Section A6.6.4 Genotoxicity in vivo

Annex Point IIA 6.6.4 **In-vivo bone marrow micronucleus study in mice**

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 A6_6_4-1. Table for Mouse Micronucleus Test In Vivo

Males	**Positive control**		**Vehicle control**		**low dose 12.5 mg/kg**		**mid dose 25 mg/kg**		**high dose 50 mg/kg**	
Sampling time (h)	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**
Total PCE scored	497	na	573	577	521	526	477	499	415	429
Total NCE scored	503	na	427	423	479	474	523	501	585	571
PCE/NCE ratio	1.01	na	1.35	1.37	1.11	1.12	0.92	1.02	0.72	0.76
Total MPCE scored	175	na	54	45	40	42	42	40	58	43
%MPCE (mean ±SD)	1.75 ± 0.47	na	0.54 ± 0.16	0.45 ± 0.14	0.40 ± 0.19	0.42 ± 0.22	0.42 ± 0.19	0.40 ± 0.20	0.58 ± 0.13	0.43 ± 0.16

Females	**Positive control**		**Vehicle control**		**low dose 12.5 mg/kg**		**mid dose 25 mg/kg**		**high dose 50 mg/kg**	
Sampling time (h)	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**
Total PCE scored	520	na	587	587	511	560	489	452	408	452
Total NCE scored	480	na	413	413	489	440	511	548	592	548
PCE/NCE ratio	1.10	na	1.42	1.43	1.06	1.28	0.98	0.86	0.69	0.85
Total MPCE scored	183	na	46	33	49	39	42	34	55	42
MPCE (mean ±SD)	1.83 ± 0.61	na	0.46 ± 0.17	0.33 ± 0.18	0.49 ± 0.10	0.39 ± 0.11	0.42 ± 0.21	0.34 ± 0.15	0.55 ± 0.29	0.42 ± 0.15

Two day treatment – sampled 24 hours after dosing completed

Males and females	**Vehicle control**		**High dose 50 mg/kg**	
Sex	**M**	**F**	**M**	**F**
Total PCE scored	594	589	435	416
Total NCE scored	406	411	565	584
PCE/NCE ratio	1.47	1.45	0.77	0.72
Total MPCE scored	32	35	40	46
%MPCE (mean ±SD)	0.32± 0.16	0.35± 0.17	0.40± 0.20	0.46± 0.12

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA 6.6.4 **In-vivo bone marrow micronucleus study in mice**

Official use only

		1 REFERENCE
1.1	**Reference**	Oláh, B. (1999); Mouse bone marrow micronucleus test of test substance Cypermethrin cis:trans/40:60; Toxicology Research Centre Ltd, report no. 98/398-013M (CYP/T309), 9 March 1999 (unpublished) Dates of experimental work: 8 December 1998 – 10 December 1998
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 authorisation
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes. OECD guideline no. 474 (1987)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Cypermethrin cis:trans/40:60
3.1.1	Lot/Batch number	1503084
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Pale brown viscous liquid / semi-solid
3.1.2.2	Purity	94.4% w/w
3.1.2.3	Stability	Stable
3.1.2.4	Maximum tolerable dose	100 mg/kg, determined in a range finding study.
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	CRL:NMRI BR
3.2.3	Source	Lab-Tech Ltd, Budapest
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	8 weeks 25.0-29.7g (males) 22.0-27.7g (females)
3.2.6	Number of animals per group	5 males + 5 females per dose and sampling time
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral (test article) Intraperitoneal (positive control)
3.3.1	Number of applications	1

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA 6.6.4 **In-vivo bone marrow micronucleus study in mice**

3.3.2	Interval between applications	Not applicable
3.3.3	Postexposure period	24 and 48 h after treatment (pos. control: 48 h after treatment; untreated control: 24 h after beginning of study)
		Oral
3.3.4	Type	Stomach tube (test substance) or gavage (vehicle control)
3.3.5	Concentration	100 mg/kg (MTD), 75 mg/kg (75% MTD) and 50 mg/kg (50% MTD)
3.3.6	Vehicle	Sunflower oil
3.3.7	Concentration in vehicle	1.0, 0.75 and 0.5% w/v
3.3.8	Total volume applied	0.1 ml/10g bw
3.3.9	Controls	Untreated control Sunflower oil (vehicle control) Cyclophosphamide 60 mg/kg (positive control)
3.4	**Examinations**	
3.4.1	Clinical signs	No
3.4.2	Tissue	bone marrow
	Number of animals:	all animals
	Number of cells:	2000
	Time points:	24 or 48 h after treatment
	Type of cells	erythrocytes in bone marrow
	Parameters:	Micronucleated PCEs polychromatic/normochromatic erythrocytes ratio
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	Not determined
4.2	**Haematology / Tissue examination**	Test substance did not induce significant increase in the number of micronucleated PCEs in either males or females at any dose level 24 hours after treatment. After 48 hours, mathematically but not biologically significant increases in the number of MPCEs were seen in male mice at the 50 and 100 mg/kg dose and in females at the 75 mg/kg dose level. No differences in the ratio of polychromatic and normochromatic erythrocytes were found after treatment and significant depression of the PCE:NCE ratio was not observed. See table A6_6_4-1
4.3	**Genotoxicity**	Cypermethrin cis:trans/40:60 proved to be negative for mutagenicity in NMRI mice
4.4	**Other**	In the positive control, cyclophosphamide caused significant increase in the number of MPCEs 48 hours after application, thus validating the

Section A6.6.4	**Genotoxicity in vivo**	
Annex Point IIA 6.6.4	**In-vivo bone marrow micronucleus study in mice**	
	test.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Potential mutagenicity of Cypermethrin cis:trans/40:60 was examined in bone marrow of NMRI mice according to OECD guideline 474. Test substance was applied at three dose levels; 100, 75 and 50 mg/kg. Following a range-finding study, the MTD was found to be 100 mg/kg. In the main study, animals were treated once via the oral route and samples were taken 24 and 48 hours after treatment. During the microscopic evaluation, 2000 PCEs were scored per animal to assess the micronucleated cells.	
5.2 Results and discussion	Single doses of 100, 75 and 50 mg/kg did not induce an increase in the frequency of micronucleated polychromatic erythrocytes (MCPEs) in male and female mice at 24 and 48 hours after treatment when compared to the vehicle control. No difference in the ratio of polychromatic to normochromatic erythrocytes occurred when compared to the vehicle control.	X
5.3 Conclusion	Cypermethrin cis:trans/40:60 proved to be negative for mutagenicity in the mouse in-vivo bone marrow micronucleus test.	
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	April, 2007.
Materials and Methods	The applicant's version is acceptable with the following amendment: 3.1.2.3 Stability: The stability data of the test substance in the vehicle was not reported.
Results and discussion	Revised version: Table A6_6_4-1 is adapted. Signs of toxicity are not reported for the main study. Single doses of 100, 75 and 50 mg/kg did not induce an increase in the frequency of micronucleated polychromatic erythrocytes (MCPEs) in male and female mice at 24 hours after treatment when compared to the vehicle control. However 48 hours after treatment, statistically significant increases in the number of MPCEs were seen in male mice at the 50 and 100 mg/kg dose and in females at the 75 mg/kg dose level. Nevertheless, the biological relevance of the seen increases is questionable.
Conclusion	The applicant's version is adopted. Under the test conditions in this study, cypermethrin cis:trans/40:60 does not produce micronuclei in the immature erythrocytes of NMRI mice, and as such proved to be negative for mutagenicity.

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA 6.6.4 **In-vivo bone marrow micronucleus study in mice**

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 A6_6_4-1. **Table for Mouse Micronucleus Test In Vivo**

Sex: Male

	Untreated control	Positive control	Vehicle control		low dose 50 mg/kg		mid dose 75 mg/kg		high dose 100 mg/kg	
No. of PCEs evaluated	**10000**	**10000**	**10000**		**10000**		**10000**		**10000**	
Sampling time (h)	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**
PCE/NCE rate	1.27	0.31	1.18	1.18	1.12	1.08	1.14	1.11	1.08	1.07
MPCE (mean ±SD)	2.80 ± 0.45	32.60** ± 5.73	3.80 ± 0.45	3.40 ± 0.55	3.40 ± 1.14	4.40* ± 0.55	4.00 ± 1.00	3.60 ± 0.55	4.80 ± 0.84	4.60* ± 0.89

Sex: Female

	Untreated control	Positive control	Vehicle control		low dose 50 mg/kg		mid dose 75 mg/kg		high dose 100 mg/kg	
No. of PCEs evaluated	**10000**	**10000**	**10000**		**10000**		**10000**		**10000**	
Sampling time (h)	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**	**24**	**48**
PCE/NCE rate	1.24	0.34	1.21	1.25	1.13	1.19	1.18	1.11	1.11	1.07
MPCE (mean ±SD)	2.80 ± 0.84	32.80** ± 3.56	3.40 ± 0.89	3.60 ± 0.55	3.40 ± 0.55	3.80 ± 0.45	4.20 ± 0.84	4.60* ± 0.55	4.40 ± 0.55	4.8 ± 1.30

* $p<0.05$

** $p<0.01$ (Kruskal-Wallis Non Parametric Anova)

Section IIIA-6.6.5 **Annex Point IIA-6.6.5**	**In-vivo mutagenicity in tissues other than bone marrow**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [√]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The results of the in-vitro gene mutation study in bacteria and the in-vitro cytogenicity study in mammalian cells were both negative for genotoxic effects (see DocIIIA_6.6..1 and DocIIIA_6.6.2 respectively). In addition, the in-vivo mouse bone marrow micronucleaus test also showed a negative result for cypermethrin (see DocIIIA_6.6.4). According to the TNG on data requirements, no further genotoxicity studies are required.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	April, 2007.	
Evaluation of applicant's justification	The applicant's justification is acceptable.	
Conclusion	The applicant's justification is acceptable.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section IIIA-6.6.6 **Annex Point IIA-6.6.6**	**Assessment of possible germ cell effects**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [√]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The results of the in-vitro gene mutation study in bacteria and the in-vitro cytogenicity study in mammalian cells were both negative for genotoxic effects (see DocIIIA_6.6..1 and DocIIIA_6.6.2 respectively). In addition, the in-vivo mouse bone marrow micronucleaus test also showed a negative result for cypermethrin (see DocIIIA_6.6.4). According to the TNG on data requirements, no further genotoxicity studies are required.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	April, 2007.	
Evaluation of applicant's justification	The applicant's justification is acceptable.	
Conclusion	The applicant's justification is acceptable.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section IIIA-6.6.7 **Annex Point IIA-6.6.7**	**Further genotoxicity studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [√]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The results of the in-vitro gene mutation study in bacteria and the in-vitro cytogenicity study in mammalian cells were both negative for genotoxic effects (see DocIIIA_6.6..1 and DocIIIA_6.6.2 respectively). In addition, the in-vivo mouse bone marrow micronucleaus test also showed a negative result for cypermethrin (see DocIIIA_6.6.4). According to the TNG on data requirements, no further genotoxicity studies are required.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	April, 2007.	
Evaluation of applicant's justification	The applicant's justification is acceptable.	
Conclusion	The applicant's justification is acceptable.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.7 **Annex Point IIA.VI.6.7**	**Carcinogenicity study – Non-rodent species**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [√] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [√]** **Other justification [ ]**	
Detailed justification:	The results of in-vitro and in-vivo genotoxicity studies all indicate that the test material is non-genotoxic. The studies covered would allow for both the assessment of clastogenicity and mutagenicity. As a result there is no evidence to suggest that the material is a genotoxic carcinogen. The results of the combined chronic toxicity and carcinogenicity study in the rat shows no evidence of carcinogenicity. This result may also be supported by a lack of preneoplastic changes evident in a subchronic study in rat. The primary site of toxic effect is the neuronal system. Toxicity does not seem to affect the proportion of neuronal type tumours. For these reasons, and in order to minimise animal testing, further testing for carcinogenicity is not considered necessary for cypermethrin. It has been acknowledged that the data for the rat carcinogenicity study was not as complete as would be expected from similar studies conducted at this present time. However this does not detract from the quality of the results that were achieved from the study, which was also reviewed and accepted under Directive 91/414/EC.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	April, 2007.	
Evaluation of applicant's justification	The applicant's justification is accepted.	
Conclusion	The applicant's justification is accepted.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	

Section A6.7 **Carcinogenicity study – Non-rodent species** **Annex Point IIA.VI.6.7**
Remarks

Section A6.8.1 (01) Teratogenicity Study

Annex Point IIA6.8.1. (01) **Teratogenicity test – rat, oral route**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Tesh, J.M., Tesh, S.A., Davies, W. (1978); WL 43467 (Cypermethrin) – Effects upon the progress and outcome of pregnancy in the rat; Life Science Research, Laboratory report no. 78/SHL2/364 (CYP/T11), 4 October 1978 (unpublished). Dates of experimental work: 8 August 1978 – 4 October 1978	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2	Companies with letter of access		
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 authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guidelines mentioned in report, however protocol is in compliance with method B.31 of Directive 87/302/EEC, corresponding OECD guideline 414 (1981).	
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	WL 43467 (Cypermethrin)	
3.1.1	Lot/Batch number	30	
3.1.2	Specification	Deviating from specification given in section 2 as follows:	
3.1.2.1	Description	Not specified in report	
3.1.2.2	Purity	98.2 % w/w	
3.1.2.3	Stability	Not mentioned in report	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	CD	
3.2.3	Source	Charles River UK Ltd.	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Not specified in report	
3.2.6	Number of animals per group	25	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Not specified in report, however the day on which a sperm positive smear or vaginal plug was detected was designated day 1 of pregnancy.	
3.3	**Administration/ Exposure**	Oral	

Section A6.8.1 (01) Teratogenicity Study

Annex Point IIA6.8.1. (01) **Teratogenicity test – rat, oral route**

3.3.1	Duration of exposure	Rats were dosed from days 6 to 15 of pregnancy
3.3.2	Postexposure period	Females killed on day 21 of gestation (i.e. post exposure period = 6 days)
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	0, 17.5, 35 or 70 mg/kg bw/d Based on the results of a preliminary range finding study to identify suitable dose levels for the main study.
3.3.5	Vehicle	corn oil
3.3.6	Concentration in vehicle	1% w/v
3.3.7	Total volume applied	Appropriate dosage volume
3.3.8	Controls	Vehicle (corn oil)
3.4	**Examinations**	
3.4.1	Body weight	Yes
3.4.2	Food consumption	No
3.4.3	Clinical signs	Yes
3.4.4	Examination of uterine content	Number of corpora lutea, number of implantations, number of resorption sites, number and distribution of live and dead foetus in each uterine horn.
3.4.5	Examination of foetuses	
3.4.5.1	General	Litter size, number of dead foetuses, foetal weight, sex.
3.4.5.2	Skeletal processing	Yes
3.4.5.3	Soft tissue	Yes
3.5	**Further remarks**	Pre-implantation and Post-implantation % loss calculated.
3.6	**Statistics**	Significance of inter group differences were examined by analysis of variance.

Section A6.8.1 (01) **Teratogenicity Study**

Annex Point IIA6.8.1. (01) **Teratogenicity test – rat, oral route**

4 RESULTS AND DISCUSSION

4.1 Maternal toxic Effects

Appearance and general condition of females in the 17.5 and 35 mg/kg dose groups was similar to that of the control animals throughout the study.

In the top dose group (70 mg/kg), 11 out of the 25 females displayed transient neurological disturbances after a minimum of three doses. Signs commenced 4-7 hours after dosing and ranged from slight splaying of the hind legs whilst walking to severe splaying of all limbs, involountary jaw movements, convulsive spasms and hypersensitivity to noise. Animals appeared to recover when observed the following morning before dosing and effects were not observed after the treatment period had been completed. One female from the top dose group was killed in extremis following severe convulsions on day 14 of gestation and a second was found dead on day 15. Both had received a total of nine doses.

Bodyweight changes in the 17.5 mg/kg were similar to the controls througout gestation. A slight dose-related depression of weight gain was noted in the 35 and 70 mg/kg groups during the dosing period ($P < 0.05$ and $P < 0.001$ respectively) however subsequently their performance was slightly better than the controls.

Examination of all females at necropsy on day 21 of gestation revealed no macroscopic changes that could be related to treatment with cypermethrin. Litter responses were unaffected by treatment of the dams (see table A6_8_1_01-1).

4.2 Teratogenic / embryotoxic effects

Examination of foetuses at necropsy, revealed a small number of abnormalities in all groups; the type and the incidences of which have previously been found to occur spontaneously in this strain of rats. No cypermethrin treatment related effects were found (see table A6_8_1_01-1).

4.3 Other effects

Litter responses were unaffected by treatment of the dams with cypermethrin.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

25 adult female CD strain rats/group were dosed with cypermethrin (B.n°30; 98.2 %) by gavage (0, 17.5, 35 and 70 mg/kg bw/d in corn oil) from day 6 of pregnancy to day 15 inclusive. All animals were examined daily for signs of adverse effects. On day 21 of gestation the females were killed and examined macroscopically for signs of adverse reactions to the treatment. Foetuses were removed for skeletal and visceral examination.

Section A6.8.1 (01) Teratogenicity Study

Annex Point IIA6.8.1. (01) **Teratogenicity test – rat, oral route**

5.2	**Results and discussion**	General condition of the dams in the two lower dose groups was similar to the controls. Transient neurological disturbances were observed in the top dose group (70 mg/kg) which in two cases resulted in death. Remaining affected animals were seen to recover the next day and showed no signs after the treatment period had ended. Slight retardation of maternal bodyweight gain was observed at the 35 and 70 mg/kg dose levels with performance improving once the treatment period had ended. No macroscopic changes were observed in females at day 21 of gestation. Litter responses were unaffected. No foetal abnormalities were observed which could be attributed to treatment with cypermethrin at any dose level. A small number of foetal abnormalities in all groups were noted at necropsy, however these were not treatment related.	
5.3	**Conclusion**	There was no embryotoxicity or teratogenicity a ssociated with oral administration of cypermethrin.	
5.3.1	LO(A)EL maternal toxic effects	35 mg/kg bw/d	X
5.3.2	NO(A)EL maternal toxic effects	17.5 mg/kg bw/d	
5.3.3	LO(A)EL embryotoxic / teratogenic effects		
5.3.4	NO(A)EL embryotoxic / teratogenic effects	>70 mg/kg bw/d	
5.3.5	Reliability	2	
5.3.6	Deficiencies	No. Study is not GLP, however it is considered robust and was performed at a recognised facility and using test substance of known purity. Study has been evaluated and accepted under Directive 91/414/EC.	

Section A6.8.1 (01) Teratogenicity Study

Annex Point IIA6.8.1. (01) **Teratogenicity test – rat, oral route**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April, 2007.
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted (minor changes in table A6_8_1_01-1).
Conclusion	The applicant's version is adopted. LOAEL maternal = ~~30~~ 35 mg/kg bw NOAEL maternal = 17.5 mg/kg bw NOAEL embryotoxic/teratogenic effects > 70 mg/kg bw
Reliability	2
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	

Section A6.8.1 (01) Teratogenicity Study

Annex Point IIA6.8.1. (01) **Teratogenicity test – rat, oral route**

Table A6_8_1_01-1 Teratogenicity of cypermethrin in rat – maternal and foetal effects

Endpoints/dose	0 mg/kg bw/d	17.5 mg/kg bw/d	35 mg/kg bw/d	70 mg/kg bw/d
Mortality				2/25 (after 9 doses)
Clinical signs				11/25 Slight to severe neurological disturbances after receiving 3 doses. Signs started 4-7 hours after dosing and regressed by the following morning.
Body weight changes:				
day 6-15			↘ *	↘ **
day 6-21				↘ *
nb. pregnant females	25	25	25	25
corpora lutea count	16.4	15.4	16	16.5
implantations	14.3	14.0	14.1	14.3
viable young ♂/♀	6.7/7.1	6.6/6.6	6.7/6.7	7.3/6.7
resorptions early/late	0.6/0.0	0.7/0.1	0.6/0.1	0.4/0.0
implantation loss % pre/post	12.7/3.9	8.6/6.0	11.8/4.8	13.5/2.5
Litter weight g	48.9	48.0	48.7	50.8
Foetal weight g	3.6	3.7	3.6	3.6

↘ significantly different from control (analysis of variance : * $p<0.05$; ** $p< 0.001$)

Section A6.8.1 (02) Teratogenicity Study

Annex Point IIA6.8.1. (02) **Teratogenicity test – rabbit, oral route**

Official use only

		1 REFERENCE
1.1	**Reference**	Tesh, J.M, Ross, F.W., Wightman, T.J. (1984); WL 43467 (Cypermethrin) – Effects upon the progress and outcome of pregnancy in the rabbit; Life Science Research, Laboratory report no.84/SHL003/014 (CYP/T12), 5 January 1984, (unpublished). Dates of experimental work: 17 May 1983 – 28 July 1983 (preliminary study) and 11 August 1983 – 4 January 1984 (main study)
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
1.2.2	Companies with letter of access	
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 authorisation
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No guidelines mention in report, however protocol appears to be in compliance with method B.31 of Directive 87/302/EEC.
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	WL 43467 (Cypermethrin)
3.1.1	Lot/Batch number	OCR/30 ST 76/001
3.1.2	Specification	Deviating from specification given in section 2 as follows
3.1.2.1	Description	Not mentioned in report
3.1.2.2	Purity	97.5 % w/w
3.1.2.3	Stability	Not mentioned in report
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White
3.2.3	Source	Ranch Rabbits, Sussex
3.2.4	Sex	Female
3.2.5	Age/weight at study initiation	21-27 weeks minimum, 3.69 - 4.44 kg
3.2.6	Number of animals per group	16 (control and 50 mg/kg dose group), 22 (20/mg/kg dose group), 17 (120 mg/kg dose group). Additional animals were allocated to the 20 and 120 mg/kg dose groups to replaced dead animals or those removed from the study or not pregnant.
3.2.7	Control animals	Yes. One vehicle control group (corn oil) and two positive control groups (thalidomide).

Section A6.8.1 (02) **Teratogenicity Study**

Annex Point IIA6.8.1. (02) **Teratogenicity test – rabbit, oral route**

3.2.8	Mating period	Not applicable. Females were artificially inseminated and subsequently injected with 25 i.u. of lutenising hormone to ensure successful ovulation. The day of insemination was designated day 0 of gestation.
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	Days 6 to 18 of pregnancy
3.3.2	Postexposure period	11 days (animals killed on day 29 of gestation)
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	

Dose group (Cypermethrin mg/kg/d)	Volume-dosage of 20% w/v cypermethrin solution (ml/kg/d)
0	0.6
20	0.1
50	0.25
120	0.6

3.3.5	Vehicle	Corn oil
3.3.6	Concentration in vehicle	Test compound was prepared twice weekly as a 20% w/v cypermethrin solution in corn oil. Quantity to be supplied to the animals in each dose group was drawn from the relevant pre-formulated batch on a daily basis.
3.3.7	Total volume applied	Not reported. Dose administered daily was dependent on bodyweight of the individual animal on the day of dosing. All individual volumes were recorded.
3.3.8	Vehicle Control	Vehicle control animals received corn-oil only at a volume-dosage equivalent to that of the 120 mg/kg dose group (i.e. 0.6 ml/kg/d)
3.3.9	Positive Control	Two positive control groups were treated with Thalidomide, formulated freshly each day as a 0.5% w/v suspension in gum tragacanth mucilage. Animals were dosed daily by gavage (5 ml/kg) from days 6 to 18 of gestation.

Dose group (Thalidomide mg/kg/d)	No. of animals/group
125	16
150	10

3.4	**Examinations**	
3.4.1	Body weight	Yes
3.4.2	Food consumption	No
3.4.3	Clinical signs	Yes
3.4.4	Examination of uterine content	Number of corpora lutea

Section A6.8.1 (02) **Teratogenicity Study**

Annex Point IIA6.8.1. (02) **Teratogenicity test – rabbit, oral route**

		Number of implantations
		Number of resorption sites
		Number and distribution of live and dead foetus in each uterine horn
3.4.5	Examination of foetuses	
3.4.5.1	General	Litter Size, numberr. of dead foetuses, foetal weight and sex.
3.4.5.2	Skeletal	Yes
3.4.5.3	Soft tissue	Yes
3.5	**Further remarks**	Pre-implantation and Post-implantation % loss calculated
3.6	**Statistics**	Inter-group differences were to be assessed using appropriate statistical methods. In this particular study the only statistical test performed was on corrected day 29 maternal bodyweight (multiple t-test).

4 RESULTS AND DISCUSSION

4.1	**Maternal toxic Effects**	The general condition of control and treated females was comparable throughout the study. One animal from the control group, three from the 20 mg/kg group and two each from the 50 and 120 mg/kg groups either died or were killed in extremis due to respiratory tract infection and/or gastro-intestinal tract infection. None of the deaths were attributed to treatment with cypermethrin. Some inter-group variations in maternal bodyweight gain were recorded but no adverse effects or treatment related trends were found. Corrected day 29 body weight showed a trend towards reduction with increased dosage, however this was not statistically significant. Examination of all females at necropsy on day 29 of gestation revealed no macroscopic changes that could be related to treatment with cypermethrin. Litter responses were unaffected by treatment of the dams (see table A6_8_1_02.1). All females successfully carried their young to term with the exception of two females in the 20mg/kg group and one in the 120 mg/kg which aborted during the post-treatment phase but none were found to be treatment related.
4.2	**Teratogenic / embryotoxic effects**	Examination of foetuses at necropsy, revealed a small number of abnormalities in all groups; the type and the incidences of which have previously been found to occur spontaneously in this strain of rabbit in other laboratory tests. (see Table A6_8_1_02-1)
4.3	**Other effects**	The number of implantations, live young and resorptions, pre- and post-implantation losses, foetal and placental weights were unaffected by treatment with cypermethrin at any dose level.

Section A6.8.1 (02) **Teratogenicity Study**

Annex Point IIA6.8.1. (02) **Teratogenicity test – rabbit, oral route**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Cypermethrin (batch number OCR/30 ST 76/001; purity 97.5%) was administered by oral gavage to pregnant New Zealand White rabbits from day 6 to day 18 of gestation inclusive at dose levels of 20, 50, and 120 mg/kg/day. A vehicle control group received corn oil at a volume dosage equal to that of the top dose group. Two positive control groups received thalidomide at 125 and 150 mg/kg/day. On day 29 of gestation the females were killed to allow examination of the uterine contents.
5.2	**Results and discussion**	The general condition of the treated females was comparable with that of the vehicle control throughout the study. Isolated deaths occurred in the control and each of the treatment groups but these were not treatment related. Some inter-group variations in bodyweight gain were recorded but no adverse effects or trends attributable to cypermethrin were found. Two females in the low dose group and one in the highest dose group aborted during the post exposure phase, however these were not treatment related. No macroscopic changes were observed in females at day 29 of gestation. Litter responses were unaffected. No foetal abnormalities were observed which could be attributed to treatment with the test substance.
5.3	**Conclusion**	There was no embryotoxicity or teratogenicity a ssociated with oral administration of cypermethrin.
5.3.1	LO(A)EL maternal toxic effects	
5.3.2	NO(A)EL maternal toxic effects	120 mg/kg bw/d
5.3.3	LO(A)EL embryotoxic / teratogenic effects	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	120 mg/kg bw/d
5.3.5	Reliability	2
5.3.6	Deficiencies	No. Study is not GLP, however it is considered robust and was carried out at a recognised facility and to an acceptable protocol using cypermethrin of known purity. Study has been evaluated and accepted under the Directive 91/414/EC.

Section A6.8.1 (02) Teratogenicity Study

Annex Point IIA6.8.1. (02) **Teratogenicity test – rabbit, oral route**

	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	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted with the following amendment: Table A6_8_1_02-1 is extended.
Conclusion	The applicant's version is adopted. There was no maternal toxicity, embryotoxicity or teratotoxicity associated with the oral administration of cypermethrin up to the dose of 120 mg/kg bw/d. NOAELmaternal = 120 mg/kg bw/d NOAELembryo/terato = 120 mg/kg bw/d
Reliability	2
Acceptability	Acceptable. Weakness of the study: MTD not reached. The dose levels have not been selected with the view to demonstrate any dosage related response. The highest dose did not induce observable maternal and/or developmental toxicity. However, the study is 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 A6_8_1_02-1. Teratogenicity of cypermethrin in rabbit – Summary of maternal and foetal effects

Endpoint/dose (mg/kg bw/d)	**0**	**20 mg/kg bw**	**50 mg/kg bw**	**120 mg/kg bw**
Mortality	1	3	2	2
Number of animals inseminated	16	22	16	17
Number pregnant with live young day 29	14	10	13	12
body weight during dosing period	Trend towards reduction (not significant)			
Body weigt day 29 (kg)	4.40	4.38	4.37	4.36
Gravid uterus weight (g)	459	474	514	495
Premature sacrifice or deaths	1	3	2	2
Animals not pregnant	1	4	1	1
% abortion and total litter loss	0	16.7	0	7.7
Corpora lutea count	9.9	11.1	11.4	11.3
Implantations	8.4	8.3	9.8	8.9
Viable young M/F	4.1/3.1	3.7/4.2	4.5/3.8	4.1/4.3
Resorptions early/late	0.4/0.8	0.1/0.3	0.2/1.3	0.1/0.5
Implantation loss % : pre/post	15.8/13.7	25.2/4.8	13.5/14.8	20.7/6.5
Foetal weight (g)	43.2	41.5	41.8	42.4
Placenta weight (g)	6.5	5.5	5.9	5.8
Litter response	Unaffected by treatment			
Foetal observations External, soft tissue, skeletal alterations	No indication of adverse response			

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 **3 Generation reproduction study – Rat**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Hend, R.W., Hendy, R., Fleming, D.J. (1978): Toxicity studies on the insecticide WL 43467(cypermethrin): A 3 generation reproduction study in rats; Shell Toxicology Laboratory, Tunstall, report no. TLGR.0188.78 (CYP/T13), unpublished.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2	Companies with letter of access		
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 authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guidelines available at the time this study was conducted. However the study appears comparable to method B.35 of Directive 87/302/EEC with some deviations.	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes Methods used not fully in compliance with method B.35 of Directive 87/302/EEC. Males and females were exposed for 5 weeks ; normally, in male rats, dosing is continued for 10 weeks prior to the mating period. Females must be dosed throughout the 3 week mating period, pregnancy and up to the weaning of the F1 offspring.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	WL 43467 (cypermethrin)	
3.1.1	Lot/Batch number	30	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Not specified in report	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	Not mentioned in report	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar (SPF)	
3.2.3	Source	Shell Toxicology Laboratory, Tunstall	
3.2.4	Sex	Male and Female	
3.2.5	Age/weight at study initiation	5 weeks, 47-50g	
3.2.6	Number of animals per group	30 males, 30 females	

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 **3 Generation reproduction study – Rat**

3.2.7	Mating	At 10 weeks of age, one female was randomly allocated to one male in the same treatments group (avoiding brother/sister pairings). Each pair were allowed to produce 2 litters (F1A and F1B). Litter F1A killed at weaning (21 days). One male and one female randomly selected from each of the weaned F1B litters and fed the appropriate diet. These rats were paired again at 10 weeks and allowed to produce 2 litters (F2A and F2B). A random selection of F2B offspring were mated to produce F3A and F3B litters.
3.2.8	Duration of mating	Each pair allowed to produce 2 offspring
3.2.9	Deviations from standard protocol	See section 2.3
3.2.10	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Animal assignment to dosage groups	30 male and 30 female animals in each treatment group
3.3.2	Duration of exposure before mating	5 weeks
3.3.3	Duration of exposure in general P, F1, F2 males, females	Dietary exposure to the test material was continuous for all generations from F0 prior to mating through to the weaning of the F2B generations.
3.3.4	Type	In food
3.3.5	Concentration	0, 10, 100 or 500 ppm Converted test article intake: 0, 1, 10, 50 mg/kg bw/d
3.3.6	Vehicle	Test article dissolved in acetone and gradually added to powdered LAD 2 diet.
3.3.7	Concentration in vehicle	For the 500 ppm dose level, 30g cypermethrin dissolved in in 60g acetone and gradually added to 60kg powdered LAD2 diet. The 100 and 10 ppm dose levels were prepared from 500ppm diet diluted with powdered LAD2.
3.3.8	Total volume applied	See above
3.3.9	Controls	Vehicle in LAD2 diet
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Body weight	Bodyweight measured weekly during pre-mating period and up to the age of 10 weeks. A further measurement was taken of male and females immediately before mating at F1 and F2.
3.4.3	Food/water consumption	Food intake measured weekly during pre-mating period and up to the age of 10 weeks.
3.4.4	Oestrus cycle	Ovaries examined microscopically
3.4.5	Sperm parameters	Testes examined microscopically

Section A6.8.2 **Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2 **3 Generation reproduction study – Rat**

3.4.6	Offspring	Date born Number of pups born alive Number of pups born dead Sex of pups alive on day 1 Number and sex of pre-weaned deaths Number and sex of pups weaned Total litter weights on days 1, 4, 7, 14 and 21 Individual pup bodyweights on day 21
3.4.7	Organ weights P and F1	Not mentioned in report
3.4.8	Histopathology P and F1	Not mentioned in report
3.4.9	Histopathology F1 not selected for mating, F2	Not mentioned in report
3.5	**Further remarks**	The majority of the microscopic findings in this study were confined to the adults of the F2B generation. Tissues examined were as follows: Brain (cerebrum, cerebellum, mid-brain, medulla), heart (ventricles), liver, spleen, kidneys, testes / ovaries, stomach, pancreas, lymph nodes, prostate / uterus, urinary bladder, thyroid, parathyroid, thymus, eye, lungs, pituitary, adrenals, small intestine (3 levels), large intestine (2 levels), oesophagus, salivary glands, sciatic nerve, any other tissues showing macroscopic lesions.

4 RESULTS AND DISCUSSION

4.1	**Effects**	
4.1.1	**Adults**	F0, F1 and F2 body weights were lower at 500 ppm; at 10 or 100 ppm no significant effects were observed. The effects were greater for females than for males. Mean food intake of all F0 test groups were lower than controls and were probably related to unpalatability of the diet containing test material, week 3 being the first week of food intake . These lower food intakes at weeks 6 and 7 were associated with adverse body weight effects and were considered to be related to exposure to 500 ppm cypermethrin (Table A6_8_2-1). Fertility, gestation, viability, and lactation indices were similar for treated and control animals within each generation.

Section A6.8.2 **Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2 **3 Generation reproduction study – Rat**

4.1.2 Litters

At 500 ppm: Litter size and weights of F0A was reduced and these findings could be attributed to significantly lower F0A litter sizes recorded at this dose group. Mean pup weights were significantly lower compared with controls for F0B females pups and pups of both sexes and for F2B male pups.

At 100 and 10 ppm: the significantly lower litter sizes recorded at 10 ppm for F0A pups a nd F1B pups were not considered to be toxicologically relevant as similar changes were not observed at 100 ppm.

The significantly higher numbers of female pups per litter for the F0B litters were regarded as anomalous.

For the F0B pups the significantly higher mean litter weight, recorded for the 10 ppm groups at day 7, was related to a large mean litter size and appears to be anomalous.

For F2B litters , mean pup weights for male only were significantly lower at 100 and 10 ppm. This effect was regarded as anomalous. These results were re-analysed using the litter as experimental unit. This is considered to be statistically preferable approach for this type of analysis. Using the litter as experimental unit, no statistically significant differences were reported at 10 and 100 ppm. Within a given litter, the weight of an individual pup can be expected to be influenced by the number and weight of other pups in the litter as well as by any effect of the test compound. Therefore, these results were not taken into account for the NOAEL.

Significantly increased mean pup weights recorded at 10 ppm for the F0A litters, reflect the significantly lower litter sizes recorded at day 7, 14, 21. No significant differences in mean pup weights were recorded for the F1A, F1B or F2A litters at any dose levels.

(See Table A6_8_2-2)

4.2 Other

At the beginning of the study each treatment group comprised 30 pairs. However, as the study progressed the following factors caused a reduction of the treatment group sizes for certain litter varieties:

1) pairings not resulting in litters produced

2) single sex litters

3) pups of one sex or no pups surviving to weaning.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Wistar rats (SPF) received in the diet for 5 weeks, cypermethrin (98% B.n° : 30) at doses of 0, 10, 100, or 500ppm, each group comprised 34 females. Males and females from each treatment group were selected at random and caged together for mating. Two litters were produced from each pair for three successive generations. The second litter of each generation was weaned, fed the appropriate diet and mated to produce 2 litters.

Dietary exposure to the test material was continuous for all generations from F0 prior to mating through to the weaning of the F2B generations.

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 **3 Generation reproduction study – Rat**

5.2	**Results and discussion**	Reductions in bodyweight and food intake were seen at intervals in male and female rats in the 500ppm dose group for each generation. Pregnancy rates of treated and control groups were similar for each generation. No changes in litter size were seen in any generation in any dose group with the exception of a reduction in litter size in the 500ppm treated F0A litters on days 0, 7 and 21. No consistent changes in mean total litter weight with the exception of the 500ppm treated F0A litters on days 4, 14 and 21. Reduction in male and female pup weaning weights were seen in the 100 and 500ppm treatment groups from F0B. No compound-related gross or microscopical pathological findings were observed over three generations.	
5.3	**Conclusion**		
5.3.1	NO(A)EL		
5.3.1.1	Parents	100ppm = 10 mg/kg bw/d	
5.3.1.2	Reproduction	~~100ppm~~ 500ppm = ~~10~~ 50 mg/kg bw/d	X
5.3.2	Reliability	2	
5.3.3	Deficiencies	Yes Methods used not fully in compliance with method B.35 of Directive 87/302/EEC. Males and females were exposed for 5 weeks, normally in male rats dosing is continued for 10 weeks prior to the mating period. Females are usually dosed throughout the 3 week mating period, pregnancy and up to the weaning of the F1 offspring. However, the study is considered scientifically sound and has been evaluated and accepted under 91/414/EC.	

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May, 2007.
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted with the following amendment: The effects observed in the pups are secondary to maternal toxicity. No effects were observed on fertility at any dose tested. NOAEL parental: 10 mg/kg bw/d NOAEL reproduction: 50 mg/kg bw/d NOAEL developmental: 10 mg/kg bw/d
Conclusion	The applicant's version is adopted with the following amendment: NOAEL parental: 10 mg/kg bw/d NOAEL reproduction: 50 mg/kg bw/d NOAEL developmental: 10 mg/kg bw/d.
Reliability	2
Acceptability	Acceptable.
Remarks	

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 **3 Generation reproduction study – Rat**

	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 A6_8_2-1. Table for reproductive toxicity – adult data

Endpoint/dose	**0**		**10 ppm**		**100 ppm**		**500 ppm**	
Adult rat F0	♂	♀	♂	♀	♂	♀	♂	♀
Food intake			↘ wk3,4,5 (4%)		↘ wk3,4 (4%)		↘ wk3wk 7 (7%)	↘ wk3,4, 5,6,7 (6-7%)
Body weight							↘	↘ (4-5%)
Adult rat F1, F2								
Food intake			↘ wk3 (F1:8%)					↘ wk4→7(F1:6-8%) ↘ wk5,7(F2:11-16%)
Body weight							↘ (F1:4-5%)	↘ (F1:4-7%) (F2:5-6%)

Table A6_8_2-2. Table Table for reproductive toxicity – litter data

Endpoint/dose	**0 ppm**	**10 ppm**	**100 ppm**	**500 ppm**
Litter survival:				↘ F0A day 0, 7,21
Litter size:				
F0A				↘ day, 7→21
FOB, F1A, F1B, F2A,F2B		no effect		
Number ♀ pups/litter				
F0A				↘ day 1& 21
F0B		↗ day 1&21	↗ day 1&21	
F1A,F1B,F2A,F2B		no effect		
Mean litter weights:				
F0A				↘ day 4, 14,21
F0B		↗ day 7		
F1A, F1B,F2A,F2B		no effect		
Mean pup weight:				
FOA		↗ day 14&21		
F0B				_: ↘ 10% day 21
F1A, F1B, F2A		no effect		
F2B		↘ 10% day 21♂	↘ 11% day 21♂	↘ day 21♂ : 9% ♀: 6%

Analysis of covariance followed by Williams or Dunnett's test () : not biologically significant

Section A6.9 (01) Delayed Neurotoxicity

Annex Point IIA6.9 **Neurotoxic potential in the hen**

Official use only

		1 REFERENCE
1.1	**Reference**	Owen, D., Butterworth, S. (1977); Toxicity of Pyrethroid Insecticides: Investigation of the Nerotoxic Potential of WL 43467 (cypermethrin) to Adult Domestic Hens; Shell Toxicology Laboratory, Tunstall, report no. TLGR.0134.77 (CYP/T8), 1977 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
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 authorisation
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No guidelines available for this type of study at the time it was conducted. However the protocol appears to be partially in compliance with OECD guideline 418 (1984).
2.2	**GLP**	No. GLP was not compulsory at the time this study was conducted.
2.3	**Deviations**	Yes Duration of treatment is not in accordance with OECD 418, birds were treated for 5 days and after 3 weeks the dosing regime was repeated and the birds killed after a further 3 weeks.
		3 MATERIALS AND METHODS
3.1	**Test material**	WL 43467 (cypermethrin)
3.1.1	Lot/Batch number	Batch no. 30 from Shell Biosciences Laboratory
3.1.2	Specification	Deviating from specification given in section 2 as follows
3.1.2.1	Description	Not specified in report
3.1.2.2	Purity	98%
3.1.2.3	Stability	Not mentioned in report
3.2	**Reference Substance (positive control)**	TOTP (Tri-o-tolyl phosphate)

Section A6.9 (01)	**Delayed Neurotoxicity**	
Annex Point IIA6.9	**Neurotoxic potential in the hen**	
3.3	**Test Animals**	
3.3.1	Species	Domestic Laying Hen (Gallus gallus domesticus)
3.3.2	Strain	Arbor Acre laying hens
3.3.3	Source	Lerwill Farm Ltd, Kent, UK.
3.3.4	Sex	Not specified in report
3.3.5	Rearing conditions	Animals were healthy free-range laying hens and were maintained for one week prior to dosing in floor pens with sawdust and woodchip litter.
3.3.6	Age/weight at study initiation	Approximately 1 year old, weight not specified in report
3.3.7	Number of animals per group	3 experimental groups of six birds: Group I – dosed with test substance Group II – Positive control group Group III – Negative control group
3.3.8	Control animals	Yes
3.4	**Administration**	Oral by gavage
3.4.1	Exposure	Repeated oral dosing by gavage, administration once daily for 5 days. Dosing regime was repeated after 3 weeks.
3.4.2	Dose Levels	One positive control (TOTP), one negative control (no treatment), one treatment group
3.4.3	Vehicle	Dimethyl sulphoxide (DMSO) (due to low water solubility of cypermethrin)
3.4.4	Concentration in vehicle	1 g/kg bw
3.4.5	Total volume applied	Not specified in report, dose was prepared as a 50% w/v solution
3.4.6	Postexposure period	3 weeks
3.4.7	Anticholinergic substances used	Not specified in report
3.4.8	Controls	Negative control group received no treatment.
3.5	**Examinations**	
3.5.1	Body Weight	Not determined
3.5.2	Signs of Toxicity	Birds were observed daily for signs of ataxia, and at intervals tested for their ability to land without staggering when forced to fly.
3.5.3	Observation schedule	All birds observed daily

Section A6.9 (01)	**Delayed Neurotoxicity**	
Annex Point IIA6.9	**Neurotoxic potential in the hen**	
3.5.4	Clinical Chemistry	No
3.5.5	Pathology	Yes
		Organs: brain and sciatic nerve
3.5.6	Histopathology	Yes
		Organs: Cervical, thoracic and lumber cords, sciatic nerve, cerebellum and medulla oblongata.
3.6	**Further remarks**	-
		4 RESULTS AND DISCUSSION
4.1	**Body Weight**	Not specified in report
4.2	**Clinical signs of toxicity**	In the group dosed with cypermethrin there were no signs of intoxication at any time during the study. See Table A6.9_01_1.
4.3	**Clinical Chemistry**	Not included in study
4.4	**Pathology**	No pathological lesions found in any of the birds dosed with cypermethrin
4.5	**Histopathology**	No histological lesions were found in the peripheral or central nervous system tissues in any of the birds treated with cypermethrin.
4.6	**Other**	-
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	6 Adult domestic hens were given 5 daily oral doses of 1 g/kg bw of cypermethrin, Wl43467 (b.n°.30; 98%) dissolved in DMSO. After 3 weeks, the dosing regime was repeated and a further 3 weeks later the birds were killed. A positive control received pralidoxine chloride and atropine sulphate and dosed once with 0.5 ml/kg of tri-ortho-tolyl phosphate.
5.2	**Results and discussion**	In the group dosed with cypermethrin there were no deaths and no signs of intoxication at any time. No histological lesions were found in the peripheral or central nervous system
5.3	**Conclusion**	
5.3.1	LOAEL	
5.3.2	NOAEL	1000 mg/kg bw
5.3.3	Reliability	2
5.3.4	Deficiencies	Yes. Duration of treatment was not strictly in accordance with the published guideline, however the study was conducted at an established facility and using cypermethrin of known purity. Study evaluated and accepted under Directive 91/414/EC.

Section A6.9 (01) Delayed Neurotoxicity

Annex Point IIA6.9 **Neurotoxic potential in the hen**

	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	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted.
Conclusion	The applicant's version is adopted. NOAEL = 1000 mg/kg bw/d
Reliability	Based on the assessment of materials and methods include appropriate reliability indicator
Acceptability	Acceptable.
Remarks	Repeated dosing regime; no body weight data; no biochemistry measurements, no vehicle control group included.
	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 A6.9 (01) **Delayed Neurotoxicity**

Annex Point IIA6.9 **Neurotoxic potential in the hen**

Table A6.9_01_1. Table for delayed neurotoxicity in the hen

	untreated control	1 g/kg cypermethrin dose group	positive control
Number of animals at the start	6	6	6
Deaths	0	0	Hens sent for pathological examination after 18, 23, 29 and 57 days after dosing
Showing lesions	0	0	6 (typical sciatic lesions)
Showing effects in behaviour	0	0	6 (ataxia and/or paresis)
Showing other effect, state other effect	0	0	Typical signs of neurological disturbance

Section A6.9 (02)	**Delayed Neurotoxicity**
Annex Point IIA6.9	**Neurobehavioral screening – Functional Observation Battery testing in the rat**

			Official use only
		1 REFERENCE	
1.1	**Reference**	McDaniel, K.L., Moser V.C. (1993); Utility of a neurobehavioral screening battery for differentiating the effect of two pyrethroids, Permethrin and cypermethrin. Neurotoxicology and Teratology 15: 71-83 (published).	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	Not applicable	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No current guidelines available for this type of study. Details of the protocol are included in the report.	
2.2	**GLP**	Not mentioned	
2.3	**Deviations**	Not applicable. Protocol was based on previous work published by the authors.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin	
3.1.1	Lot/Batch number	Not specified (supplied by FMC Corp., Princeton, NJ)	
3.1.2	Specification	97% purity, approx. equal quantities of cis and trans isomers	
3.2	**Reference Substance (positive control)**	No positive control used in this study. However the synthetic pyrethroid Permethrin was also investigated in this study.	
3.3	**Test Animals**		
3.3.1	Species	Rat	
3.3.2	Strain	Long Evans	
3.3.3	Source	Charles River Laboratories, Raleigh, NC	
3.3.4	Sex	Males and Females	
3.3.5	Rearing conditions	Prior to study initiation, rats used in the FOB study were singly housed in polycarbonate cages. Rats used in the motor activity study were housed in wire mesh cages. All rats were allowed access to feed and deionised water *ad libitum*. Temperature was maintained at 22 ± 1 °C, 55 ± 5% humidity with a 12 hour light / 12 hour dark photoperiod.	
3.3.6	Age/weight at study initiation	70-90 days old	
3.3.7	Number of animals per group	8 rats of each sex	
3.3.8	Control animals	Yes (vehicle only)	
3.4	**Administration**		
3.4.1	Exposure	Oral (gavage)	

Section A6.9 (02)		**Delayed Neurotoxicity**
Annex Point IIA6.9		**Neurobehavioral screening – Functional Observation Battery testing in the rat**
3.4.2	Dose Levels	0 (vehicle only), 20, 60 or 120 mg/kg bw (FOB study) 0 (vehicle only), 20, 60 or 100 mg/kg bw (motor activity study)
3.4.3	Vehicle	Corn oil
3.4.4	Concentration in vehicle	Not specified
3.4.5	Total volume applied	1 ml/kg
3.4.6	Post exposure period	48 hours
3.4.7	Anticholinergic substances used	Not applicable
3.4.8	Controls	Vehicle only
3.5	**Examinations**	
3.5.1	Body Weight	Yes, measured during study
3.5.2	Signs of Toxicity	Effects on specific domains of neurological function: autonomic (salivation, increased urination), excitability (removal resistance, decreased arousal, choreoathetosis), neuromuscular (splayed limbs, flattened posture, decreased grip strength, altered righting, increased landing foot splay) and sensorimotor (increased click response, decreased touch response, decreased tail pinch response) based on a 1-4 severity scoring system.
3.5.3	Observation schedule	In the FOB study, animals were observed 1.5 and 3 hours after dosing (times of peak effect determined during preliminary arousal and gait score assessments) and after 24 and 48 hours.
3.5.4	Motor activity study	Motor activity experiments were performed separately. Eight rats of each sex were dosed with either 0 (vehicle only), 20, 60 or 100 mg/kg bw (the top dose was reduced due to a lethality noted in the FOB study). Motor activity testing took place after 3, 24 and 48 hours after dosing using a maze composed of interconnecting alleys in a figure of eight design with two blind alleys projecting from the centre. Six phototransmitter/diode pairs were equally spaced around the maze, including each of the blind alleys. Motor activity was recorded by a microprocessor as the number of photocell interruptions over a 1 hour session.
3.5.5	Statistical analysis	Two-way ANOVA followed by Dunnett's t-test
3.6	**Further remarks**	-

Section A6.9 (02)	**Delayed Neurotoxicity**
Annex Point IIA6.9	**Neurobehavioral screening – Functional Observation Battery testing in the rat**

4 RESULTS AND DISCUSSION

4.1 Body Weight

Body weight loss was seen in rats of both sexes and was still evident 48 hours after dosing. Rats in the high dose group losing a maximum of 7% and 9% of the pre-dosing weight in male and female rats respectively at 24h.

4.2 Signs of toxicity

In the FOB study, 1 male and 6 females died in the top dose group (120 mg/kg). These rats were subsequently replaced in order to allow the study to continue. The top dose was reduced to 100 mg/kg for the motor activity test, where 2 males died at this dose.

4.3 Functional Changes

Two phases of toxicity were evident. Salivation and increased removal activity (males) were more evident at 1.5 hours. At 3 hours these signs were subsiding and pronounced motor and sensory effects were apparent.

See Table A6_9_02-1

4.4 Motor Activity

Motor activity was markedly depressed on the day of dosing and the effects on total counts showed that 3 h after dosing, all doses significantly decreased activity in both sexes, and the high dose was still effective at 24 h. Furthermore, the low dose (20 mg/kg) was closed to the ED50 value for this measure, in that it decreased activity by 46% and 43% in males and females, respectively. No effects were obtained at 48 h.

4.5 Behavioural effects

After cypermethrin administration, rats displayed pawing and burrowing behavior even while on the open field. The swollen muzzles may have been a direct effect of cypermethrin or due to irritation produced by the excessive burrowing actions. Spontaneous vocalization was noted.

The behavior changes included: increased sensitivity to external stimuli, splayed hindlimbs and abnormal locomotion, decrease of sensorimotor reactivity, with exeption of increased click response. Neuromuscular changes were decreased grip strengths, gait changes and altered righting ability. Muscle tone was decreased as evidenced by splayed leggs, increased landing foot spread, and flattened posture.

The behavioral effects were significant at both the middle and the high dose.

4.6 Other

Hypothermia was observed in rats of both sexes.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A functional observation battery (FOB) study was performed to assess the effects of cypermethrin on neurological function and behaviour in the rat. 8 Adult Long-Evans rats/sex/dose received by gavage, cypermethrin (97%) in corn oil at 20, 60 or 120 mg/kg bw and were observed 1.5 and 3 h , 24 and 48 h after dosing. Motor activity experiments were preformed at 20, 60 and 100 mg/kg bw after 3, 24 and 48 h after dosing.

Section A6.9 (02)	**Delayed Neurotoxicity**
Annex Point IIA6.9	**Neurobehavioral screening – Functional Observation Battery testing in the rat**

5.2	**Results and discussion**	After cypermethrin administration, rats displayed pawing and burrowing behavior excessive burrowing actions, spontaneous vocalization, increased sensitivity to external stimuli, splayed hindlimbs and abnormal locomotion, decrease of sensorimotor reactivity, with exeption of increased click response. Neuromuscular changes were decreased: grip strengths, gait changes and altered righting ability. Muscle tone was decreased as evidenced by splayed legs, increased landing foot spread, and flattened posture. The behavioral effects were significant at both the middle and the high dose.
5.3	**Conclusion**	Behavior representing all of the functional domains assessed were affected, indicating the broad neurological activity of cypermethrin.
5.3.1	LOAEL	Not determined
5.3.2	NOAEL	20 mg/kg bw
5.3.3	Reliability	2
5.3.4	Deficiencies	Although this is a non-guideline study, the published report contains a high level of detail including the protocol used, purity and source of the test substance and full details of the experimental conditions. This report was identified in the monograph for cypermethrin under Directive 91/414/EC and an NOAEL of 20 mg/kg assigned by the RMS.

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	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted with the following amendments: Neuromuscular dysfunction produced by cypermethrin was evident at even the low dose. Two phases of toxicity were evident. Salivation and increased removal reactivity (males) were most evident at 1.5h. By 3h these signs were subsiding, and the pronounced motor and sensory effects were apparent. Several motor effects (gait changes, lowered grip strenghts, decreased motor activity) were still evident 1-2 days later (especially females).
Conclusion	The applicant's version is adopted. NOAEL = 20 mg/kg bw
Reliability	2
Acceptability	Acceptable.
Remarks	

Section A6.9 (02)	**Delayed Neurotoxicity**
Annex Point IIA6.9	**Neurobehavioral screening – Functional Observation Battery testing in the rat**

	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 A6_9_02-1: Effects of Cypermethrin on measures of the FOB

Data for only the time at wich the peak effect occurred								
FOB tests /dose	**0 mg/kg bw**		**20 mg/kg bw**		**60 mg/kg bw**		**120 mg/kg bw**	
	♂	♀	♂	♀	♂	♀	♂	♀
Salivation 1.5 h	1	1	1	1	1.5*	2*	2.5*	3*
Urination 1.5 h	0.13	0.13	0.13	0	0.63	0.38	0.86*	0.38
Arousal 3 h	3	4	3	4	2.5*	2.5*	2*	2*
Abnormal motor movements: 1.5 h	0/8	0/8	1/8	0/8	6/8*	4/8*	7/8*	7/8*
3 h	0/8	0/8	1/8	0/8	7/8*	4/8*	7/8*	8/8*
24 h	0/8	0/8	0/8	0/8	2/8*	0/8	2/8*	1/6
Forelimb grip strength 3h	1.012± 0.69	0.993± 0.044	0.973± 0.074	1.022± 0.053	0.654± 0.155*	0.835± 0.114	0.533± 0.152*	0.275± 0.051*
Hindlimb grip strength 3 h	0.966± 0.054	0.866± 0.033	0.851± 0.034	0.825± 0.054	0.521± 0.066*	0.582± 0.063*	0.511± 0.107*	0.379± 0.05*
Landing foot splay 24 h	66.1±4.1	59.3±4.4	65.2±4	58.5±3.9	81.6±8.4*	62.4±4	80.8±6.1*	78.5±4.5*
Righting reflex 3 h	1	1	1	1	3.5*	3*	4*	4*
Touch response 3 h	3	3	3	2*	1.5*	2.5	1.5*	1*
Tail pinch response 3 h	4	3	4	2	1.5*	4	2*	1*

* Statistically different from vehicle control group

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

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1 REFERENCE

1.1	**Reference**	Schneider , E. (1997); Hydrolysis in water at 3 pH values; Krebs Analytik GmbH, report no. PR97/003 (CYP/C52), 22 July 1997 (unpublished) Dates of experimental work: 18 March 1997 – 24 June 1997
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 111 (12/05/81), Directive 92/69/EEC method C.7. (1992)
2.2	**GLP**	Yes
2.3	**Deviations**	Yes. As two geometrical cypermethrin isomers (cis and trans) could be separated by HPLC, no further GC-MS investigations were carried out concerning the isomer distribution.

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	30716
3.1.2	Specification	As given in section 2
3.1.3	Purity	91%
3.1.4	Further relevant properties	The water solubility of cypermethrin is very low, therefore each of the buffer solutions used in the study were mixed with acetonitrile (95% buffer, 5% acetonitrile v/v)
3.2	**Reference substance**	Not used in this study
3.2.1	Initial concentration of reference substance	Not applicable
3.3	**Test solution**	A solution of 100 µg/ml cypermethrin dissolved in acetonitrile was prepared. pH 4, 7 and 9 buffer solutions were prepared as described in table A7_1_1_1_1-1. 16 vials (32 vials for pH 7) were prepared containing 100ml buffer solution and 100 µl of the cypermethrin solution added (10 µg cypermethrin per sample or 100 µg/L). See table A7_1_1_1_1-2
3.4	**Testing procedure**	
3.4.1	Test system	16 vials were prepared for pH 4 and 9 and 32 for pH 7 (see table A7_1_1_1_1-3)

Section A7.1.1.1.1 **Hydrolysis as a function of pH and identification of breakdown products**

Annex Point IIA7.6.2.1

3.4.2	Temperature	pH 4: 50°C pH 7: room temperature (in the dark) and 50°C pH 9: 50°C	X
3.4.3	pH	See table A7_1_1_1_1-1	
3.4.4	Duration of the test	480 minutes (pH 9) to 29 days (pH 4 and 7)	
3.4.5	Number of replicates	16 vials for each experiment	
3.4.6	Sampling	pH 4 (50°C): Samples taken at 0, 4, 6, 15, 18, 22 and 29 days. pH 7 (50°C): Samples taken at 0, 2, 4, 5, 6, 8, 10 and 15 days. pH 7 (room temp.): Samples taken at 0, 4, 6, 10, 15, 22 and 29 days. pH 9 (50°C): Samples taken at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360 and 480 mins. In each case 1 ml of concentrated hydrochloric acid was added to the samples before analysis. If sample preparation could not be performed on the same day the vials were stored in a refiridgerator.	
3.4.7	Analytical methods	Each sample was extracted three times with dichloromethane. Extracts were unified into a 50ml conical flask and 5ml acetonitrile added. Samples were then evaporated in a rotary evaporator to approximately 3ml, a further 10ml of acetonitrile added and the sample further evaporated to around 0.5ml. The exact volume of the sample was then measured using a 1ml syringe and transferred to a HPLC vial, and the volume brought up to 1ml with 0.1% H_3PO_4. HPLC analysis was carried out at 220nm using the Spectra Physics SP 8100 with autosampler. The system was first calibrated using the test substance and also the metabolites mentioned in section 3.2. Three concentrations of each reference substance were analysed (0.1, 1.0 and 10 µg/ml) to product a calibration curve for each substance. Recovery experiments were performed using samples of pH 7 buffer solution fortified with cypermethrin and each of the four reference substances. The LOD was found to be 1 µg/L for each substance.	
3.5	**Preliminary test**	No. Cypermethrin is known to readily hydrolyse in alkaline conditions and be relatively stable under acidic conditions.	X

Section A7.1.1.1.1 **Hydrolysis as a function of pH and identification of breakdown products**

Annex Point IIA7.6.2.1

4 RESULTS

4.1	**Concentration and hydrolysis values**	See table A7_1_1_1_1-4. At pH 4 (50°C) no significant decrease of the initial cypermethrin concentration was observed and the half life was estimated at >1year under environmental conditions. At pH 7 (50°C) the cypermethrin concentration decresed significantly and the half-life under these conditions was calculated to be 4.73 days (1st order). No significant decrease in cypermethrin concentration was observed at this pH at room temperature. At pH 9 (50°C) the cypermethrin concentration decreased rapidly and the half-life was calculated to be 114 min or 1.9 hours (1st order). The estimated half-life under environmental conditions can be considered less than 1 day.	X
4.2	**Hydrolysis rate constant (k_h)**	Not mentioned in report	
4.3	**Dissipation time**	See DT50 values in table A7_1_1_1_1-5.	
4.4	**Concentration – time data**	See Table A7_1_1_1_1-4	
4.5	**Specification of the transformation products**	When hydrolysis did occur, 2 metabolites were formed in equimolar amounts (see table A7_1_1_1_1-6). No further metabolites were formed in the study. As the mass balance showed that all the initial cypermethrin concentration was recovered it can be concluded that during hydrolysis the cypermethrin molecule is divided into two parts – the dichlorovinylcyclopropane moiety and the phenoxybenzyl moiety.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The stability of cypermethrin with respect to hydrolysis behaviour in water was investigated at pH 4, pH 7 and pH 9 according to EEC method C7. Test samples were prepared using an initial concentration of 100 µg/L cypermethrin (cis:trans/40:60). Test vials were maintained at 50 °C with the exception of one of the two pH 7 vials which was kept at room temperature. Vials were sampled at regular time intervals, depending on the pH, and the extract anaylsed by HPLC to determine the concentration of parent compound. Metabolites were identified by comparison with known reference substances and the mass balance calculated. Where hydrolysis occurred, the half life was calculated using the IVA computer model.	

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

5.2	**Results and discussion**	Cypermethrin was stable at pH4, with no degradation observed up to a period of 29 days. Similarly cypermethrin as stable at pH7 at room temperature over a period of 29 days. At pH7 and 50°C, cypermethrin was degraded significantly over 15 days. In alkaline media (pH 9) degradation was rapid over an 8 hour observation period. Where hydrolysis occurred, the same two metabolites were identified – namely 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCVC acid) and 3-phenoxybenzaldehyde. These two metabolites were formed in equimolar amounts suggesting cleavage of the parent compound via the ester linkage to give the two halves of the molecule, the dichlorovinylcyclopropane moiety and the phenoxybenzyl moiety. As cypermethrin is a mixture of –cis and –trans isomers, it was seen that the –trans isomer was degraded more rapidly than the –cis isomer, probably due to less steric hinderance of the –trans isomer to the attack of the hydroxyl ion.
5.2.1	k_H	Not mentioned in report
5.2.2	DT_{50}	>1year atpH4 (50°C) 4.73 days at pH7 (50°C) 1.9 hours at pH9 (50°C)
5.2.3	r^2	1st order kinetics
5.3	**Conclusion**	Cypermethrin is stable under acidic conditions (up to 29 days) but is hydrolysed in alkaline media with a half life of 1.9 hours at pH9. The trans isomer degrades more rapidly than the cis isomer. Hydrolysis of the ester linkage forms the two metabolites 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCVC acid) and 3-phenoxybenzaldehyde in equimolar amounts.
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is adopted. Please note that dark controls of photolysis in water show hydrolysis reactions between 12 and 18% at 20±3°C after 4 days at pH 4. There fore, cypermethrin can not be regarded as stableà pH4, but only relatively stable.
Conclusion	Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable
Remarks	4.1: Room temperature varies between 23 and 26 °C No test were performed for temperature under 40°C except for pH 7 This test should therefore be regarded as a preliminary test completed by a test 1 for pH7 according to test guideline C7
	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_1_1_1_1-1: Type and composition of buffer solutions

pH	Type of buffer (final molarity)	Composition
4	KH_2PO_4 / H_3PO_4- buffer	9 g/L KH_2PO_4 ; H_3PO_4 (10% in water) added until pH 4 reached (ca. 0.5 ml). 2 L prepared.
7	KH_2PO_4 / Na_2HPO_4- buffer	400 ml KH_2PO_4 solution (0.071M = 9.65 g/L) + 600 ml Na_2HPO_4- solution (0.01 M); pH adjusted by adding 1 N NaOH (4L prepared)
9	$NaHCO_3$ / NaOH buffer	4.2 g/L (0.05M) $NaHCO_3$; 1 N NaOH added until pH 9 reached (ca. 2.5 ml). 2 L prepared.

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	HPLC grade water.
Preparation of test medium	100 ml of the appropriate buffer (pH 4, 7 or 9) poured into each vial and 100µl of the 100µg/l cypermethrin solution added (10µg a.s./sample).
Test concentrations (mg a.i./L)	100 µg/L cypermethrin
Temperature (°C)	50
Identity and concentration of co-solvent	Acetonitrile (HPLC-grade) added to each buffer solution (95% buffer, 5% acetonitrile v/v)
Replicates	16 head space vials for pH 4 and 7 32 head space vials for pH7 (16 for room temperature and 16 for 50°C)

Table A7_1_1_1_1-3: Description of test system

Glassware	100ml brown glass vessels with screw cap
Other equipment	Microlitre syringes, pH-meter (WTW), volumetric flasks, drying cupboard, thermostated oven.
Method of sterilization	pH 4 and pH 7 buffersolutions cooked under reflux for 40 mins (this did not affect the pH). All glass vials were heated in a drying cupboard at 180°C for 3 hours. Benches and syringes were cleaned wih 70% ethanol

Table A7_1_1_1_1-4: Hydrolysis of test compound and transformation products expressed as percentage of initial concentrations, at pH 4, pH 7 and pH 9.

pH 4 (50°C)

Compound	**Sampling times (*days*)**							
	0	T_2	T_4	T_6	T_{15}	T_{18}	T_{22}	T_{29}
Parent compound (mean % of day 0 value)	100	101	99	100	98	96	91	95
Transformation product 1	No further investigation necessary, <10% hydrolysis observed after 5 days							
Transformation product 2								
Total % recovery								

pH 7 (50°C)

Compound	**Sampling times (*days*)**							
	0	T_2	T_4	T_5	T_6	T_8	T_{10}	T_{15}
Parent compound	100	74	55	45	39	31	24	10
Transformation product (M1)	0	15	19	29	31	32	37	44
Transformation product (M2)	4	16	16	28	32	29	34	39
Total % recovery	104	105	90	102	102	92	95	93

M1 = 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid

M2 = 3-Phenoxybenzaldehyde

pH 7 (room temperature)

Compound	Sampling times (*days*)						
	0	T_4	T_6	T_{10}	T_{15}	T_{22}	T_{29}
Parent compound (mean % of day 0 value)	100	98	99	99	94	97	93
Transformation product 1	No further investigation necessary, no degradation observed						
Transformation product 2							
Total % recovery							

pH 9 (50°C)

Compound	Sampling times (*hours*)												
	0	$T_{0.5}$	T_1	$T_{1.5}$	T_2	$T_{2.5}$	T_3	$T_{3.5}$	T_4	$T_{4.5}$	T_5	T_6	T_8
Parent compound	100	78	63	54	43	40	28	30	21	19	14	10	6
Transform-ation product (M1)	0	12	16	19	24	33	37	38	37	44	37	45	47
Transform-ation product (M2)	4	14	18	22	24	33	37	37	32	41	37	41	44
Total % recovery	104	104	97	95	91	106	102	105	90	104	88	96	97

M1 = 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid

M2 = 3-Phenoxybenzaldehyde

Table A7_1_1_1_1-5: Dissipation times of parent compound, transformation products and reference compound at pH 5, pH 7 and pH 9

	pH 4 (50°C)	pH 7 (50°C)	pH 9 (50°C)
	DT_{50}	DT_{50}	DT_{50}
Parent compound	ND	4.73 days	1.9 hours
Transformation product 1	ND	ND	ND
Transformation product 2	ND	ND	ND

ND = Not Determined

Table A7_1_1_1_1-6: Specification and amount of transformation products

CAS-Number	CAS and/or IUPAC Chemical Name(s)	Amount [%] of parent compound measured at		
		pH 4	pH 7 (50°C)	pH 9(50°C)
55701-05-8	3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (-cis and -trans isomers) (DCVC acid)	ND	44 (day 15)	39 (day 15)
39515-51-0	3-Phenoxybenzaldehyde	ND	47 (8 hours)	44 (8 hours)

ND = Not Determined

Section A7.1.1.1.2 (01) Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Direct Phototransformation in purified water

Official use only

1 REFERENCE

1.1	**Reference**	Swales, S. (2003); ^{14}C-Cypermethrin : Photodegradation in sterile, aqueous solution; Covance Laboratories Ltd., Report N° 40/35 (CYP/M70), 24 April 2003 (unpublished)
		Dates of experimental work: 16 August 2002 – 7 February 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

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes
		EC Directive 94/37EC, Section 2.9.2 (July 1994)
		SETAC Procedures for assessing the Environmental Fate and Ecotoxicity of Pesticides, Section 10 (March 1995)
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Cypermethrin (cis:trans 40:60) - radiolabelled sample
3.1.1	Lot/Batch number	Radiolabelled cypermethrin supplied by Blychem Ltd: see section 3.1.4
		Non-radiolabelled cypermethrin cis:trans/40:60: 2001060167
3.1.2	Specification	Non-radiolabelled cypermethrin: As given in section 2
3.1.3	Purity	Non-radiolabelled cypermethrin: 96.5%

Section A7.1.1.1.2 (01) Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Direct Phototransformation in purified water

3.1.4	Radiolabelling	[^{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 % [^{14}C cyclopopane] trans-cypermethrin Batch 01BLY095A Specific activity: 57 mCi/mmole (5.066 MBq/mg) Radiochemical purity: > 98 % Prior to dosing, the two –phenoxy labels were mixed together to give a cis:trans ratio of 40:60. The two –cyclopropane labels were similarly mixed to form a second dosing solution.
3.1.5	UV/VIS absorption spectra and absorbance value	Not determined in this study
3.1.6	Further relevant properties	Solubility in water: 0.004 mg/L (pH7)
3.2	**Reference substances**	Not used in this study
3.3	**Test solution**	[^{14}C-phenoxy] cypermethrin and [^{14}C-cyclopropane] cypermethrin were used in separate experiments. The radiolabelled test materials were dispensed in acetonitrile (<1% by volume) into sterile aqueous buffer solution at pH 4 (the pH at which cypermethrin is most stable to chemical hydrolysis), to give a nominal concentration of 4 µg/l, the water solubility of cypermethrin. See table A7_1_1_1_2_01-1
3.4	**Testing procedure**	
3.4.1	Test system	Nine units were treated with the (^{14}C phenoxy) cypermethrin and a further nine units were treated with the (^{14}C cyclopropane) cypermethrin, to allow four units to be used as dark controls, four units to be irradiated and one remaining unit for analysis at time zero. See table A7_1_1_1_2_01-2

Section A7.1.1.1.2 (01) Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Direct Phototransformation in purified water

3.4.2	Properties of light source	The units were exposed to simulated sunlight using an Atlas Suntest CPS+ accelerated exposure machine which filtered radiation to remove wavelengths below 290 nm. Units were continuously irradiated such that the amount of light illuminating the samples during a 12 hour period was approximately equivalent to one summer sunlight day
3.4.3	Determination of irradiance	Spectral properties and intensity of the lamp were measured at the height of the buffer surface and at the position of the irradiated units using a LI-1800 spectroradiometer.
3.4.4	Temperature	Both the irradiated and the dark control samples were maintained at 20±3°C.
3.4.5	pH	pH 4
3.4.6	Duration of the test	4 days (equivalent to 7 summer sunlight days).
3.4.7	Number of replicates	Four units to be used as dark controls, four units to be irradiated and one remaining unit for analysis at time zero.
3.4.8	Sampling	Analysis was carried out at time 0 (immediately after test article application) and after 0.25, 1, 2 and 4 days continuous irradiation, approximately equal to 0.5, 2, 4 and 7 summer sunlight days; values were also calculated in terms of the standardised Florida summer sunlight.
3.4.9	Analytical methods	Aqueous buffer samples were acidified with HCl followed by sequential extraction with dichloromethane. The aqueous phases were quantified by LSC. DCM phases were rotary evaporated and reconstituted into acetonitrile prior to analysis by HPLC and TLC.
3.5	**Transformation products**	Transformation products tested: Yes Identification of transformation products which at any sampling time accounted for > 10 % of a.s. added unless the half-life of the transformation product is < 6 days and the a.s. is not continuously released to the environment.
3.5.1	Method of analysis for transformation products	HPLC and TLC
		4 RESULTS
4.1	**Screening test**	Not performed
4.2	**Actinometer data**	Not applicable
4.3	**Controls**	The percentage of Applied Radioactivity (%AR) present as parent compound in the total extracts for the dark controls is given in table A7_1_1_1_2_01-3.
4.4	**Photolysis data**	
4.4.1	Concentration values	The percentage of Applied Radioactivity (%AR) present as parent compound in the total extracts for the irradiated units is given in table A7_1_1_1_2_01-3.

Section A7.1.1.1.2 (01) Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Direct Phototransformation in purified water

4.4.2	Mass balance	Overall recoveries of applied radioactivity ranged from 96 to 105% (^{14}C phenoxy) and 96 to 107% (^{14}C cyclopropane) for the irradiated units, and from 90 to 105% (^{14}C phenoxy) and 99 to 107% (^{14}C cyclopropane) for the dark controls.
4.4.3	k^{e}_{p}	Degradation rate, assuming first order kinetics (expressed as equivalent summer sunlight days): irradiated : $k = 0.0783\ d^{-1}$; $t_{1/2} = 8.85$ d (^{14}C phenoxy) $k = 0.0976\ d^{-1}$; $t_{1/2} = 7.10$ d (^{14}C cyclopropane) (cis-isomers are degraded 1.3 to 1.7 times faster than trans-isomers) dark control : $k = 0.0314\ d^{-1}$; $t_{1/2} = 22.1$ d (^{14}C phenoxy) $k = 0.0419\ d^{-1}$; $t_{1/2} = 16.5$ d (^{14}C cyclopropane) Sunlight accelerates the rate of degradation.
4.4.4	Kinetic order	Degradation process in both the irradiated solution and controls was First order.
4.4.5	Reaction quantum yield (ϕ^{e}_{E})	ϕ : 0.0308 (determined in a separate study, see Doc IIIA7.1.1.1.2_02)
4.4.6	k_{pE}	From the rate constants obtained for irradiated samples and dark controls, the net photolysis rate constant and corresponding half lives were calculated to be $0.0469\ d^{-1}$ and 14.8 d for ^{14}C phenoxy label and $0.0557\ d^{-1}$ and 12.4 d for ^{14}C cyclopropane label.
4.4.7	Half-life ($t_{1/2E}$)	The half-life in the irradiated solution was 8.85 summer sunlight days for the (^{14}C phenoxy) cypermethrin and 7.10 summer sunlight days for the (^{14}C cyclopropane) cypermethrin. The corresponding dark control samples had half-lives of 22.1 and 16.5 days respectively. All figures are quoted as equivalent to Florida summer sunlight days. DT90 values were estimated as 29.2 days for the (^{14}C phenoxy) cypermethrin and 23.2 days for the (^{14}C cyclopropane) cypermethrin. The corresponding dark control samples had DT90 values of 73.3 and 54.8 days respectively.

Section A7.1.1.1.2 (01) Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Direct Phototransformation in purified water

4.5 Specification of the transformation products

Two photolysis products were formed in amounts > 10% of applied radioactivity (one from each label) during irradiation and were identified as DCVC acid and 3-phenoxybenzoic acid. DCVC acid was formed at a maximum level of 18% of applied radioactivity and the maximum level of 3-phenoxybenzoic acid formed was 15% of applied radioactivity. The amounts of both degradation products formed were still increasing at the end of the study. One photolysis product at < 10% of applied radioactivity, formed from the (^{14}C phenoxy) cypermethrin, and was identified as 3-phenoxybenzaldehyde. Maximum levels were 3% of applied radioactivity at the end of the photoperiod.

A further 16 unidentified photolytic degradation products containing < 10% of applied radioactivity at any time point (maximum 5.6% at 7 day sunlight equivalent) were detected, 6 following irradiation of (^{14}C phenoxy) cypermethrin and 10 following irradiation of (^{14}C cyclopropane) cypermethrin. In the aqueous phase, after extraction, 8% of applied radioactivity remained from irradiation of the (^{14}C cyclopropane) cypermethrin, whereas only 2% remained unextracted for the (^{14}C phenoxy) label after the equivalent of 7 days sunlight.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The Photodegradation rate of [^{14}C] cypermethrin (cis:trans 40:60) was studied at 20°C in pH 4 buffer (sterile conditions, < 1% acetonitrile co-solvent) with continuous irradiation for up to 100 hrs (equivalent to ca. 7 days of summer sunlight).

5.2 Results and discussion

Degradation process in both the irradiated solution and controls was first order. The half-life in the irradiated solution was 8.85 summer sunlight days for the (^{14}C phenoxy) cypermethrin and 7.10 summer sunlight days for the (^{14}C cyclopropane) cypermethrin. The corresponding dark control samples had half-lives of 22.1 and 16.5 days respectively. All figures are quoted as equivalent to Florida summer sunlight days. DT90 values were estimated as 29.2 days for the (^{14}C phenoxy) cypermethrin and 23.2 days for the (^{14}C cyclopropane) cypermethrin. The corresponding dark control samples had DT90 values of 73.3 and 54.8 days respectively. Two photolysis products were formed in amounts > 10% of applied radioactivity (one from each label) during irradiation and were identified as DCVC acid and 3-phenoxybenzoic acid. DCVC acid was formed at a maximum level of 18% of applied radioactivity and the maximum level of 3-phenoxybenzoic acid formed was 15% of applied radioactivity.

Proposed degradation pathway: Photolysis of Cypermethrin proceeds via cleavage of the ester linkage to form DCVC acid and 3-phenoxybenzaldehyde, and subsequent oxidation of the CHO group resulting in 3-phenoxybenzoic acid. The DCVC acid is further degraded into unidentified polar compounds and subsequently to CO_2.

Section A7.1.1.1.2 (01) Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Direct Phototransformation in purified water

5.3	**Conclusion**	Photolysis of cypermethrin in sterile aqueous conditions is a route of degradation of cypermethrin. Hydrolysis also occurs, as proven by degradation in the dark control samples. Half-life values of 8.85 days for the (^{14}C phenoxy) cypermethrin and 7.10 days for the (^{14}C cyclopropane) cypermethrin were estimated. The corresponding hydrolysis (dark control) samples had half-lives of 22.1 and 16.5 days respectively.	X
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	April 2007
Materials and Methods	Applicant's version is acceptable
Results and discussion	Applicant's version is adopted
Conclusion	Applicant's From the rate constants obtained for irradiated samples and dark controls, the net photolysis rate constant and corresponding half lives were calculated to be 0.0469 d^{-1} and 14.8 d for ^{14}C phenoxy label and 0.0557 d^{-1} and 12.4 d for ^{14}C cyclopropane label.
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_1_1_1_2_01-1: Description of test solution and controls

Criteria	Details
Purity of water	Buffer solution: 0.02M potassium hydrogen phthalate buffer in HPLC grade water (pH4)
Preparation of test chemical solution	[^{14}C Phenoxy]-cypermethrin: 240µl, 80µg, 356.4 kBq [^{14}C Phenoxy]-cis-cypermethrin, 384µL, 120µg 533.4KBq [^{14}C Phenoxy]-trans-cypermethrin diluted to 2 ml with acetonitrile. Immediately prior to dose application, an aliquot of 98µl, 10µg 44.5 kBq was diluted to 25ml with acetonitrile. [^{14}C Cyclopropane]-cypermethrin: 275µl, 80µg, 405.6 kBq [^{14}C Phenoxy]-cis-cypermethrin, 478µL, 120µg 603.8KBq [^{14}C Phenoxy]-trans-cypermethrin diluted to 2 ml with acetonitrile. Immediately prior to dose application, an aliquot of 100µl, 10µg 50.88 kBq was diluted to 25ml with acetonitrile Non-radiolabelled cypermethrin (7.92µg/ml) was further diluted in acetonitrile to produce a 0.4µg/ml dosing solution for the pH/sterility control units
Test concentrations	The application rate of ca. 0.1µg per unit was calculated to give a final concentration of 4 µg/L
Temperature	20±3°C
Preparation of a.s. solution	See above
Controls	Yes, incubation in the dark and pH/sterility
Co-solvent	Acetonitrile

Table A7_1_1_1_2_01-2: Description of test system

Criteria	Details
Test vessels	Irradiation vessels were glass vials with quartz glass lids, air inlet and outlet ports (fitted with bacterial filters) and septum-sealed injection port. Dark control vessels were glass vials sealed with crimped PTFE-lined rubber caps
Buffer solution	25ml of Potassium hydrogen phthalate buffer (0.02M) at pH 4 (the pH at which cypermethrin is most hydrolytically stable).
Preparation of test article	Radiolabelled cypermethrin (cis:trans/40:60) in acetonitrile was dispensed aseptically onto the buffer solution to give a final concentration of 4µg/L
Light Source	Atlas Suntest CPS+ Accelerated Exposure Machine which filters wavelengths below 290nm

Table A7_1_1_1_2_01-3: Percentage of Applied Radioactivity (%AR) present as parent compound

Timepoint	(^{14}C phenoxy)-cypermethrin (cis:trans 40:60) (%AR)		(^{14}C cyclopropane)-cypermethrin (cis:trans 40:60) (%AR)	
	Irradiated	Dark	Irradiated	Dark
0 day	99.2	99.2	91.9	91.9
0.25 day	94.9	90.7	89.9	84.2
1 day	84.2	97.0	81.1	95.5
2 day	66.4	94.0	83.4	95.7
4 day	56.7	87.6	47.3	81.6

Section A7.1.1.1.2 (02) Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Quantum Yeild of Direct Phototransformation in purified water

			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) Dates of experimental work: 11 December 2002 – 10 April 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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes Draft OECD guideline on phototransformation of chemicals in sterile water and EPA OPPTS 835.2210	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[^{14}C-cyclopropane] Cypermethrin (cis:trans 40:60) - radiolabelled sample, see 3.1.4	
3.1.1	Lot/Batch number	Non-radiolabelled test article (used for pH/sterility testing): 2001060167	
3.1.2	Specification	Non-radiolabelled test article (used for pH/sterility testing): As given in section 2	
3.1.3	Purity	Non-radiolabelled test article (used for pH/sterility testing): 96.5%	

Section A7.1.1.1.2 (02) Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Quantum Yeild of Direct Phototransformation in purified water

3.1.4	Radiolabelling	[^{14}C cyclopropane] cis-cypermethrin Batch 01BLY095 Specific activity: 57 mCi/mmole (5.066 MBq/mg) Radiochemical purity: > 98 % [^{14}C cyclopopane] trans-cypermethrin Batch 01BLY095A Specific activity: 57 mCi/mmole (5.066 MBq/mg) Radiochemical purity: > 98 %
3.1.5	UV/VIS absorption spectra and absorbance value	Not determined in this study
3.1.6	Further relevant properties	Low water solubility and tendancy to adsorb onto glassware. Both addressed in study design.
3.2	**Test solutions**	A radiolabelled stock solution was prepared by mixing the separate cis- and trans-isomers of cypermethrin in acetonitrile to give an initial concentration of 98.8 µg/ml. The dose solution was prepared by removing a 100µl aliquot and diluting in 25ml acetonitrile to give a final concentration of 4µg/ml. 150µl (0.06µg) of this solution was then added to each test unit. An aqueous binary actinometer solution was prepared containing p-nitroacetophenone (PNAP, 2.87 µg/mL, 1.54 x 10^{-5}M) and pyridine (534.1 µg/mL, 0.0068 M). Two portions of the solution were analysed for PNAP immediately after preparation and a further two after 90 hours irradiation. Non-radiolabelled cypermethrin (0.06 µg) in acetonitrile (150 µL) was injected into four samples of sterile buffer (15 mL, pH 4) giving concentrations of 0.004 µg/mL. Two of the samples were irradiated for 90 hours and the other two kept in the dark. Samples were analysed for sterility (one irradiated and one dark) and pH (one irradiated and one dark) at the end of the study.
3.3	**Testing procedure**	

Section A7.1.1.1.2 (02) Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Quantum Yeild of Direct Phototransformation in purified water

3.3.1	Test system	(^{14}C cyclopropane) cypermethrin (0.06 µg) in acetonitrile (150 µL) was injected into six samples of sterile buffer (15 mL, pH 4) to give concentrations of 0.004 µg/mL. Duplicate samples were analysed immediately after test article application and a further two after 90 hours irradiation. A further two units were similarly treated but were kept dark and analysed after 90 hours. Samples for irradiation were contained in vessels with quartz glass lids and were continuously exposed to simulated sunlight for 90 hours. The samples were held in a carousel that rotated at 1 rpm, thereby ensuring that all samples were exposed to the same levels of irradiation. The incident photon flux was measured at 2 nm intervals using a spectroradiometer
3.3.2	Properties of light source	Hanau Suntest CPS+ Accelerated Exposure Machine. The light used was filtered to remove wavelengths below 290 nm to produce a similar ultra violet and visible light spectrum to natural sunlight.
3.3.3	Temperature	The sample temperature was maintained at 25 ± 2°C by a cooling system.
3.3.4	Duration of the test	90 hours
3.3.5	No. of replicates	2
3.3.6	Sampling	(^{14}C cyclopropane) Cypermethrin was measured at the start and end of the incubation period by a thin layer chromatography (TLC) method and the results were used to determine the test article degradation rate constant. PNAP was measured by a validated HPLC method at the end of the incubation period and was used to determine the actinometer degradation rate constant. The extinction coefficients of the test article and actinometer in aqueous solution was determined at 2 nm intervals. The light adsorption rate constants were calculated at each 2 nm wavelength interval as the product of the extinction coefficient and the photon flux at each wavelength. They were then summed using the trapezoidal rule to obtain the light adsorption rate constants for the actinometer and test article over the wavelength 290 to 800 nm.
3.3.7	Analytical methods	TLC and HPLC

4 RESULTS

4.1	**Screening test**	Not performed

Section A7.1.1.1.2 (02) Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Quantum Yeild of Direct Phototransformation in purified water

4.2	**Actinometer data**	After 90 hours irradiation means of 68 and 91% of cypermethrin and PNAP, respectively, remained. There was no measurable hydrolysis of the cypermethrin samples in the dark over this time-period and therefore no correction had to be applied to the observed degradation rate in the light. The quantum yield of the actinometer solution was calculated from the molar concentration of pyridine using a standard equation. Analysis by Thin Layer Chromatography (TLC), illustrated that after 90 hours irradiation, the majority of radioactivity detected was cypermethrin, 68% of applied radioactivity at 90 hours. In the dark control units, 95% of applied radioactivity was identified as cypermethrin after 90 hours incubation. The levels of origin material increased throughout the study, reaching 16% and 2% of applied radioactivity at 90 hours in the irradiated and dark controls units respectively, confirming that photodegradation of cypermethrin had taken place. See Tables A7_1_1_1_2_02-1 and A7_1_1_1_2_02-2.	X
4.2.1	Reaction quantum yield (ϕ^{c}_{E})	The quantum yield of cypermethrin was calculated from the quantum yield of the actinometer, the rate constants for test article and PNAP degradation and the light adsorption rate constants for the actinometer and the test article. The value calculated was 0.0308.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The quantum yield for direct photolysis of cypermethrin in sterile pH4 aqueous buffer was determined after 90 hours continuous irradiation. Aqueous buffer solutions containing cypermethrin, in quartz lidded vials, were mounted on a carousel and irradiated from above with artificial sunlight using a Xenon lamp. The photon flux as a function of wavelength of the light source was measured using a spectroradiometer. A binary chemical actinometer solution, of known quantum yield, was simultaneously irradiated with the test samples. The losses of test substance and of a component of the binary actinometer were measured analytically at the end of the exposure period. The ultra violet/visible absorbance spectrum of the test substance and the actinometer were recorded using a spectrophotometer. The quantum yield was then calculated from the information generated.	
5.2	**Results and discussion**	Analysis by Thin Layer Chromatography (TLC), illustrated that after 90 hours irradiation, the majority of radioactivity detected was cypermethrin, 68% of applied radioactivity at 90 hours. In the dark control units, 95% of applied radioactivity was identified as cypermethrin after 90 hours incubation. The levels of origin material increased throughout the study, reaching 16% and 2% of applied radioactivity at 90 hours in the irradiated and dark controls units respectively, confirming that photodegradation of cypermethrin had taken place.	X

Section A7.1.1.1.2 (02) Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Quantum Yeild of Direct Phototransformation in purified water

5.3	**Conclusion**	The quantum yield of cypermethrin was calculated from the quantum yield of the actinometer, the rate constants for test article and PNAP degradation and the light adsorption rate constants for the actinometer and the test article. The value calculated was 0.0308.
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	April 2007
Materials and Methods	Applicant's version is acceptable
Results and discussion	Under 4.2 and 5.2, in the sentence : "The level of orignin material, ,... the word "increase" should be replaced by "decrease"
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_1_1_1_2_02-1: Concentration of (^{14}C cyclopropane) cypermethrin

	Concentration of(^{14}C cyclopropane) cypermethrin (percentage of applied radioactivity)		
	Time Zero	Dark Control (90 hours)	Irradiated (90 hours)
Replicate 1	97.41	96.56	68.40
Replicate 2	97.82	92.49	68.38
Mean	97.61	94.52	68.39

Table A7_1_1_1_2_02-2: Concentration of PNAP

	Concentration of PNAP (µg/mL)		% PNAP Remaining
	Time Zero	Irradiated (after 90 hours)	
Replicate 1	2.868	2.616	91%
Replicate 2	2.864	2.615	91%
Mean	2.866	2.616	91%

Section A7.1.1.2.1
Annex Point IIA7.6.1.1

Ready Biodegradability

Modified Sturm Test

			Official use only
		1 REFERENCE	
1.1	**Reference**	Klein, W. (1990); Biodegradation – The modified sturm test; Fraunhofer Institute für Umweltchemie und Ökotoxicologie, report no. FEI-001/3-11 (CYP/M50), 15 June 1990 (unpublished) Dates of experimental work: 18 April 1990 – 22 May 1990	
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. EEC Directive 79/831, Annex V, part C 5.2, Biotic degradation: modified sturm test. (corresponds to OECD guideline 301B)	
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	Not specified in report	
3.1.2	Specification	As given in section 2	X
3.1.3	Purity	94%	
3.1.4	Further relevant properties	Water solubility <1 mg/L	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	EC50 = 163 mg/L (from respiration inhibition study)	
3.1.7	Specific chemical analysis	Not applicable	
3.2	**Reference substance**	Aniline	
3.2.1	Initial concentration of reference substance	10 mg/L and 20 mg/L	

Section A7.1.1.2.1
Annex Point IIA7.6.1.1

Ready Biodegradability

Modified Sturm Test

3.3	**Test ing procedure**	
3.3.1	Inoculum / test species	Activated sludge from a small municipal sewage works fed predominantly with household waste. Activated sludge was aerated for 4 hours and then homogenised for 2 minutes and left to stand for 30 mins. The optical density was adjusted to 0.2 extiction units with Ringer Solution. See Table A7_1_1_2_1-2
3.3.2	Test system	Test vessels were aerated with CO_2-free air. The air washer consisted of 2 flasks connected in series and containing 50% sodium hydroxide. 6 cultivation pots were used (2 innoculum blanks, 2 reference substance, 2 test substance) each connected to 3 conical flasks filled with 100ml Ba (OH)2 as the CO_2 absorbing fluid.
3.3.3	Test conditions	Test was carried out at 20 °C (± 3°C)
3.3.4	Method of preparation of test solution	Due to low water solubility, test substance (30mg and 60mg) was weighed on Teflon scales and used immediately.
3.3.5	Initial TS concentration	Initial concentration 10 mg a.s./L and 20 mg a.s./L
3.3.6	Duration of test	18.04.90 – 22.05.90 (35 days)
3.3.7	Analytical parameter	CO_2 evolution
3.3.8	Sampling	Total CO_2 generated in the test receptacles throughout the study was determined by titration
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	Aniline was used as a reference substance to ensure validity of the test (degradation being >60% over 28 days). An innoculum blank was also used to calculate CO_2 generated from the test substance.
	4 RESULTS	
4.1	**Degradation of test substance**	
4.1.1	Graph	See Fig. A7_1_1_2_1-3
4.1.2	Degradation	Biodegradation of cypermethrin was between 0.6 and 1.4% at day 33.
4.1.3	Other observations	No inhibition effects were reported.
4.1.4	Degradation of TS in abiotic control	Not reported
4.1.5	Degradation of reference substance	Biodegradation of aniline was 94.4-100.7% after 28 days incubation.

Section A7.1.1.2.1
Annex Point IIA7.6.1.1

Ready Biodegradability

Modified Sturm Test

4.1.6	Intermediates/ degradation products	Not determined	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Cypermethrin (initial concentration: 10 and 20 mg a.s./l) was used. The vessels were aerated with CO_2-free air. The extent of biodegradation was determined by titrating the total CO_2 evolved during the incubation for 33 days.	
5.2	**Results and discussion**	% biodegradation of cypermethrin = 0.4 - 1.4% after 33 days incubation. % biodegradation of aniline (positive control) = 94.4-100.7% after 28 days incubation	X
5.3	**Conclusion**	Cypermethrin is not readily biodegradable.	
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	April 2007
Materials and Methods	Applicant's version is acceptable
Results and discussion	Applicant's version is adopted
Conclusion	Under 5.2 : "% biodegradation of cypermethrin = 0.6 – 1.4% after 33 days incubation" instead of 0.4-1.4%
Reliability	1
Acceptability	Acceptable
Remarks	*Setion 3.1.2 Specification: (RS)-a-cyano-3-phenoxybenzyl-(1r,1s)-cis, trans-3-(2.2-dichlorovinyl)-2.2-dimethyl-cyclopropan-carboxylat.* No further specification provided.
	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

Section A7.1.1.2.1
Annex Point IIA7.6.1.1

Ready Biodegradability

Modified Sturm Test

Remarks

Table A7_1_1_2_1-1: Guidline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

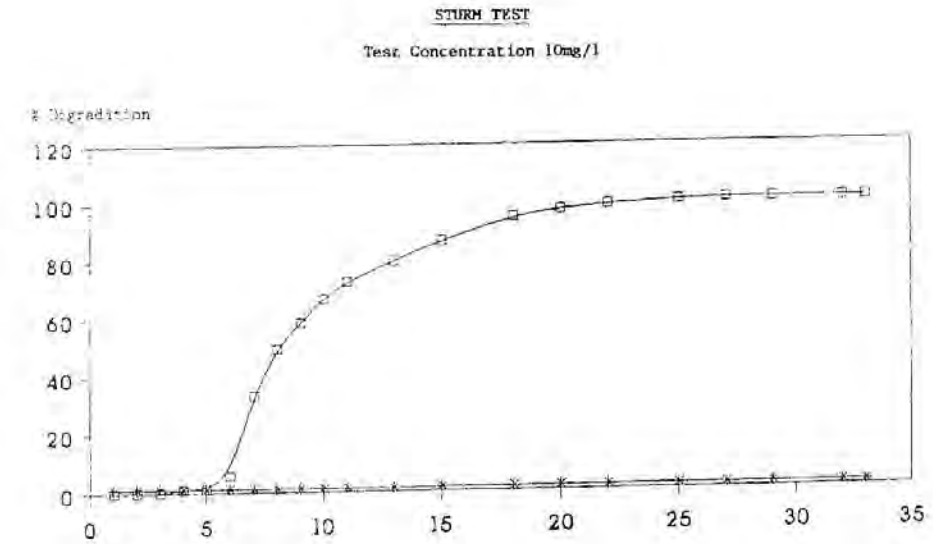
Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	**C.4-C**	**301B**	**ready**
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready
MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	C.9	302B	Inherent
Modified MITI-Test (II)	-	302C	Inherent
Modified SCAS-Test	C.12	302A	Inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	Simulation Test[1)]

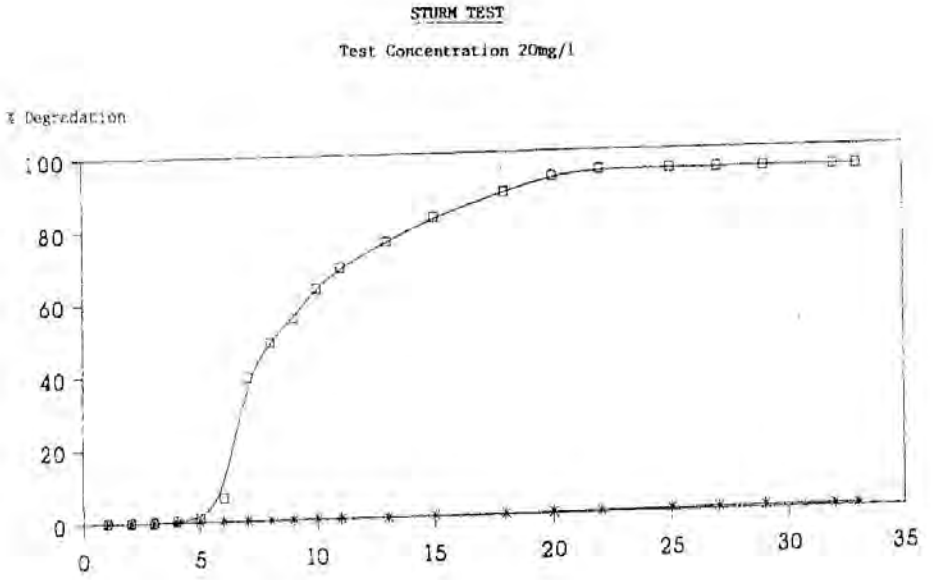
[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Table A7_1_1_2_1-2: Innoculum / test organism

Criteria	Details
Nature	Activated sludge
Species	Not specified
Strain	Not specified
Source	Small municipal sewage works treating predominantly household waste
Sampling site	Not specified in report
Laboratory culture	No
Method of cultivation	Not specified in report
Preparation of innoculum	Aerated for 4 hours then homogenized for 2 mins and left to stand for 30 mins. Final optical density 0.2 extinction units (436nm)
Pre-treatment	No adaptation
Initial cell concentration	CFU= 1.33×10^5 microbes/ml

Fig A7_1_1_2_1-3: Graphs of degradation vs time





□ Aniline (control)

* Cypermethrin

Section A7.1.1.2.2 Biodegradability (inherent)

Annex Point IIA7.6.1.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	Burwood, C. (2005); Cypermethrin cis:trans/40:60: Assessment of Inherent Biodegradability by measurement of CO_2 evolution; Covance Laboratories Ltd., report no. 1699/017-D2149, 10 August 2005 (unpublished) Dates of experimental work: 10 March 2005 – 8 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 The study was designed to meet the requirements of the OECD 302 series "Inherent Biodegradability". The only OECD method available for assessing the inherent biodegradation of insoluble chemicals is OECD Guideline 302C, the modified MITI test (II). The Modified MITI test (II) requires the use of a complex composite inoculum and specialist electrical equipment (a manometric respirometer). As an alternative to this method, the study reported here adopted the methodology of the OECD Guideline 301B, CO_2 evolution test but used the medium composition and test substance and inoculum concentrations from OECD Guideline 302C. In this way, the less stringent conditions required for an assessment of inherent biodegradation were provided, without the requirement for a composite inoculum or specialist equipment.	X
2.2	**GLP**	Yes	
2.3	**Deviations**	No (other than mentioned above)	
		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	

Section A7.1.1.2.2 Biodegradability (inherent)

Annex Point IIA7.6.1.2

3.1.4	Further relevant properties	Cypermethrin is insoluble in water, therefore the modified MITI test was not considered to be appropriate (see section 2.1 above).
3.1.5	Composition of Product	Not applicable, test was carried out on the active substance
3.1.6	TS inhibitory to microorganisms	In the activated sludge respiration inhibition test the EC50 value was determined as 163 mg cypermethrin / Litre (see DocIIIA_7.4.1.4)
3.1.7	Specific chemical analysis	Not performed
3.2	**Reference substance**	Yes, Sodium Benzoate
3.2.1	Initial concentration of reference substance	30 mg/L A separate treatment group was also established containing both test and reference substance (30 mg/L each) to serve as a toxicity control.
3.3	**Test ing procedure**	
3.3.1	Inoculum / test species	See table A7_1_1_2_2-2
3.3.2	Test system	See table A7_1_1_2_2-3
3.3.3	Test conditions	See table A7_1_1_2_2-4
3.3.4	Method of preparation of test solution	The test substance was insufficiently soluble to permit dosing of the test system with a concentrated aqueous stock solution. The test substance was therefore added directly to the two test vessels and the toxicity control as individual weighings on PTFE discs. Each individual weighing was approximately 90 mg, to give a nominal test substance concentration of 30 mg/L. A blank PTFE boat was also added to each of the other vessels in the test, for consistency.
3.3.5	Initial TS concentration	30 mg/L
3.3.6	Duration of test	28 days
3.3.7	Analytical parameter	CO_2 evolution
3.3.8	Sampling	At appropriate intervals, the air supply to each vessel was stopped and the trap bottle nearest to the test vessel was removed for sampling. The remaining two bottles of the series were moved up along towards the test vessel, and a fresh trap bottle placed on the end of the train. Once the train of trap bottles was connected to the test vessel, the air supply was restarted. The initial barium hydroxide stock concentrations and the residual concentrations in detached trap bottles were determined by titration

Section A7.1.1.2.2 **Biodegradability (inherent)**

Annex Point IIA7.6.1.2

against standard hydrochloric acid (nominally 0.05M) against 0.5% ethanolic phenolphthalein indicator solution. Titrations were performed on 20 mL trap solution volumes until two matching (± 0.1 mL) titres were obtained. As evolved CO_2 is trapped in the trap bottle, barium carbonate is precipitated and the concentration of barium hydroxide in the bottle decreases. Consequently, the amount of CO_2 absorbed by each trap was calculated from the reduction in the concentration of barium hydroxide solution in the trap bottle determined by the titration.

On Day 28 of the test, each culture vessel was opened and 1 mL concentrated hydrochloric acid added. The vessels were then reconnected to the train of trap bottles and aeration continued until the following day. The acidification and aeration procedure drives off generated carbon dioxide remaining in solution. Final sampling and titrations were carried out on Day 29 when all of the traps in each train were sampled.

3.3.9 Intermediates/ degradation products

Not identified

3.3.10 Nitrate/nitrite measurement

No

3.3.11 Controls

Control without test substance (inoculated mineral salts medium only)

Toxicity control (inoculated mineral salts medium plus test and reference substance at 30mg/L each)

3.3.12 Statistics

The theoretical yield of carbon dioxide (TCO_2 in mg) from cultures containing the test and/or reference substance was calculated as follows:

$TCO_2 = D_{abs} \times P_c \times 3.667$

D_{abs} = the absolute dose ie: the amount in mg of test or reference substance added to the culture, as appropriate

P_c = the percentage carbon content (by weight) of the test or reference substance as appropriate

3.667 = the weight of CO_2 in mg produced from 1 mg of carbon

Biodegradation (D_t) of Cypermethrin *cis:trans* 40:60 expressed in terms of percentage theoretical CO_2 yield (TCO_2) was calculated by applying the formula:

$$D_t = \frac{\text{cumulative mg } CO_2 \text{ produced at time t}}{TCO_2} \times 100$$

All cumulative CO_2 values were corrected for the mean CO_2 generated by the blanks.

Section A7.1.1.2.2 Biodegradability (inherent)

Annex Point IIA7.6.1.2

4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Graph	See fig. A7_1_1_2_2-5
4.1.2	Degradation	Cypermethrin cis:trans 40:60 did not show any evidence of biodegradation during the test and biodegradation was 0% on Day 28. Final CO_2 yields for the test substance, expressed as a percentage of theoretical, were 0% in both replicates throughout the test. The total CO_2 evolution from the replicate cultures containing the test substance were 253.4 and 267.0 mg CO_2 on Day 28 equating to a difference of 5%. See table A7_1_1_2_2-6
4.1.3	Other observations	CO_2 evolution was lower in cultures containing the test substance than in the controls which suggests cypermethrin had a slightly toxic effect on the activated sludge microbes.
4.1.4	Degradation of TS in abiotic control	Not performed
4.1.5	Degradation of reference substance	Rapid CO_2 generation commenced immediately and declined to a more gradual rate around Day 2. The rate of degradation began to plateau around Day 14 as shown in Figure 1. The mean level of degradation on Day 28 was 89%. See table A7_1_1_2_2-6
4.1.6	Degradation of toxicity control	Assessment of degradation in the toxicity control was confined to the sodium benzoate fraction where vigorous CO_2 production began immediately. The rate of CO_2 production followed the same trend as that observed in the two reference vessels containing sodium benzoate alone, but with a slightly lower percentage biodegradation value. Biodegradation of sodium benzoate in this vessel exceeded 60% on Day 11 and was 75% at the end of the test on Day 28. The rate of degradation began to plateau around Day 14. Despite the noted suppression in CO_2 evolution, the level of biodegradation achieved shows that the test substance did not significantly inhibit the degradative activity of the inoculum. See table A7_1_1_2_2-6
4.1.7	Intermediates/ degradation products	Not performed.

Section A7.1.1.2.2 **Biodegradability (inherent)**

Annex Point IIA7.6.1.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The inherent biodegradability of Cypermethrin *cis:trans* 40:60 was assessed by measuring carbon dioxide evolution. The study reported here adopted the methodology of the OECD Guideline 301B, CO_2 evolution test but used the medium composition and test substance and inoculum concentrations from OECD Guideline 302C.

The test material, which provided the sole source of carbon and energy, was suspended in a buffered mineral salts medium at a concentration nominally equivalent to 30 mg/L. The medium was inoculated with microorganisms derived from a sample of activated sludge not previously exposed to the test substance. Test vessels were incubated in darkness within a specified temperature range for 28 days and the medium continually sparged with a supply of CO_2-free air. The exhaust air from each vessel was passed through a series of dedicated CO_2 traps containing a barium hydroxide ($Ba(OH)_2$) solution.

At intervals during the incubation, traps were detached and their contents titrated with acid to determine the quantity of CO_2 purged from the respective test vessels. At the end of incubation, the test vessel contents were acidified to release any residual CO_2 that had remained in solution. After correcting yields for the CO_2 generated from a pair of blank vessels containing only inoculated medium, the extent of biodegradation was determined by expressing the cumulative recovered yield as a percentage of the theoretical, calculated from the carbon content of the test substance. The procedure and the activity of the inoculum were checked by a pair of vessels containing a reference substance. An additional vessel contained a combination of the test and reference substances, and served as a toxicity control to assess whether the test substance was inhibitory at the concentration at which it was applied.

5.2 Results and discussion

To be considered to be inherently biodegradable a test substance must achieve 20% biodegradation by the end of the test. Cypermethrin cis:trans 40:60 did not show any evidence of biodegradation during the test and biodegradation was 0% on Day 28. Cypermethrin cis:trans 40:60 cannot therefore, be considered to be inherently biodegradable. It was noted that CO_2 production was lower in cultures containing the test substance than in control cultures. This shows that the test substance had a slightly toxic effect on the activated sludge microbes used to inoculate the cultures. This is also supported by a slight suppression of biodegradation in the toxicity control group.

Degradation of the reference substance, sodium benzoate, exceeded 60% on Day 6, and was 89% at the end of the test. These data show that the inoculum was viable and exerting normal degradative activity.

In the toxicity control group, degradation of sodium benzoate was slightly suppressed relative to cultures containing sodium benzoate alone and exceeded 60% on Day 11 and was 75% at the end of the test on Day 28. The level of biodegradation achieved shows that the test

Section A7.1.1.2.2 **Biodegradability (inherent)**

Annex Point IIA7.6.1.2

substance did not significantly inhibit the degradative activity of the inoculum.

5.3 Conclusion

The validity criteria stated in the protocol were satisfied. Biodegradation of the reference substance reached 40% by day 7 and 65% by day 14. Duplicate CO_2 production values in cultures containingthe test substance differed by less than 20%. Therefore results of this study are considered valid and showed that cypermethrin is not inherently biodegradable.

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	April 2007
Materials and Methods	Applicants version is acceptable. However the thest guideline have been adapted. In place of studing the O^2 consuption, the CO^2 production was followed. This change in methodology is not regarded as a deficiency.
Results and discussion	Applicant's version is adopted
Conclusion	Applicant's version is adopted
Reliability	2
Acceptability	Acceptable
Remarks	The test was performed according to OECD 301B guideline. For the miti (II) test, the temperature sould be 25+-1°C However for the OECD 301B guideline, the temperature should be 22+-2°C. In the test, temperature was 21±1 °C instead of 25±1 °C as recommended by OECD 302 C(Miti(II)). This deviation is regarded acceptable Additionally, the inoculum is not composed of samples from 10 different places but this deviation can be accepted. *Due to these deviations rms set a reliability of 2*

Section A7.1.1.2.2 Biodegradability (inherent)

Annex Point IIA7.6.1.2

	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_1_1_2_2-1: Guidline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready
MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	**C.9**	**302B**	**Inherent**
Modified MITI-Test (II)	-	**302C**	**Inherent**
Modified SCAS-Test	C.12	302A	Inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	Simulation Test[1)]

[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Table A7_1_1_2_2-2: Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Species	Not applicable
Strain	Not applicable
Source	sewage treatment plant treating predominantly domestic sewage
Sampling site	Burley Menston sewage works, West Yorkshire, UK
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Sample was aerated with a compressed air supply. A 25ml subsample was filtered through a pre-weighed glass microfibre filter. This was then dried and re-weighed to determine the suspended solids concentration.
Pretreatment	The activated sludge was not adapted or acclimitised to cypermethrin before exposure.
Initial cell concentration	Based on the above determination, the test medium was inoculated with active sludge at 300 mg suspended solids/L

Table A7_1_1_2_2-3: Test system

Criteria	Details
Culturing apparatus	Test vessels (3 litre volume), sealed and connected to a series of barium hydroxide traps
Number of culture flasks/concentration	Duplicate vessels for the test, reference and control groups. Single vessel for the toxicity control (test plus reference).
Aeration device	CO_2-free air regulated in 2 stages. Initial control was provided by a gas regulator and the air flow to each vessel controlled by individual needle valves.
Measuring equipment	Measurements were made of the flow rate exiting each test vessel, at intervals not exceeding eight days, with a bubble flow meter and stop watch. Adjustments were made as necessary to maintain a flow rate of approximately 50 mL per minute.
Test performed in closed vessels due to significant volatility of TS	Test performed in closed vessels but not due to volatility of test substance.

Table A7_1_1_2_2-4: Test conditions

Criteria	Details
Composition of medium	The test was conducted in an aqueous synthetic mineral salts medium containing 3 mL/L of each of the following solutions: (i) 8.50 g potassium dihydrogen phosphate; 21.75 g dipotassium hydrogen phosphate; 21.175 g disodium hydrogen phosphate dihydrate; 1.7 g ammonium chloride, all dissolved in and made up to 1 L with reverse-osmosis water; (ii) 36.4 g calcium chloride dihydrate, dissolved in and made up to 1 L with reverse-osmosis water; (iii) 22.5 g magnesium sulphate heptahydrate, dissolved in and made up to 1 L with reverse-osmosis water; (iv) 0.25 g ferric chloride hexahydrate plus one drop concentrated hydrochloric acid, dissolved in and made up to 1 L with reverse-osmosis water.
Additional substrate	No
Test temperature	21 ± 1°C
pH	Measured at the start of incubation in the blank and reference vessels only (to avoid removal of any undissolved test substance onto the pH probe). Final measurements made on day 28 in all vessels. Measured values ranged from 6.9-7.1 (day 0) and from 6.3-6.7 (day 28)
Aeration of dilution water	Yes, air-flow
Suspended solids concentration	Nominal final solids concentration of 100 mg/L in each test vessel.
Other relevant citeria	At appropriate intervals, the air supply to each vessel was stopped and the trap bottle nearest to the test vessel was removed for sampling. The remaining two bottles of the series were moved up along towards the test vessel, and a fresh trap bottle placed on the end of the train. Once the train of trap bottles was connected to the test vessel, the air supply was restarted. The initial barium hydroxide stock concentrations and the residual concentrations in detached trap bottles were determined by titration against standard hydrochloric acid (nominally 0.05M) against 0.5% ethanolic phenolphthalein indicator solution

Fig. A7_1_1_2_2-5: Percentage Biodegradation in the CO_2 Evolution Test

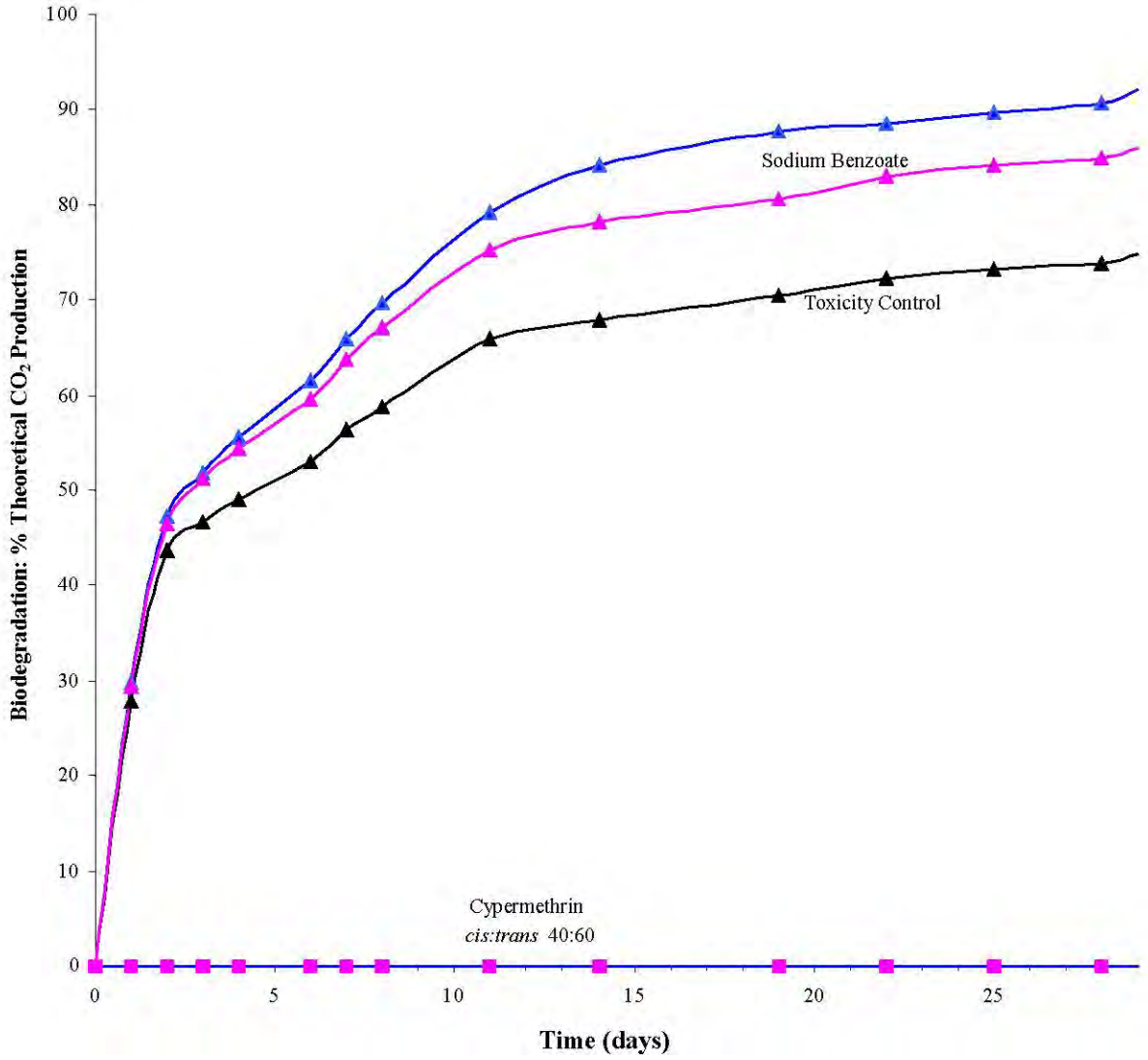


Table A7_1_1_2_2-6: Percentage Biodegradation

	Biodegradation (%)												
	Day 1	Day 2	Day 3	Day 4	Day 6	Day 7	Day 8	Day 11	Day 14	Day 19	Day 22	Day 25	Day 28
Test: Replicate 1	0	0	0	0	0	0	0	0	0	0	0	0	0
Test: Replicate 2	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	0	0	0	0	0	0	0
Reference: Replicate 1	30	47	52	56	62	66	70	79	84	88	89	90	92
Reference: Replicate 2	29	46	51	54	60	64	67	75	78	81	83	84	86
Mean	30	47	51	55	61	65	68	77	81	84	86	87	89
Toxicity control	28	44	47	49	53	56	59	66	68	70	72	73	75

Section 7.1.2.1.2 Anaerobic biodegradation
Annex Point IIIA XII 2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Barnes S. (2005); Cypermethrin cis:trans/40:60 Evaluation of ultimate anaerobic biodegradability by measurement of biogas production; Huntingdon Life Sciences Ltd., report no. HZL 010/053287, 11 November 2005, (unpublished). Dates of experimental work: 11 April 2005 – 27 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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, British Standard (BS) method 6068 (1996) and International Organisation for Standardisation (ISO) method 11734 (1995) "Water quality – Section 5.21; Evaluation of the 'ultimate' biodegradability of organic compounds in digested sludge – Method by measurement of the biogas production.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
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	Further relevant properties	Due to the low water solubility of cypermethrin a preliminary solubility and formulation trial was carried out which showed the test substance was sufficiently soluble in acetone to allow preparation of a stock solution.	
3.1.5	Composition of Product	Not applicable, test carried out on the a.s.	
3.1.6	TS inhibitory to microorganisms	The potential inhibition of the inoculum by cypermethrin cis:trans 40:60 at the test concentration was assessed in an inhibition assay which assessed biogas evolution from cultures containing the test and reference substances.	
3.1.7	Specific chemical analysis	Not applicable, method of measurement was biogas production.	
3.2	**Reference substance**	Yes, Polyethylene glycol (PEG 400 AR grade product number P/3676/08, batch 0255601) Fisher Scientific UK.	X

Section 7.1.2.1.2 Anaerobic biodegradation
Annex Point IIIA XII 2.1

3.2.1	Initial concentration of reference substance	Nominally 20 mg as carbon [C]/L
3.3	**Test ing procedure**	
3.3.1	Inoculum / test species	See table A7_1_2_1_2-1
3.3.2	Test system	See table A7_1_2_1_2-2
3.3.3	Test conditions	See table A7_1_2_1_2-3
3.3.4	Method of preparation of test solution	The results of preliminary solubility and formulation trials showed that the test substance was sufficiently soluble in acetone to allow the preparation of a stock solution. The trials showed that an adequate dispersion was formed in an aqueous system, initially, by the deposition of the material from the solvent solution onto the walls of empty culture vessels. Therefore on the day before test initiation, a solution was prepared at a nominal concentration of 3.47 g/l and aliquots (1 ml) were added to appropriate empty culture vials to establish a final nominal concentration equivalent to 20 mgC/l. Care was taken to ensure that the solution remained in the neck of the vial, as this was consistent with ensuring maximum contact between residue and inoculum in the inverted orientation of the culture vessels during incubation. The solvent was evaporated in a gentle stream of nitrogen and any lingering vapour then removed at least one hour later by reflushing each vial. An appropriate volume of freshly prepared MSM was added to the calibration mark of each vial then ca. 25 g of glass balls (ca. 4 mm diameter) were added. This weight of glass balls was chosen to achieve dispersal of the test substance and provide an occupied volume by the balls, which equated to ca. 10 ml in the cultures. The headspace volume of each culture was considered to be 40 ml.
3.3.5	Initial TS concentration	Nominally 20 mg as Carbon [C]/L
3.3.6	Duration of test	60 days
3.3.7	Analytical parameter	Biogas production (CH_4 and CO_2)
3.3.8	Sampling	Headspace pressure measurements were performed at least once each week. On Day 60, the cultures were allowed to settle and inorganic carbon (IC) analysis performed on the supernatant to give an estimate of the total mineralization of the test substance.
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Controls	Blank and positive control cultures comprised inoculated MSM and MSM plus the reference substance, polyethylene glycol (PEG) 400 (nominally, 20 mg as Carbon [C]/l), respectively. The potential inhibition of the inoculum by cypermethrin cis:trans 40:60 at the test concentration was assessed in an inhibition assay which assessed biogas evolution from cultures containing the test and reference substances.

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3.3.11 Statistics

The level of biodegradation of the test and reference substances was calculated from blank-corrected measurements of headspace pressure made during the course of the test and IC analysis of samples of the supernatant taken at the end in the following way:

Since 1 mole of methane (CH_4) and 1 mole of carbon dioxide (CO_2) each contain 12 g of carbon, the carbon content, m, of a given volume of evolved biogas for n moles of gas is given by:

$$m = 12 \times 10^3 \times n$$

where:

m is the mass of carbon in mg in a given volume of evolved gas;

12 is the relative atomic mass of carbon;

n is the number of moles of gas in the given volume calculated from the gas;

$$n = \frac{\rho V}{RT}$$

where:

ρ is the pressure of the gas in Pascals (1 Pascal = 0.01 millibar);

V is the volume of the gas in cubic metres;

R is the molar gas constant (8.314 J/(mol K));

T is the incubation temperature in kelvin.

The cumulative net mass of carbon (m_h) from the test compound produced as biogas in the headspace, corrected for the corresponding blank values, is calculated using the following equation:

$$m_h = \frac{12 \times 10^3 \times 0.1 (\Delta\rho \times V_h)}{RT}$$

where:

$\Delta\rho$ is the difference between the initial and final blank corrected pressures in millibars;

V_h is the volume in litres of the headspace in the vessel;

0.1 is the conversion factor for both newtons/m^2 and cubic meters to litres.

For a normal incubation temperature of 35°C (308k) this can be written more simply as:

$$m_h = 0.468 (\Delta\rho \times V_h)$$

The inorganic carbon that exists in solution (m_I) is calculated as follows:

$$m_I = ICnet \times V_I$$

where:

ICnet is the blank corrected level of inorganic carbon present in the test liquor;

V_I is the liquor volume of the test

The total amount of carbon formed as a result of biodegradation (m_t) is derived from:

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4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Degradation of TS in abiotic control	Not reported
4.1.2	Degradation	Biodegradation of cypermethrin cis:trans 40:60 had achieved a mean total level equivalent to 17% by the end of the test on Day 60. See table A7_1_2_1_2-4
4.1.3	Graph	See Figure A7_1_2_1_2-1
4.1.4	Other observations	The biodegradation of PEG400 in the presence of cypermethrin cis:trans 40:60 was had achieved 62% after 34 days, which indicated that the cypermethrin cis:trans 40:60 was not inhibitory to the activity of the inoculum at the test concentration. See table A7_1_2_1_2-5
4.1.5	Degradation of reference substance	The biodegradation of PEG400 had achieved 62% after 45 days of incubation. By the end of the test on Day 60, the mean level of total biodegradation for the test system was 78% of the theoretical level (2.2 mgC/culture). This confirmed that the inoculum was viable and that the test was valid (recommended level of biodegradation >60% within 60 days). See table A7_1_2_1_2-6
4.1.6	Intermediates/ degradation products	Degradation products not determined in this study.

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The anaerobic biodegradability of cypermethrin cis:trans 40:60 was assessed using recommendations of the British Standard (BS) method 6068 (1996) and International Organisation for Standardisation (ISO) method 11734 (1995). The test cultures contained cypermethrin cis:trans 40:60 (nominally, 20 mg as Carbon [C]/l), mineral salts medium (MSM) and pre-digested anaerobic sludge inoculum (solids content, 2.31 g/l), obtained six days before test initiation from a plant treating predominantly domestic waste water. The culture vessels were Wheaton vials (nominal capacity, 160 ml) with butyl rubber septa and aluminium crimp seals and contained a headspace volume equivalent to 40 ml. Blank and positive control cultures comprised inoculated MSM and MSM plus the reference substance, polyethylene glycol (PEG) 400 (nominally, 20 mg as Carbon [C]/l), respectively. The potential inhibition of the inoculum by cypermethrin cis:trans 40:60 at the test concentration was assessed in an inhibition assay which assessed biogas evolution from cultures containing the test and reference substances.

The cultures were prepared and handled during the test using bench-top, anaerobic gassing techniques and incubated inverted in darkness at 35 ± 2°C for 60 days.

At the start of the test, the pH of one replicate of the controls, test and inhibition assay series of cultures was determined and the culture discarded. Five replicates of each culture series were incubated and biogas evolution was determined at intervals during the test using a handheld pressure meter. After 60 days of incubation, the pH of each mixture was determined and the inorganic carbon (IC) content of the settled culture medium was measured to provide an estimate of total mineralisation of the test and reference substances.

5.2 Results and discussion

Biogas evolution in cultures containing PEG400 increased steadily during the incubation period and a mean level of biodegradation, based on biogas evolved, was equivalent to 62% of the theoretical level after 45 days of incubation. Biodegradation was equivalent to 78% by the end of the test on Day 60, based on the sum of net biogas and inorganic carbon evolution. These results confirm that the inoculum was viable and that the test precision was adequate. Validity criteria concerning the inorganic carbon concentration of the inoculated medium at the start of the test (3.63 mgC/l) and pH control in the test system (pH 7.0 on Days 0 and 60) were fulfilled (acceptable ranges, 10 mgIC/l on Day 0 and pH 7.0 ± 0.2 on Day 0 and 7.0 ± 1 on Day 60, respectively).

Biodegradation of cypermethrin cis:trans 40:60 had achieved a mean total level equivalent to 17% by the end of the test on Day 60.

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5.3	**Conclusion**	Biodegradation of cypermethrin cis:trans 40:60 had achieved a mean total level equivalent to 17% by the end of the test on Day 60. Substances are considered to be ultimately biodegradable in this test if the level of biodegradation achieves 60% of the theoretical level by the end of the test. Cypermethrin cis:trans 40:60 was not, therefore, considered ultimately biodegradable under these test conditions. The biodegradation of PEG400 in the presence of the test substance was monitored in order to assess whether any inhibitory effects were exerted on the activity of the inoculum. None were observed.
5.3.1	Reliability	2
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	April 2007
Materials and Methods	Applicant's version is acceptable.
Results and discussion	The strong deviation from replicates to replicates is not explained.
Conclusion	Applicant's version is adopted.
Reliability	2
Acceptability	acceptable
Remarks	The study was performed at 35 +/- 1 °C and the PEG used was polyethylene glycol (PEG) 400 (nominally, 20 mg as Carbon [C]/l).
	COMMENTS FROM ... (specify)
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_1_2-1: Inoculum / Test organism

Criteria	Details
Nature	Primary anaerobic digestor sludge
Species	Not determined
Strain	Not determined
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	Caister Sewage Treatment works, Norfolk, UK.
Laboratory culture	Yes.
Method of cultivation	All transfers of sludge in the laboratory were performed using 'bench-top' anaerobic gassing techniques with oxygen free nitrogen. Sub-samples from the laboratory scale digesters were removed and pooled for the analysis of the pre-digestion mixed liquor suspended solids (MLSS) concentration by filtration through pre-weighed then dried, glass-fibre (GF/C) filters. The filters were placed in an oven (*ca.* 105°C) and the dry weight determined. The laboratory scale digester vessels were re-weighed and placed in a water bath at 35 ± 2°C. Outlet tubes from the vessels were fitted to graduated glass cylinders (internal diameter, 25 mm) containing water and endogenous biogas evolution was monitored for six days.
Preparation of inoculum for exposure	At test initiation (Day 0), samples of sludge from two laboratory scale digesters was transferred into two centrifuge buckets. The sludge was centrifuged at *ca.* 3000 x *g* for five minutes, the supernatant was removed and the pellet re-suspended in mineral salts medium (MSM). This procedure was repeated three times, but on the third occasion, the supernatant was retained. A sample from each centrifuge bucket was pooled then analysed using an OI Model 700 organic carbon analyser to determine the inorganic carbon content. The anaerobic sludge in each centrifuge bucket was pooled and suspended in MSM to achieve the target MLSS concentration of 22 g /l, based on the pre-digestion concentration. The MLSS content of the pooled sample of sludge was determined and used to verify the concentration in the test system.
Initial cell concentration	Mixed liquor suspended solids was 22 g/L

Table A7_1_2_1_2-2: Test system

Criteria	Details
Culturing apparatus	Wheaton vials, nominal capacity 160ml
Number of replicates/concentration	6
Measuring equipment	The headspace pressure measurements on each culture were performed using a Watson-Smith Model 300 pressure meter.
Oxidation reduction indicator	Yes, the mineral salts medium contained Resazurin as a redox potential indicator

Table A7_1_2_1_2-3: Test conditions

Criteria	Details
Composition of medium	Anhydrous potassium dihydrogenphosphate = 0.27 g Disodium hydrogenphosphate diihydrate = 0.56 g Ammonium chloride = 0.53 g Calcium chloride dehydrate = 0.075 g Magnesium chloride hexahydrate = 0.1 g Iron (II) chloride = 0.02 g Resazurin redox potential indicator = 0.001 g Sodium sulphide nonahydrate = 0.1 g Trace element solution = 10 ml Deoxygenated ultrapure water to 1 litre pH 6.8 – 7.2 (pH adjusted with NaOH or HCl)
Additional substrate	No
Solvent	Acetone
Preparation of medium	The dilution water used to prepare components of the mineral salts medium was tap water that had been softened and treated by reverse osmosis (Elgastat, Prima 4 reverse osmosis unit, Vivendi Water Systems) and then purified (Elgastat UHP unit, Vivendi Water Systems) nominal resistivity, ≥ 18 MegOhm.cm. The water was autoclaved and cooled using a gentle stream of nitrogen when used in the preparation of deoxygenated mixtures.
Test temperature	Maintained at 35 ± 2°C in a water bath. The incubation temperature during the test ranged from 34.3-36.4 °C.
pH	At day 0 (test initiation), the pH of one of the replicates from each group was determined and the mixture discarded. No adjustment was required. The pH of each culture was determined at the end of the study (day 60). pH measurements were 7 ± 0.2 on day 0 and pH 7 ± 1 on day 60.
Suspended solids concentration	2.31 g/L
Other relevant citeria	The cultures were prepared and handled during the test using bench-top, anaerobic gassing techniques and incubated inverted in darkness at 35 ± 2°C for 60 days

Figure A7_1_2_1_2-1: Biodegradation of the test and reference substances

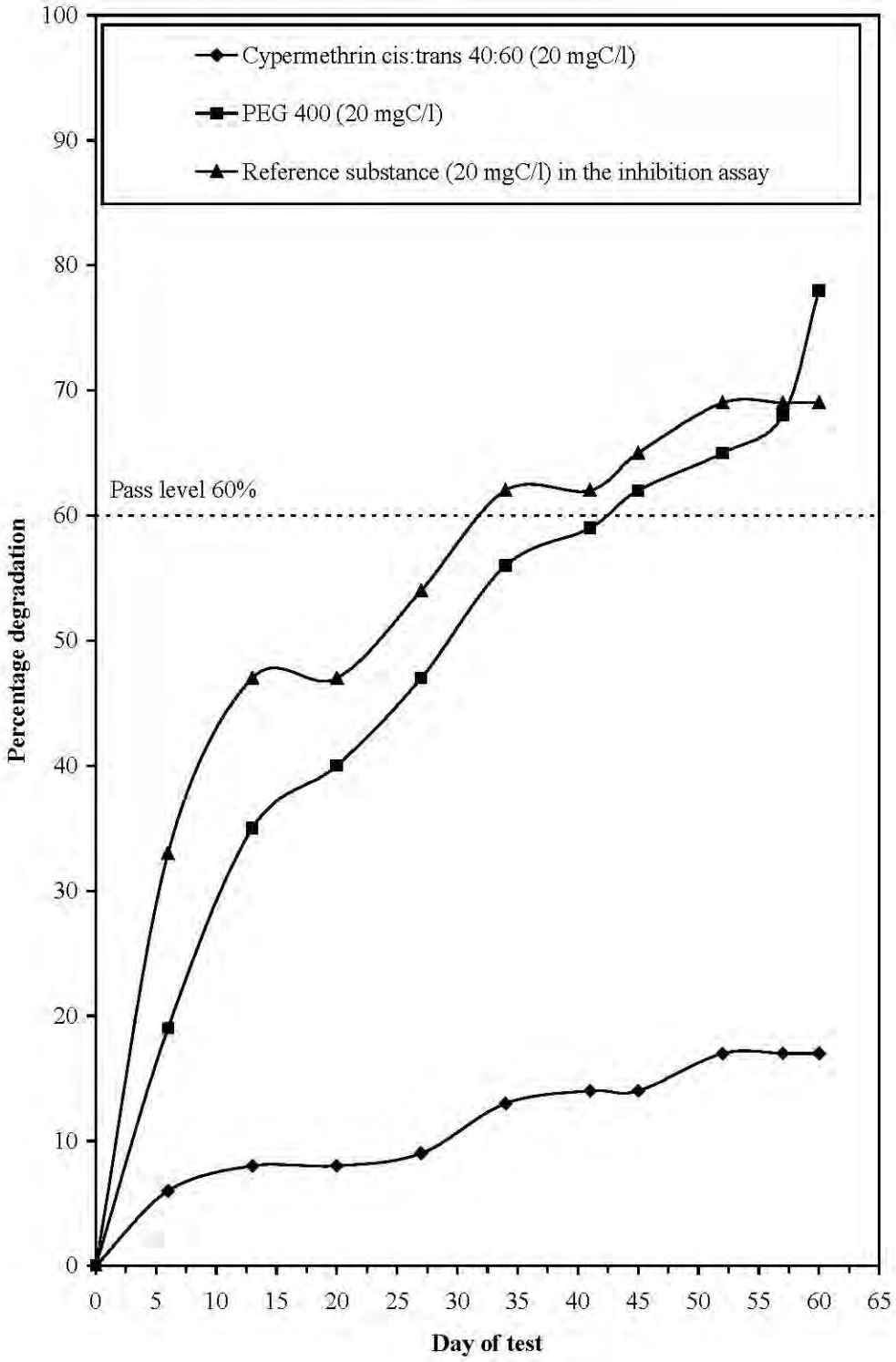

Table A7_1_2_1_2-4: Cypermethrin *cis:trans* 40:60 - Cumulative biogas production and percentage biodegradation

Day	Replicate 1			Replicate 3			Replicate 4			Replicate 5			Replicate 6			Mean %Th
	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	
6	0.001	0.026	1	0.034	0.644	29	0.000	0.000	0	0.000	0.000	0	0.000	0.000	0	6
13	0.001	0.026	1	0.034	0.644	29	0.000	0.007	0	0.000	0.000	0	0.010	0.195	9	8
20	0.001	0.026	1	0.034	0.644	29	0.000	0.007	0	0.000	0.000	0	0.010	0.195	9	8
27	0.001	0.026	1	0.034	0.644	29	0.000	0.007	0	0.000	0.000	0	0.014	0.262	12	9
34	0.001	0.026	1	0.034	0.644	29	0.012	0.217	10	0.000	0.000	0	0.031	0.584	27	13
41	0.004	0.082	4	0.034	0.644	29	0.012	0.217	10	0.000	0.000	0	0.031	0.584	27	14
45	0.004	0.082	4	0.034	0.771	35	0.012	0.217	10	0.000	0.000	0	0.031	0.584	27	14
52	0.005	0.097	4	0.041	0.771	35	0.020	0.382	17	0.000	0.000	0	0.033	0.618	28	17
57	0.006	0.109	5	0.041	0.771	35	0.020	0.382	17	0.000	0.000	0	0.033	0.618	28	17
60	0.006	0.109	5	0.041	0.771	35	0.020	0.382	17	0.000	0.000	0	0.033	0.618	28	17

$\Sigma\Delta p$ – net cumulative biogas pressure (bar)
m_h - cumulative mass of carbon in the biogas (mgC)
%Th - biodegradation expressed as a percentage of the theoretical carbon content of the substance in the culture (mgC)

Table A7_1_2_1_2-5: Cumulative biogas production and percentage biodegradation in the inhibition assay

Day	Replicate 1			Replicate 2			Replicate 3			Replicate 5			Replicate 6			Mean %Th
	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	
6	0.049	0.922	42	0.029	0.547	25	0.038	0.716	33	0.000	0.000	0	0.075	1.408	64	33
13	0.055	1.039	47	0.051	0.964	44	0.053	1.001	46	0.019	0.360	16	0.096	1.806	82	47
20	0.055	1.039	47	0.051	0.964	44	0.053	1.001	46	0.020	0.379	17	0.096	1.806	82	47
27	0.066	1.242	56	0.051	0.964	44	0.054	1.018	46	0.035	0.657	30	0.109	2.047	93	54
34	0.073	1.364	62	0.051	0.964	44	0.069	1.289	59	0.047	0.873	40	0.123	2.300	105	62
41	0.073	1.364	62	0.051	0.964	44	0.069	1.289	59	0.047	0.873	40	0.123	2.300	105	62
45	0.080	1.495	68	0.051	0.964	44	0.079	1.473	67	0.047	0.873	40	0.124	2.319	105	65
52	0.080	1.495	68	0.051	0.964	44	0.089	1.667	76	0.056	1.045	47	0.130	2.434	111	69
57	0.080	1.495	68	0.051	0.964	44	0.089	1.667	76	0.056	1.045	47	0.130	2.434	111	69
60	0.080	1.495	68	0.051	0.964	44	0.089	1.667	76	0.056	1.045	47	0.130	2.434	111	69

$\Sigma\Delta p$ – net cumulative biogas pressure (bar)
m_h - cumulative mass of carbon in the biogas (mgC)
%Th - biodegradation expressed as a percentage of the theoretical carbon content of the substance in the culture (mgC)

Table A7_1_2_1_2-6 PEG 400 - Cumulative biogas production by cultures and percentage biodegradation

Day	Replicate 1			Replicate 2			Replicate 3			Replicate 5			Replicate 6			Mean %Th
	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	$\Sigma\Delta p$	m_h	%Th	
6	0.018	0.344	16	0.041	0.775	35	0.031	0.588	27	0.009	0.176	8	0.013	0.251	11	19
13	0.020	0.371	17	0.047	0.876	40	0.072	1.344	61	0.029	0.539	25	0.037	0.689	31	35
20	0.028	0.520	24	0.055	1.026	47	0.072	1.344	61	0.038	0.708	32	0.043	0.801	36	40
27	0.051	0.962	44	0.055	1.026	47	0.088	1.655	75	0.038	0.708	32	0.044	0.831	38	47
34	0.067	1.247	57	0.056	1.048	48	0.104	1.939	88	0.043	0.805	37	0.058	1.078	49	56
41	0.076	1.415	64	0.061	1.142	52	0.107	1.996	91	0.043	0.805	37	0.059	1.097	50	59
45	0.076	1.415	64	0.064	1.198	54	0.114	2.127	97	0.053	0.992	45	0.059	1.097	50	62
52	0.076	1.415	64	0.064	1.198	54	0.114	2.127	97	0.056	1.045	47	0.075	1.411	64	65
57	0.082	1.539	70	0.068	1.265	58	0.114	2.127	97	0.057	1.075	49	0.077	1.441	66	68
60	0.086	1.614	73	0.068	1.265	58	0.114	2.127	97	0.057	1.075	49	0.077	1.441	66	68

$\Sigma\Delta p$ – net cumulative biogas pressure (bar)
m_h - cumulative mass of carbon in the biogas (mgC)
%Th - biodegradation expressed as a percentage of the theoretical carbon content of the substance in the culture (mgC)

Section A7.1.2.2.2 Water/sediment degradation study

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Official use only

		1 REFERENCE
1.1	**Reference**	Brice A, Cooke C (2005); [^{14}C]-Cypermethrin cis:trans 40:60: Degradation and retention in water-sediment systems. Covance Laboratories Ltd, Report No. 1669/014-D2149, 23 March 2006 (unpublished). Dates of work: 14 February 2005 – 22 September 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 308 (April 2002)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Radiolabelled Cypermethrin (two isomers per label) supplied by BlyChem Ltd : [^{14}C-cyclopropyl]cypermethrin-cis, [^{14}C-phenyl]cypermethrin-cis, [^{14}C-cyclopropyl]cypermethrin-trans and [^{14}C-phenyl]cypermethrin-trans.
3.1.1	Lot/Batch number and radiochemical purity	See Table A7_1_2_2_2-1
3.2	**Degradation products**	The identity of metabolites were confirmed by TLC using authentic reference standards
3.3	**Reference substance**	No
3.4	**Sediment Properties**	See Table A7_1_2_2_2-2
3.5	**Water properties**	See Table A7_1_2_2_2-3
3.6	**Testing procedure**	
3.6.1	Test system	The rate of degradation of cypermethrin was studied in two water-sediment systems at 20 ± 2°C over a period of 100 days. 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. There were four incubation groups. 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

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		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. See Table A7_1_2_2_2-4
3.6.2	Test solution and Test conditions	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. Acetonitrile (92 µL for incubation groups A and B, 90 µL for incubation groups C and D) was added to six water-sediment units per incubation group at the same time as treatment with the radiolabelled cypermethrin. These units were to provide samples for determination of microbial biomass at day 0 and the end of the study.
3.7	**Sampling**	
3.7.1	Sampling timing	Microbial biomass of the sediments was determined at day 0 and at the end of the incubation, using units dosed with acetonitrile only. Water-sediment samples were taken for analysis of cypermethrin and radiolabelled degradation products at zero-time and 1, 3, 10, 29, 45 (incubation groups A and B only), 58 (incubation groups C and D only), and 100 days after application. Traps attached to units for the later timepoints were additionally quantified for radioactivity at 29, 45, 58 and 75 days, where applicable. The traps were replenished with fresh reagents before being returned to the incubation system.
3.7.2	Water extraction	The water was separated from the sediment by aspiration and the two phases were separately analysed. Following the addition of concentrated hydrochloric acid (1.5 mL), the water samples were partitioned three times with dichloromethane to give aqueous and organic phases. The organic phases were reduced to dryness by rotary evaporation and then under nitrogen. The samples were reconstituted in acetonitrile for chromatography.
3.7.3	Sediment extraction	Sediment samples were shaken four times with acetonitrile (90 mL) and acidified with acetonitrile:water (1:1 v/v, 100 mL). The extracts were combined, filtered, reduced to dryness and were reconstituted in acetonitrile for chromatography. The extract was concentrated to ca 1 mL under a stream of nitrogen and sonicated to aid reconstitution. Following clarification by centrifugation (850 g, 2000 rpm for 10 minutes), the extract was quantified by LSC.
3.7.4	Bound residues	Bound residue fractionation. The residues resulting from the acetonitrile:water extraction were base extracted to further separate them into fulvic acid, humic acid and humin fractions. Each sample was shaken with sodium hydroxide solution (0.5 M, 100 mL, 24 hours) and the residue (humin fraction) and supernatant were