3 hours and EL-7 at 6 hours, replication between results was

Section A 7.1.3, 7.2.3.1 Adsorption / desorption screening test

Annex Point IIA7.7, , XII.1.2

Adsorption / desorption in four soils and one sediment

poor. This was due to the relatively low levels of applied radioactivity detected in the supernatant making it difficult to accurately determined levels of radioactivity in each supernatant. Similarly it was not possible to assess the purity of the cypermethrin in the stability samples by HPLC analysis, however this data did confirm that cypermethrin was unstable over 48 hours in the test system.

4.3 Adsorption equilibrium time (sterile conditions)

Under sterile conditions, the mean amount of applied radioactivity remaining in the supernatants for each soil ranged from 0.6 to 1.3%. The amount of applied radioactivity in the soil ranged from 96.8 to 101.1%.

The purity of the cypermethrin in each soil and selected supernatant samples confirmed that the cypermethrin was unstable over the reduced 3 hours incubation time and was assumed to undergo degradation to a more water-soluble metabolite.

See Table A7.2.3.1-2 and Table A7.2.3.1-3

4.4 Calculations

Due to the relatively low water solubility of cypermethrin and its instability in the test units, it was not possible to accurately determine adsorption and desorption isotherms in this study. Freundlich adsorption coefficient (K) values could not therefore be determined.

In order to calculate the minimum distribution coefficient for adsorption (K_d) and the minimum organic carbon normalised adsorption coefficient (K_{oc}) values for cypermethrin in each soil, it was assumed that any radioactivity determined in each adsorption supernatant and soil/sediment sample was cypermethrin.

In order to make an estimated assessment of the mobility of cypermethrin the minimum K_{oc} values of this compound in each soil/sediment were used.

See Table A7.2.3.1-4

These results characterise cypermethrin as 'non-mobile' (Hollis, J M (1991)) and 'immobile' (McCall) in each of the four soils and in the sediment.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The adsorption characteristics of [^{14}C]-cypermethrin were determined in four soil types and one sediment according to OECD Guideline 106 (January 2000). Soil samples (0.5 g dry weight equivalent) were pre-equilibrated with 0.01 M calcium chloride solution (25 mL) overnight. They were then treated with solutions of [^{14}C]-cypermethrin prepared in acetonitrile (20 µL) to produce duplicate samples per soil, with an initial nominal concentration in the aqueous phase of 2.0 µg/mL. Recovery of applied radioactivity was determined by radioassay of the adsorption supernatants and remaining soil/sediment. In an attempt to reduce the degradation of cypermethrin, tests were repeated in the dark at 20 ± 2°C under sterile conditions.

5.2 Results and discussion

The initial ratio of soil to aqueous phase test under non-sterile conditions demonstrated that only very low levels of radioactivity were present in the supernatant. This test also demonstrated that only trace levels of the radioactivity in the supernatant corresponded to cypermethrin

Section A 7.1.3, 7.2.3.1 Adsorption / desorption screening test

Annex Point IIA7.7, , XII.1.2		**Adsorption / desorption in four soils and one sediment**
		Due to the relatively low water solubility of cypermethrin and its instability in the test system, it was not possible to determine adsorption and desorption isotherms for this compound. Freundlich adsorption coefficient (K) values could not therefore be determined. In order to make an estimated assessment of the mobility of cypermethrin the minimum K_{oc} values of this compound in each soil/sediment were used.
5.2.1	Asorption coefficient normalised for organic carbon content, K_{OC}	80653 to 574360 mL/g
5.3	**Conclusion**	These results characterise cypermethrin as 'non-mobile' (Hollis, J M (1991)) and 'immobile' (McCall) in each of the four soils and in the sediment
5.3.1	Reliability	1
5.3.2	Deficiencies	No. Test procedures were in line with the requirements of OECD guideline 106, despite the fact that the full study could not be performed.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	May 2007
Materials and Methods	3.1.2: The water solubility value given here doesn't correspond to the value given in the listing of endpoints! Which one should be used? As regards to other summary, the 0.004 mg/l value has been chosen.
Results and discussion	Applicant's version is accepted
Conclusion	Applicant's conclusions are accepted. The molecule will be considered as immobile.
Reliability	1
Acceptability	Acceptable
Remarks	The molecule is known to degrade in soil and in water. It would be interesting to have an idea of the mobility of the degradation product.

COMMENTS FROM ...

Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A 7.2.3.1-1: Classification and physico-chemical properties of soils used as adsorbents

Parameter / Soil name	Site C1 sediment	Soil EL-7	Soil SK 566696	Soil SK 961089	Soil Matanuska
Source	SK268699, Derbyshire, UK	149N, Range 54 West, NW section 7, USA	SK 566696, Derbyshire, UK	SK 961089, Rutland, UK	Makenzie, Alaska
Textural classification, UK/BBA	Sandy loam	Clay loam	Loamy sand	Clay loam	Sandy silt loam
Sand (%)	76	31	84	36	28
Silt (%)	15	45	5	36	63
Clay (%)	9	24	11	28	9
Organic matter (%)	2.9	5.2	1.4	8.3	5.5
Maximum water holding capacity (pF0)	42.6	69.0	29.0	77.6	64.1
pH ($CaCl_2$)	5.4	6.3	4.2	7.5	4.7
Cation exchange capacity (MEQ/100 g)	10.1	38.7	13.4	41.6	29.7

Table A 7.2.3.1-2: Results of the ratio test under sterile conditions

Unit	Ratio	Soil type	% applied radioactivity in supernatant	Mean % applied radioactivity in supernatant	% applied radioactivity in soil
SSR11	1:50 w/v	Site C1 sediment	0.6	0.6	96.8
SSR12	1:50 w/v	Site C1 sediment	0.6	-	NA
SSR13	1:50 w/v	EL-7	1.3	1.1	96.8
SSR14	1:50 w/v	EL-7	0.8	-	NA
SSR15	1:50 w/v	SK566696	1.1	1.1	101.1
SSR16	1:50 w/v	SK566696	1.1	-	NA
SSR17	1:50 w/v	SK961089	1.3	1.3	99.9
SSR18	1:50 w/v	SK961089	1.3	-	NA
SSR19	1:50 w/v	Matanuska	0.8	1.1	97.6
SSR20	1:50 w/v	Matanuska	1.3	-	NA
NA = Not Analysed					

Table A 7.2.3.1-3: Purity in soil and supernatant samples over 3 hours (sterile conditions)

Soil type	Cypermethrin purity in soil			Cypermethrin purity in supernatant			Cypermethrin purity in the total system
	CCP	TCP	Total	CCP	TCP	Total	
Site C1 sediment	37.6	55.9	93.5	NA	NA	NA	93.5
EL-7	34.6	52.5	87.1	ND	ND	ND	87.1
SK566696	40.8	53.6	94.4	NA	NA	NA	94.4
SK961089	41.4	55.3	96.7	NA	NA	NA	96.7
Matanuska	40.0	55.3	95.3	NA	NA	NA	95.3

Recovery values are expressed as a percent of applied radioactivity
CCP = *cis*-cypermethrin
TCP = *trans*-cypermethrin
Total = Total cypermethrin
NA = Not analysed
ND = Not detected

Table A 7.2.3.1-4: Assessment of mobility (minimum Koc)

Soil name	Minimum K_d	Minimum K_{oc}
Site C1 sediment	8976	527972
EL-7	4858	202418
SK566696	4595	574360
SK961089	3871	80653
Matanuska	4876	152388

Section A7.3.1
Annex Point IIA7.3.1

Phototransformation in air (estimation method), including identification of breakdown products

Photochemical Oxidative Degradation

Official use only

1 REFERENCE

1.1	**Reference**	Greenwood, J., Maudsley, L. (2003); Cypermethrin cis:trans/40:60 (purified active substance): Quantum yield analysis; Covance Laboratories Ltd, study number 0040/034 (CYP/M70), 24 April 2003 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1 **Guideline study** Not applicable, test is non experimental

3 MATERIALS AND METHODS

3.1 The chemical structure of the test substance was derived in SMILES format and entered into the computer programme AopWin v1.90, contained within the suite in EpiWin v3.05. From the structure, this programme estimates the possibility of whether photochemical oxidative degradation may occur, which pathways may be involved, and where appropriate, the likely rates of such degradation pathways.

4 RESULTS

4.1 The structure of cypermethrin under SMILES format was derived to be as follows:

C(CL) (CL)=CC3C(C) (C)C3C(=O)OC(C(#N))c1cc(Oc2ccccc2)ccc1

Atmospheric oxidation (25°C)

Hydroxyl Radicals Reaction:

Overall OH Rate constant = 21.4274 E-12 cm3/molecule-sec
Half-Life = 0.499 Days (12-hr day; 1.5 E6 OH/cm3)
Half-Life = 5.990 Hrs

Ozone Reaction:

Overall Ozone Rate constant = 0.023261 E-17 cm3/molecule-sec
Half-Life = 49.268 Days (at 7 E11 mol/cm3)

Section A7.3.1
Annex Point IIA7.3.1

Phototransformation in air (estimation method), including identification of breakdown products

Photochemical Oxidative Degradation

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The Photochemical Oxidative Degradation was calculated using the computer programme AopWin v1.90, contained within the suite in EpiWin v3.05 (Atkinson method).

5.2 Results and discussion

Atmospheric oxidation (25°C)

Hydroxyl Radicals Reaction:

Overall OH Rate constant = 21.4274 E-12 cm3/molecule-sec
Half-Life = 0.499 Days (12-hr day; 1.5 E6 OH/cm3)
Half-Life = 5.990 Hrs

Ozone Reaction:

Overall Ozone Rate constant = 0.023261 E-17 cm3/molecule-sec
Half-Life = 49.268 Days (at 7 E11 mol/cm3)

5.3 Conclusion

5.3.1 Reliability 1

5.3.2 Deficiencies No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2007
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Photolysis in air is calculated using 12-hour days to a half-life of 6 hours. We suggest including a remark that TGD recommends using 24-hour days and $5.0x10^5$ OH/cm^3. **Atmospheric oxidation (25°C)** **Hydroxyl Radicals Reaction:** Overall OH Rate constant = 21.4274 E-12 cm3/molecule-sec Half-Life = 0.749 Days (24-hr day; 0.5 E6 OH/cm3) Half-Life = 17.990 Hrs
Conclusion	Applicant's version is adopted
Reliability	2
Acceptability	Acceptable

Section A7.3.1 **Annex Point IIA7.3.1**	**Phototransformation in air (estimation method), including identification of breakdown products** **Photochemical Oxidative Degradation**

Remarks	This method only gives indications of the degradation rate of cypermethrine and is not an experimental test. However, as the molecule is not intended to reach the air compartment, it is accepted.
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

Official use only

		1 REFERENCE
1.1	**Reference**	Manson, P. (2005); Cypermethrin cis:trans/40:60: Acute toxicity to *Oncorhynchus mykiss*; Covance Laboratories Ltd; study no. 1669/018, 10 January 2006 (unpublished) Dates of experimental work: 21 April 2005 – 21 July 2005
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD guideline 203 and Annex V to EC Directive 92/69/EEC, Part C1
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	SL25163S63
3.1.2	Specification	As given in section 2
3.1.3	Purity	93.05 % w/w
3.1.4	Composition of Product	Not applicable, study was carried out on the active substance
3.1.5	Further relevant properties	Cypermethrin has a very low water solubility and was difficult to maintain at concentrations in the flow through range finding test. For this reason the test design in the main study was changed to semi-static and the test system was preconditioned for 96 hours before the addition of fish.
3.1.6	Method of analysis	Concentrations of cypermethrin in the test media were determined by Gas Chromatography with Electron Capture detection (GC-ECD) (method ref. CLE(E)1669/022-01V). Each stereoisomer of cypermethrin cis:trans 40:60 was measured individually and the total cypermethrin cis:trans 40:60 concentration was calculated by summing the four individual stereoisomer concentrations. The method was fully validated in a separate study by Covance Laboratories. Cypermethrin was first extracted from the fish dilution water by adding saturated aqueous sodium chloride followed by hexane. The flask was shaken for *ca.* 1 minute and the layers were allowed to separate. The lower aqueous layer was drained into a 1-litre glass bottle and the upper hexane layer was passed through a plug of anhydrous sodium sulphate, into a 150-mL glass bottle. The aqueous layer was returned to the separating funnel. The aqueous sample was extracted with a further two portions of hexane rinsing the glass bottle which had

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

		contained the aqueous layer with hexane each time. The hexane extracts were combined, evaporated to dryness under a stream of nitrogen at 40°C and reconstituted in 1 mL of toluene, with the aid of ultrasound treatment.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see table A7_4_1_1-1
3.3	**Reference substance**	No
3.4	**Range Finder**	Two separate range finding tests were performed. The first was performed as a flow through test, at concentrations of 0.1, 1, 10 and 100 µg/L. Due to problems in maintaining the concentrations of test substance under flow-through conditions, the test design was changed to semi-static. The second range-finding test was performed using fewer concentrations to minimise the number of fish used. The concentrations used were 0.01, 1 and 100 µg/L.
3.5	**Testing procedure**	
3.5.1	Dilution water	See table A7_4_1_1-2
3.5.2	Test organisms	See table A7_4_1_1-3
3.5.3	Test system	See table A7_4_1_1-4
3.5.4	Test conditions	See table A7_4_1_1-5
3.5.5	Duration of the test	96 hours
3.5.6	Test parameter	Mortality
3.5.7	Sampling	At 0 and 72 hours, a sample (100 mL) of each freshly prepared test media was taken from each vessel for chemical analysis of cypermethrin *cis:trans* 40:60. Samples (1000 mL) of the freshly prepared control and solvent control were also taken for analysis. At 24 and 96 hours, a sample (100 mL) of old media from each individual vessel was taken for chemical analysis of cypermethrin *cis:trans* 40:60. Samples (1000 mL) of the freshly prepared control and solvent control were also taken for analysis. Samples of the acetone stock solutions used to prepare the test media were also analysed. The 4.01 and 1000 mg/L acetone stock solutions were analysed at the start and end of the test, in order to demonstrate the stability of cypermethrin *cis:trans* 40:60 in acetone. Samples of the remaining acetone stock solutions were analysed at the end of the test only. All extracts were stored refrigerated (1-10°C) where necessary
3.5.8	Monitoring of TS concentration	Yes, see section 3.4.7 above
3.5.9	Statistics	LC_{50} values were calculated using a Probit method

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

4 RESULTS

4.1	**Limit Test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal cypermethrin concentrations used in the main study were 0.401, 0.882, 1.94, 4.27, 9.39, 20.7, 45.5 and 100 µg/L
4.2.2	Actual concentrations of test substance	See table A7_4_1_1-8
4.2.3	Effect data (Mortality)	See tables A7_4_1_1-9 and A7_4_1_1-10
4.2.4	Concentration / response curve	Not determined
4.2.5	Other effects	After *ca.* 5 hours, it was observed that all of the fish in the 20.7, 45.5 and 100 µg/L test media were displaying signs of pain and distress. Symptoms being displayed included flared opercular, nervous and erratic darting, barrel rolling, immobility, hyperventilation, convulsions/tremors, severe head shaking and loss of normal pigmentation; their bodies were very pale compared to the fish at the lower concentrations and in the control treatments. Therefore, these fish were killed *in extremis*.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	See table A7_4_1_1-9 After 48 hours, in the solvent control treatment, 1 of the fish was found dead outside of the vessel, having escaped through the side of the lid.
4.3.2	Nature of adverse effects	Not applicable
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute toxicity of cypermethrin cis:trans 40:60 to the Salmonid fish , Onchorhynchus mykiss was determined at approximately 14°C in a 96-hour, semi-static definitive test. The definitive test was conducted at nominal cypermethrin cis:trans 40:60 exposure concentrations of 0.401, 0.882, 1.94, 4.27, 9.39, 20.7, 45.5 and 100 µg/L, based on the results of two range-finding tests. In the definitive test, initially, a solvent (acetone) stock solution was prepared at a nominal cypermethrin cis:trans 40:60 concentration of

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

		1000 mg/L. A concentration series of solvent (acetone) stock solutions was then prepared at 455, 207, 93.9, 42.7, 19.4, 8.82 and 4.01 mg/L by serial dilution of the 1000 mg/L solvent stock solution. The test media at nominal cypermethrin cis:trans 40:60 concentrations of 100, 45.5, 20.7, 9.39, 4.27, 1.94, 0.882 and 0.401 µg/L, were prepared by measuring a 1.4 mL aliquot of the appropriate acetone stock solution into a 15-litre constructed glass aquarium (test vessel) part filled with treated mains water. Each test vessel was made up to 14 litres with treated mains water and stirred for homogeneity. Appropriate solvent (1.4 mL of acetone into 15 litres of treated mains water) and dilution water control media (15 litres of treated mains water only) were also prepared.
5.2	**Results and discussion**	The overall geometric mean measured concentrations of cypermethrin cis:trans 40:60 in the 0.401, 0.882, 1.94, 4.27, 9.39, 20.7, 45.5 and 100 µg/L test media were 0.509, 0.874, 1.944, 4.11, 8.74, 22.2, 44.8 and 79.3 µg/L respectively (corresponding to 127, 99.1, 100, 96.3, 93.1, 107, 98.5 and 79.3% of the nominal concentration). As some values were outside the 80 to 120% of nominal range, the toxicity values were therefore expressed in terms of the geometric mean measured concentrations. The 24-hour LC_{50} toxicity value was calculated to be 4.26 µg/L (95% CI not determined). The highest concentration at which no mortality occurred was 1.94 µg/L. The lowest concentration at which 100% mortality occurred was 8.74 µg/L. The 48, 72 and 96-hour LC_{50} toxicity values were all calculated to be 2.83 µg/L (95% CI not determined). The highest concentration at which no mortality occurred was 1.94 µg/L. The lowest concentration at which 100% mortality occurred was 4.11 µg/L.
5.2.1	LC_0	1.94 µg/L
5.2.2	LC_{50}	2.83 µg/L (95% CI not determined)
5.2.3	LC_{100}	4.11 µg/L
5.3	**Conclusion**	The validity criteria of control mortality being less than 10% and maintaining a dissolved oxygen concentration above 60% of the air saturation value were satisfied
5.3.1	Other Conclusions	
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2007
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is adopted.
Conclusion	Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable
Remarks	Calculated toxicity value, very close to the water solubility value of the active. In the table 7_4_1_1_4, test system: the fish stock density was 1g/l and not 0.8g/l (cf Appendix 1 of the study report).
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes, Acetone was used as the vehicle
Concentration of vehicle	A 1000 mg/L stock solution was prepared (0.1009g cypermethrin in 100ml acetone). This was then used to prepare a concentration series of stock solutions of 1000, 455, 207, 93.9, 42.7, 19.4, 8.82 and 4.01 mg/L which were then used to prepare the nominal test media concentrations in the glass aquaria (1.4ml of concentration stock added to 14 L water)
Vehicle control performed	Yes, 1.4ml of acetone alone was added to 15 L water in the solvent control aquarium.
Other procedures	A semi-static test design was used in order to help maintain the appropriate test media concentrations.

Table A7_4_1_1-2: Dilution water – measurements taken in holding tanks

Criteria	Details
Source	De-chlorinated laboratory mains supply.
Alkalainity[1]	27.3 mg/L (HCO_3)
Residual chlorine	0.00-0.06 mg/L
Hardness	79-98 mg/l (as $CaCO_3$)
pH	7.1-7.5
Oxygen content	90-98% (air saturation volume)
Conductance[1]	165 uS/cm
Holding water different from dilution water	No

[1] Not measured in holding tanks, values taken from certificate of analysis for laboratory mains supply water

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	*Onchorhynchus mykiss*
Source	Recognised external supplier
Wild caught	No
Age/size	Mean fork length of 5.3 cm and mean wet of 1.605g
Kind of food	The fish were fed a proprietary food, which was supplied to the fish in quantities and grades of fineness dependent on the size of the fish. The food was not considered to contain contaminants likely to affect the outcome of the study.
Amount of food	Appropriate to size of fish.
Feeding frequency	Not specified in report
Pre-treatment	Fish were acclimated for at least 12 days
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Semi static
Renewal of test solution	Two sets of aquaria were used to allow for daily renewal of test solution.
Volume of test vessels	15L-constructed glass aquaria containing 14L test media.
Volume/animal	Fish stock density was 0.80 g/L (weight of fish per litre of test media)
Number of animals/vessel	7
Number of vessels/ concentration	Two sets of vessels were prepared for each concentration but only one set was used at any one time during the test.
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	14.1-14.9 °C, see table A7_4_1_1-6 below
Dissolved oxygen	See table A7_4_1_1-7 below
pH	7.2-7.5, see table A7_4_1_1-6 below
Adjustment of pH	No
Aeration of dilution water	Yes, using an oil free supply of compressed air, bubbled through test media via a glass tube (preconditioned for 96h)
Intensity of irradiation	Not specified in report
Photoperiod	16 hour light, 8 hour dark

Table A7_4_1_1-6: Temperature and pH measurement

Parameter	Nominal cypermethrin concentration (µg/L)	0-hour (new)	24-hour (old)	24-hour (new)	48-hour (old)	48-hour (new)
#Temperature range over the duration of the study (°C)		14.1 - 14.9				
Test media temperature (°C)	Control	14.3	14.7	14.1	14.7	14.1
	Solvent control	14.2	14.7	14.2	14.7	14.3
	0.401	14.3	14.7	14.2	14.7	14.3
	0.882	14.3	14.7	14.2	14.7	14.3
	1.94	14.3	14.8	14.2	14.7	14.3
	4.27	14.3	14.7	14.2	14.7	-
	9.39	14.3	14.7	-	-	-
	20.7	14.3	14.7	-	-	-
	45.5	14.3	14.7	-	-	-
	100	14.3	14.7	-	-	-
pH	Control	7.4	7.3	7.3	7.5	7.4
	Solvent control	7.4	7.3	7.3	7.5	7.4
	0.401	7.4	7.2	7.3	7.5	7.4
	0.882	7.4	7.3	7.3	7.5	7.4
	1.94	7.4	7.3	7.3	7.4	7.4
	4.27	7.4	7.3	7.3	7.5	-
	9.39	7.4	7.3	-	-	-
	20.7	7.4	7.4	-	-	-
	45.5	7.4	7.3	-	-	-
	100	7.4	7.3	-	-	-
Total hardness (mg/L as $CaCO_3$)		98	-	75	-	75
Residual chlorine (mg/L)		0.00	-	0.04	-	0.06

- Value not required

Temperature range recorded using a digital minimum / maximum thermometer

Table A7_4_1_1-6 (continued): Temperature and pH measurement

Parameter	Nominal cypermethrin concentration (µg/L)	72-hour (old)	72-hour (new)	96-hour (old)
Test media temperature (°C)	Control	14.7	14.3	14.6
	Solvent control	14.7	14.3	14.6
	0.401	14.7	14.3	14.6
	0.882	14.8	14.3	14.7
	1.94	14.8	14.3	14.8
	4.27	-	-	-
	9.39	-	-	-
	20.7	-	-	-
	45.5	-	-	-
	100	-	-	-
pH	Control	7.3	7.3	7.3
	Solvent control	7.3	7.4	7.3
	0.401	7.3	7.4	7.3
	0.882	7.3	7.4	7.3
	1.94	7.4	7.4	7.3
	4.27	-	-	-
	9.39	-	-	-
	20.7	-	-	-
	45.5	-	-	-
	100	-	-	-
Total hardness (mg/L as $CaCO_3$)		-	73	-
Residual chlorine (mg/L)		-	0.05	-

- Value not required

Table A7_4_1_1-7 Dissolved oxygen measurements in test media

Parameter	Nominal cypermethrin *cis:trans* 40:60 concentration (µg/L)	0-hour (new)	24-hour (old)	24-hour (new)	48-hour (old)
Dissolved oxygen (% ASV/mg/L)	Control	100/10.37	94/9.71	97/9.74	103/10.43
	Solvent control	99/10.30	94/9.69	91/9.40	94/9.54
	0.401	100/10.36	95/9.75	93/9.51	96/9.82
	0.882	100/10.36	93/9.60	93/9.53	98/10.03
	1.94	98/10.28	97/9.95	92/9.61	92/9.44
	4.27	102/10.42	94/9.71	91/9.67	97/9.95
	9.39	100/10.35	95/9.71	-	-
	20.7	100/10.34	96/9.80	-	-
	45.5	103/10.50	93/9.58	-	-
	100	101/10.41	95/9.76	-	-

- Value not required
% ASV - percentage air saturation value

Parameter	Nominal cypermethrin *cis:trans* 40:60 concentration (µg/L)	48-hour (new)	72-hour (old)	72-hour (new)	96-hour (old)
Dissolved oxygen (% ASV/mg/L)	Control	102/10.38	92/9.46	99/10.17	93/9.57
	Solvent control	101/10.01	91/9.40	98/10.12	93/9.60
	0.401	101/10.01	92/9.51	96/9.97	93/9.59
	0.882	99/10.00	93/9.59	97/10.04	92/9.48
	1.94	100/9.95	95/9.87	97/10.05	93/9.61
	4.27	-	-	-	-
	9.39	-	-	-	-
	20.7	-	-	-	-
	45.5	-	-	-	-
	100	-	-	-	-

- Value not required
% ASV - percentage air saturation value

Table A7_4_1_1-8 Concentration of test substance during the study

Nominal cypermethrin *cis:trans* 40:60 conc. (µg/L)	Measured cypermethrin *cis:trans* 40:60 concentration (µg/L)				[$]Mean measured concentration (µg/L)	Mean as % of nominal
	0-hour (new)	24-hour (old)	72-hour (new)	96-hour (old)		
Control	-	-	-	-	N/A	N/A
Solvent Control	-	-	-	-	N/A	N/A
0.401	0.502	0.362	0.702	0.527	0.509	127
0.882	1.22	0.693	0.964	0.717	0.874	99.1
1.94	2.24	1.44	2.23	1.98	1.94	100
4.27	4.74	3.57	#	#	4.11	96.3
9.39	9.25	8.25	#	#	8.74	93.1
20.7	23.5	20.9	*	*	22.2	107
45.5	42.3	47.5	*	*	44.8	98.5
100	80.3	78.3	*	*	79.3	79.3
Fort 0.401	0.887	0.386	0.402	0.430	0.493	123
Fort 0.882	N/A	N/A	0.861	0.935	0.897	102
Fort 1.94	N/A	N/A	2.07	2.08	2.08	107
Fort 9.39	9.61	8.66[##]	N/A	N/A	9.12	97.1
Fort 100	92.2	84.0	N/A	N/A	88.0	88.0

N/A Not applicable

- Test substance not detected above the limit of determination (0.01 µg/L)

No analysis performed, all fish had died

* No analysis performed, fish had already been killed in extremis

[$] Mean value determined as the geometric mean of all measured concentrations. Example: Geometric mean for the 0.401 µg/L media was calculated as: 'Exp ((ln(0.502)+ln(0.362)+ln(0.702)+ln(0.527))/4)'.

Mean of two sample results. The results for the 1.94 and 100 µg/L fortification standards at 24 hours were not acceptable due to non-homogenous preparations. All standards were re-analysed following additional ultrasound treatment (to improve homogeneity), including the 9.39 µg/L fortification standard. The 24-hour value for the 9.39 fortification standard is therefore the geometric mean of the initial result (8.96 µg/L) and the re-analysed sample (8.37 µg/L).

Table A7_4_1_1-9: Mortality data

Nominal cypermethrin *cis:trans* 40:60 conc. (µg/L)	No. of Fish	Observation time and symptom classification *											
		ca. 1.5 hours				4-hour				24-hour			
		a	b	c	d	a	b	c	d	a	b	c	d
Control	7	7	-	-	-	7	-	-	-	7	-	-	-
Solvent control	7	7	-	-	-	7	-	-	-	7	-	-	-
0.401	7	7	-	-	-	6	1	-	-	6	1	-	-
0.882	7	5	2	-	-	3	4	-	-	4	3	-	-
1.94	7	4	3	-	-	4	3	-	-	3	4	-	-
4.27	7	4	2	1	-	2	3	2	-	-	2	2	3
9.39	7	-	3	4	-	-	1	6	-	-	-	-	7
20.7	7	-	1	6	-	-	-	7	-	-	-	-	7
45.5	7	-	-	7	-	-	-	7	-	-	-	-	7
100	7	-	-	7	-	-	-	7	-	-	-	-	7
Nominal cypermethrin *cis:trans* 40:60 conc. (µg/L)	**No. of Fish**	**48 hour**				**72-hour**				**96-hour**			
		a	b	c	d	a	b	c	d	a	b	c	d
Control	7	7	-	-	-	7	-	-	-	7	-	-	-
Solvent control	7	6	-	-	[$]1	6	-	-	[$]1	6	-	-	[$]1
0.401	7	5	2	-	-	5	2	-	-	7	-	-	-
0.882	7	4	3	-	-	3	4	-	-	4	3	-	-
1.94	7	4	3	-	-	1	6	-	-	3	4	-	-
4.27	7	-	-	-	7	-	-	-	7	-	-	-	7
9.39	7	-	-	-	7	-	-	-	7	-	-	-	7
20.7	7	-	-	-	7	-	-	-	7	-	-	-	7
45.5	7	-	-	-	7	-	-	-	7	-	-	-	7
100	7	-	-	-	7	-	-	-	7	-	-	-	7

* Symptom classification

a - No toxic effects

b - Mild toxic effects: swimming normally but exhibiting mild effects e.g. increased cough frequency, swimming position in test vessel different to controls

c - Severe toxic effects: eg swimming abnormally or lying on bottom of tank

d - Fish dead

- Value not required

[$] Fish found dead outside the vessel between 24 and 48-hour timepoints

Table A7_4_1_1-10: Effect data

	24 h [µg/l][1]	95 % c.l.	48, 72, 96 h [µg/l][1]	95 % c.l.
LC_0	1.94	Not determined	1.94	Not determined
LC_{50}	4.26	Not determined	2.83	Not determined
LC_{100}	8.74	Not determined	4.11	Not determined

[1] Effect data are based on the geometric mean measured concentration. 95% C.L. could not be determined.

Table A7_4_1_1-11: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fulfilled
Mortality of control animals <10%	Y	
Concentration of dissolved oxygen in all test vessels > 60% saturation	Y	
Concentration of test substance ≥80% of initial concentration during test		**Results based on geometric mean measured concentration**

Criteria for poorly soluble test substances	Y	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 **Daphnia magna**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Manson P (2005) Cypermethrin cis:trans 40:60: Acute toxicity to Daphnia magna. Covance Laboratories Ltd., Report no. 1669/019-D2149, 14 February 2006 (unpublished). Dates of experimental work: 21 March 2005 – 9 May 2005	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, study was carried out in accordance with OECD Guideline No. 202 Daphnia sp. Acute Immobilisation Test (April 2004), Annex V to EC Directive 92/69/EEC Methods for the Determination of Ecotoxicity Part C: C.2. Acute Toxicity for Daphnia and OPPTS Guideline 850.1010, Aquatic Invertebrate Acute Toxicity Test, Fresh Daphnids.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	SL 25163S63	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	93.05% w/w	
3.1.4	Composition of Product	Not applicable, test conducted on the a.s.	
3.1.5	Further relevant properties	Due to the low water solubility of cypermethrin (c. 4 µg/L), the maximum dose tested was 100 µg/L and the test substance was administered in acetone.	X
3.1.6	Method of analysis	The Analytical Procedure CLE (E) 1669/022-01V, used for the quantification of cypermethrin in aquatic test medium was previously validated by Covance laboratories Ltd . The concentrations of cypermethrin cis:trans 40:60 in samples of test media were determined by GC with electron capture detection (ECD).	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_2-1	
3.3	**Reference substance**	No	

Section A7.4.1.2 **Acute toxicity to invertebrates**

Annex Point IIA7.2 **Daphnia magna**

3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_2-2
3.4.2	Test organisms	See table A7_4_1_2-3
3.4.3	Test system	See table A7_4_1_2-4
3.4.4	Test conditions	The pH, temperature (°C) and concentration of dissolved oxygen (% air saturation value and mg/L) were measured in freshly prepared control and test media at the start of the test, and from pooled samples of old test media at each nominal concentration at the end of the test. Total hardness and alkalinity of the ASTM medium used to prepare the test media was measured prior to test media preparation. See table A7_4_1_2-5 and table A7_4_1_2-6
3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Immobility
3.4.7	Sampling	After 24 and 48 hours, each of the test vessels was observed and the numbers of immobilised D. magna were recorded. The D. magna were considered immobile if, when the contents of the test vessel were briefly swirled, they did not swim during a 15-second period of observation. The observations differentiated between those animals that were immobile at the bottom of the vessel and those trapped/held in the surface tension of the test media.
3.4.8	Monitoring of TS concentration	Yes. Six additional control test vessels were prepared for each of the control and solvent control treatments, to give sufficient sample volume for the analysis. All additional control vessels were treated in the same manner as the initial four replicates in each control treatment. Samples were taken from the freshly prepared control treatments (1000 mL) and test media (100 mL) at the start of the test and from pooled replicate vessels of the control (930 mL), solvent control (920 mL) and test media (100 mL) at 48 hours (old), and analysed on each occasion for cypermethrin cis:trans 40:60.
3.4.9	Statistics	Toxicity values were calculated using the probit method of analysis.
	4 RESULTS	
4.1	**Limit Test**	Not performed . A preliminary range-finder was conducted to determine a suitable range of concentrations for the definitive study.

Section A7.4.1.2 **Acute toxicity to invertebrates**

Annex Point IIA7.2 **Daphnia magna**

4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Based on the results of the range-finder study, nominal concentrations of cypermethrin in the definitive study were 1.94, 4.27, 9.39, 20.7, 45.5 and 100 µg/L. See Table A7_4_1_2-7
4.2.2	Actual concentrations of test substance	See Table A7_4_1_2-7
4.2.3	Effect data (Immobilisation)	See Table A7_4_1_2-8 and Table A7_4_1_2-9 for immobilisation data after 24 and 48 hours respectively. Based on the mean measured cypermethrin cis:trans 40:60 concentrations, the 24-hour EC_{50} toxicity value was calculated to be outside of the experimental range (971 µg/L) and 95% confidence limits could not be determined. The corresponding no observed effect concentration (NOEC) was 1.05 µg/L (5% immobility occurred at this concentration but is not considered to be of biological significance). The lowest concentration causing 100% immobility could not be determined. The 48-hour EC_{50} toxicity value was calculated to be 4.71 µg/L (equivalent to 6.87 µg/L nominal), 95% confidence limits could not be determined. The corresponding NOEC and the lowest concentration causing 100% immobility could not be determined.
4.3	**Results of controls**	At both 24 and 48 hours, no immobility was observed in the control.
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute toxicity of cypermethrin cis:trans 40:60 to Daphnia magna was determined at approximately 20°C in a 48-hour static test, without renewal of the test media. The definitive test was conducted at nominal cypermethrin concentrations of 1.94, 4.27, 9.39, 20.7, 45.5 and 100 µg/L. These concentrations were selected based on the results of a range-finding limit test (presented in Appendix 1) conducted at 0.1, 1, 10 and 100 µg/L. For the definitive test, the test media were prepared by transferring 10 µL aliquots of 1000, 455, 207, 93.9 and 42.7 and 19.3 mg/L acetone stock media (prepared by serial dilution of a 1000 mg/L acetone stock medium) into four replicate test vessels, (150 mL glass crystallising dishes containing 100 mL of daphnia dilution media) at each of the nominal concentrations. Solvent and dilution media control vessels were also prepared.

Section A7.4.1.2 **Acute toxicity to invertebrates**

Annex Point IIA7.2 **Daphnia magna**

		Five juvenile D.magna were added to each replicate test vessel and the number of immobile D.magna was recorded at 24 and 48 hours. Samples of each test medium were sampled for chemical analysis at 0 and 48 hours.
5.2	**Results and discussion**	The overall geometric mean measured concentrations of cypermethrin cis:trans 40:60 in samples of test media were 1.05, 2.55, 7.20, 14.2, 32.7 and 56.1 µg/L respectively, corresponding to 54.1, 59.7, 76.7, 68.6, 71.9 and 56.1% of the nominal cypermethrin cis:trans 40:60 concentrations respectively. As the mean measured concentrations were outside of 80 to 120% of the nominal concentrations, the toxicity values are expressed in terms of mean measured concentrations. The 24-hour EC_{50} toxicity value was calculated to be outside of the experimental range (971 µg/L) and 95% confidence limits could not be determined, with a corresponding no observed effect concentration (NOEC) of 1.05 µg/L (5% immobility occurred at this concentration but is not considered to be biologically significant). The lowest concentration causing 100% immobility at 24 hours could not be determined. The 48-hour EC_{50} toxicity value was calculated to be 4.71 µg/L (equivalent to 6.87 µg/L nominal), 95% confidence limits could not be determined. The corresponding NOEC and the lowest concentration causing 100% immobility could not be determined.
5.2.1	EC_0	The lowest concentration causing 100% immobility could not be determined.
5.2.2	EC_{50}	The 48-hour EC_{50} toxicity value was calculated to be 4.71 µg/L (equivalent to 6.87 µg/L nominal), 95% confidence limits could not be determined.
5.3	**Conclusion**	The 48-hour EC_{50} toxicity value was calculated to be 4.71 µg/L (equivalent to 6.87 µg/L nominal), 95% confidence limits could not be determined. The corresponding NOEC and the lowest concentration causing 100% immobility could not be determined, however a NOEC value is available from the chronic daphnia study (Doc IIIA_7.4.3.4). The validity criteria of a maximum of 10% immobility in the control treatment and achieving dissolved oxygen concentrations of ≥ 60% in the control and test vessels at the end of the test were both satisfied.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 **Daphnia magna**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2007
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is adopted.
Conclusion	Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable.
Remarks	Under 3.1.5, the water solubility of the test substance is not the same as the result in the physic-chem part of the dossier (i.e.< 9µg/l) Belgium propose to use the value 4µg/l also in the LOE.
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Acetone
Concentration of vehicle	A concentrated stock medium at a nominal cypermethrin cis:trans 40:60 concentration of 1000 mg/L was prepared by weighing 10.0 mg of cypermethrin into a 10-mL volumetric flask and making up to volume with acetone. The 1000 mg/L acetone stock medium was then used to prepare a series of acetone stock media at nominal concentrations of 455, 207, 93.9 42.7 and 19.3 mg/L which were used to make up the final test concentrations (10 µl aliquots in 100mL of daphnia dilution media).
Vehicle control performed	Yes. Solvent controls were prepared using 10 µL acetone in four replicate test vessels each containing 100 mL of daphnia dilution media.
Other procedures	Test vessels were preconditioned prior to test initiation. Test media was added to each vessel and allowed to stand for 2 days prior to addition of D.magna.

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Standard hard water prepared according to ASTM (American Society for Testing and Materials) prepared with analytical grade reagents and RO water.
Alkalinity[1]	79-85 mg/l (as $CaCO_3$)
Hardness[1]	165-175 mg/l (as $CaCO_3$)
pH[1]	7.3-7.8
$NaHCO_3$	192 mg/L
$CaSO_4 . 2H_2O$	120 mg/L
$MgSO_4 . 7H_2O$	246 mg/L
KCl	8 mg/L
Oxygen content[1]	97 % air saturation
Conductance	Not specified
Holding water different from dilution water	No

[1]Results given for the D.magna holding culture prior to the definitive test (last 2 water changes)

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	Straus
Source	Cultures maintained at Covance Laboratories (original culture obtained from The Environment Agency, Portsmouth, UK)
Age	< 24 hours old
Breeding method	Juveniles were removed when present in cultures at least three times per week using a sieve. Cultures were maintained up to a maximum of 28 days. Juveniles for use in acute toxicity tests were collected from the second brood onwards.
Kind of food	Concentrated suspension of *Chlorella vulgaris*. (Seaweed extract also added at each medium renewal)
Amount of food	Not specified
Feeding frequency	Daily.
Pre-treatment	Approximately 24 hours before a test was set up, juveniles present in the cultures were removed and discarded. Over the next 24 hours, juveniles for use in the test were then removed from the culture and transferred to fresh culture medium, using a wide bore pipette. The juveniles were then left for at least 1 hour before selecting actively swimming individuals for use.
Feeding of animals during test	No

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	No
Volume of test vessels	150ml glass dish containing 100ml daphnia dilution media
Volume/animal	20ml
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	See table A7_4_1_2-6
Dissolved oxygen	See table A7_4_1_2-6
pH	See table A7_4_1_2-6
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	
Photoperiod	16-hour light; 8-hour dark

Table A7_4_1_2-6: Water quality (measurements during definitive test)

Parameter	Nominal concentration (µg/L)	0-hour (new media)	48-hour (old media)
Alkalinity as $CaCO_3$ (mg/L)	Control	76	-
Total hardness as $CaCO_3$ (mg/L)	Control	168	-
Temperature (°C)	Control	20.8	20.5
	Solvent control	20.7	20.4
	1.94	20.8	20.4
	4.27	20.6	20.5
	9.39	20.6	20.5
	20.7	20.6	20.4
	45.5	20.7	20.5
	100	20.7	20.5
pH	Control	7.6	7.9
	Solvent control	7.6	8.0
	1.94	7.6	8.0
	4.27	7.6	8.1
	9.39	7.6	8.1
	20.7	7.6	8.1
	45.5	7.6	8.2
	100	7.6	8.2
Dissolved oxygen	Control	98/8.5	98/8.7
(% air saturated	Solvent control	99/8.7	94/8.3
value/mg/L)	1.94	98/8.6	94/8.4
	4.27	95/8.5	94/8.4
	9.39	99/8.7	94/8.4
	20.7	98/8.6	93/8.3
	45.5	97/8.6	92/8.2
	100	99/8.8	93/8.3

Table A7_4_1_2-7: Analysis of test substance during the definitive test

Nominal cypermethrin *cis:trans* 40:60 concentration (µg/L)	Measured cypermethrin *cis:trans* 40:60 concentration (µg/L)			Mean conc. as % of nominal conc.
	0-hour (new media)	48-hour (old media)	*Mean	
Control	-	-	N/A	N/A
Solvent control	-	-	N/A	N/A
1.94	1.34	0.822	1.05	54.1
4.27	3.67	1.77	2.55	59.7
9.39	10.7	4.83	7.20	76.7
20.7	20.6	9.80	14.2	68.6
45.5	39.8	26.9	32.7	71.9
100	46.6	67.4	56.1	56.1

- Not detected above LOD (Limit of Determination = 0.1 µg/L)

* Mean value determined as the geometric mean of the 0 a nd 48-hour measured concentrations (Geometric mean calculated as: e.g. at 100 µg/L, mean = 'Exp ((ln(46.6)+ln(67.4))/2)'.

N/A - Not applicable

Table A7_4_1_2-8: Immobilisation data after 24 hours exposure

Nominal cypermethrin *cis:trans* 40:60 concentration (µg/L)	Number of *Daphnia magna* exposed	Mobile *Daphnia*		Immobile *Daphnia*	
		Bottom	Surface	Bottom	Surface
Control	20	20	-	-	-
Solvent control	20	20	-	-	-
1.94	20	18	$1	1	-
4.27	20	16	$2	2	-
9.39	20	14		6	-
20.7	20	17	-	3	-
45.5	20	15	-	5	-
100	20	15	-	5	-

- Values not appropriate

$ - Surface held *D. magna* resubmerged by single drop of test media applied using a Pasteur pipette

Table A7_4_1_2-9: Immobilisation data after 48 hours exposure

Nominal cypermethrin *cis:trans* 40:60 concentration (mg/L)	Number of *Daphnia magna* exposed	Mobile *Daphnia*		Immobile *Daphnia*	
		Bottom	Surface	Bottom	Surface
Control	20	20	-	-	-
Solvent control	20	20	-	-	-
1.94	20	13	-	7	-
4.27	20	11	-	7	2
9.39	20	7	1	11	1
20.7	20	7	-	13	-
45.5	20	5	1	13	1
100	20	8	3	7	2

- Values not appropriate

Table A7_4_1_2-10: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h	971 µg/L	ND	ND	ND
48 h	4.71 µg/L	ND	ND	ND

[1] Based on measured (m) concentrations

ND= not determined

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Official use only

		1 REFERENCE
1.1	**Reference**	Manson, P. (2005); Cypermethrin cis:trans/40:60: Inhibition of growth to the alga *Pseudokirchneriella subcapitata*; Covance Laboratories Ltd, Harrogate, UK, study no. 1669/020, 11 January 2006 (unpublished). Dates of experimental work: 31 March 2005 – 29 April 2005
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD guideline 201 (July 1992), Annex V to EC Directive 92/69/EEC Part C;C.3, OPPTS guideline 850.5400 tiers I and II.
2.2	**GLP**	Yes
2.3	**Deviations**	Minor deviation only. In the range finding test, two temperatures were outside the specified range stated in the study protocol for approximately 30 minutes on two occasions but these were not considered to have impacted upon the integrity of the study.
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	SL25163S63
3.1.2	Specification	As given in section 2
3.1.3	Purity	93.05% w/w
3.1.4	Composition of Product	Not applicable, study performed on active substance
3.1.5	Further relevant properties	Due to the low water solubility of cypermethrin, acetone was used as the solvent.
3.1.6	Method of analysis	Concentrations of cypermethrin cis:trans 40:60 in algal media were extracted by partitioning with hexane, and quantified using GC using ECD detection. Each stereoisomer of cypermethrin cis:trans 40:60 was measured individually and the total cypermethrin cis:trans 40:60 concentration was calculated by summing the four individual stereoisomer concentrations. A sample (100 or 1000 mL) of cypermethrin cis:trans 40:60 in the appropriate media was measured into a separating funnel. Saturated aqueous sodium chloride (20 or 50 mL) was added, followed by hexane (25 mL). The flask was shaken for ca. 1 minute and the layers were allowed to separate. The lower aqueous layer was drained into a 1-litre glass bottle and the upper hexane layer was passed through a plug of anhydrous sodium sulphate, into a 150-mL glass bottle. The aqueous layer was

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		returned to the separating funnel. The aqueous sample was extracted with a further two portions of hexane (2 x 25 mL), rinsing the glass bottle which had contained the aqueous layer with hexane each time. The hexane extracts were combined, evaporated to dryness under a stream of nitrogen at 40°C and reconstituted in 1 mL of toluene, with the aid of ultrasound treatment. All extracts were stored refrigerated (1 to 10°C) where necessary.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Due to the low water solubility, the test substance was dissolved in Acetone (see table A7_4_1_3-1)
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Culture medium	Stock solutions of the various nutrients were prepared with reverse osmosis water and then autoclaved. Once cooled, an aliquot of $NaCO_3$ was added via a sterile filter. See table A7_4_1_3-2 At the start of the test, the pH in the control vessels (algal nutrient medium only) was 7.4
3.4.2	Test organisms	See table A7_4_1_3-3
3.4.3	Test system	See table A7_4_1_3-4
3.4.4	Test conditions	See table A7_4_1_3-5 and A7_4_1_3-6
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Algal cell concentration (inhibition of algal cell growth)
3.4.7	Sampling	At approximately 24-hour intervals during the test, samples of test media were taken for cell counting from all flasks containing algae, and from those flasks not inoculated with algal cells for background cell counts.
3.4.8	Monitoring of TS concentration	Yes At 0 hours, samples of each control and test media were taken for chemical analysis of cypermethrin cis:trans 40:60, before addition to the test vessels. At 96 hours, pooled samples (1000 mL) from the control and solvent control treatments, from inoculated (with algal cells) and non-inoculated (blank) vessels, were taken for analysis. Samples (100 mL) from the 100 µg/L test media, both inoculated and blank vessels, were also taken for analysis.

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3.4.9 Statistics

The algal cell concentration data were evaluated using two approaches:

(a) Comparison of areas under the growth curve (A):

$$A = \frac{N_1 - N_0}{2} x\ t_1 + \frac{N_1 + N_2 - 2N_0}{2} x\ (t_2 - t_1) + \frac{N_{n-1} + N_n - 2N_0}{2} x\ (t_n - t_{n-1})$$

(b) Comparison of the average specific growth rate (μ):

$$\mu = \frac{\mathrm{Log}_e N_n - \mathrm{Log}_e N_0}{t_n}$$

Where:
N_0 = initial measured cell concentration at time t_0 (cells/mL)
N_1 = measured cell concentration at time t_1 (cells/mL)
N_n = measured cell concentration at time t_n (cells/mL)
N_2 = measured cell concentration at time t_2 (cells/mL)
t_1 = time of first measurement after the beginning of the test (h)
t_2 = time of second measurement after the beginning of the test (h)
t_n = time of n^{th} measurement after the beginning of the test (h)

Using A and μ, the percentage inhibition of cell growth at each concentration of test substance (I_A) was calculated as the difference between the mean of A or μ for controls (A_c) and A or μ at each concentration of test substance (A_s) using the formula:

$$I_A(\%) = \frac{A_c - A_s}{A_c} x\ 100$$

To distinguish between EC_{50} values determined using areas under the growth curve and those determined using growth rates, the symbols E_bC_{50} and E_rC_{50} were used respectively.

The highest no observed effect concentrations (NOEC) were determined from observations of the results of the definitive limit test

4 RESULTS

4.1 Limit Test — Performed

4.1.1	Concentration	100 µg/L (nominal)
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable

4.2 Results test substance

4.2.1	Initial concentrations of test substance	100 µg/L (nominal)