Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

4.2.2	Actual concentrations of test substance	See table A7_4_1_3-7
4.2.3	Growth curves	See fig. A7_4_1_3-1
4.2.4	Concentration / response curve	Not applicable
4.2.5	Cell concentration data	See table A7_4_1_3-8
4.2.6	Effect data (cell multiplication inhibition)	As the geometric mean concentration of cypermethrin cis:trans 40:60 was below 80% of the nominal exposure concentration, the toxicity (E_bC_{50} and E_rC_{50}) values and no observed effect concentrations of cypermethrin cis:trans 40:60 to P. subcapitata were based on the geometric mean measured concentration. Based on the geometric mean measured concentration of cypermethrin cis:trans 40:60, the 24, 48, 72 and 96-hour E_rC_{50} and the E_bC_{50} toxicity values could not be calculated as no significant inhibition of algal cell growth occurred during the definitive test in either of the test parameters, (the area under the growth curves (A) or the average specific growth rates (μ)) relative to the control. Therefore, the 24, 48, 72 and 96-hour E_rC_{50} and E_bC_{50} toxicity values are considered to be >33.0 μg/L, the geometric mean measured concentration. The corresponding NOEC values were observed to be ≥ 33.0 μg/L. See table A7_4_1_3-9.
4.2.7	Other observed effects	None reported
4.3	**Results of controls**	See table A7_4_1_3-8
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The objective of this study was to determine the effects of cypermethrin cis:trans 40:60 on the growth of the green alga, Pseudokirchneriella subcapitata (formerly known as Selenastrum capricornutum). A range-finding test was conducted at 0.01, 0.1, 1.0, 10 and 100 μg/L nominal concentrations of cypermethrin cis:trans 40:60, no significant effects on growth were detected. Therefore, a definitive limit test was conducted at a nominal concentration of 100 μg/L, with an appropriate control and solvent control group. A stock medium at a nominal cypermethrin cis:trans 40:60 concentration of 1000 μg/L was prepared by rinsing 1000 μg into a 100-mL volumetric flask and making to volume with acetone. The nominal 100 μg/L test medium was prepared from the 1000 μg/L stock medium. A control treatment of algal nutrient medium only was also prepared. A solvent control treatment was prepared by adding 10 μL of acetone to a

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

		100-mL test vessel, filled to 100-mL with algal nutrient media (max. solvent loading 0.1 mL/L).
5.2	**Results and discussion**	The overall geometric mean measured concentration of cypermethrin cis:trans 40:60 in the 100 µg/L test media inoculated with algal cells was 33.0 µg/L, corresponding to 33.0% of the nominal concentration. As the geometric mean concentration was below 80% of the nominal exposure concentration, the E_rC_{50} (average specific growth rates) and E_bC_{50} (area under the growth curves) toxicity values and corresponding no observed effect concentrations (NOEC) of cypermethrin cis:trans 40:60 to P. subcapitata were based on the geometric mean measured concentration. Based on the geometric mean measured concentration of cypermethrin cis:trans 40:60, the 24, 48, 72 and 96-hour E_bC_{50} and E_rC_{50} toxicity values could not be calculated as no significant inhibition of algal cell growth occurred at 100 µg/L (relative to the control) during the definitive test. Therefore, the 24, 48, 72 and 96-hour E_rC_{50} and E_bC_{50} toxicity values are considered to be >33.0 µg/L, the geometric mean measured concentration. The corresponding NOEC values were observed to be ≥ 33.0 µg/L.
5.2.1	NOE_rC	≥ 33.0 µg/L
5.2.2	E_{r50}	>33.0 µg/L
5.2.3	E_bC_{50}	>33.0 µg/L
5.3	**Conclusion**	The validity criterion of a logarithmic increase in cell concentrations over the duration of the test (96 hours) was achieved
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	November 2007
Materials and Methods	Applicant's version is acceptable
Results and discussion	Applicant's version is adopted
Conclusion	Applicant's version is adopted
Reliability	1
Acceptability	Acceptable
Remarks	This is a limited test. NOEC ErC50 and EbC50 were estimated at a value far above the solubility value of the active substance.

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

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

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes, Acetone
Concentration of vehicle	A 1000 µg/L stock solution was prepared by direct addition of 1000µg cypermethrin into a 100 ml glass weighing vessel and making up to volume with acetone. This was then used to make up the nominal 100 µg/L test medium in the main study.
Vehicle control performed	Yes. A solvent control medium was prepared by adding 10 µL of acetone to a 100 mL test vessel filled to volume with algal nutrient medium (maximum solvent loading 0.1 mL/L).
Other procedures	At the start and end of the test, the behaviour of the test substance in the test system was observed and recorded (e.g. whether it dissolved, formed a surface film or adhered to the walls of the vessel).

Table A7_4_1_3-2: Preparation of algal medium

Nutrient	Concentration (mg/L)
NH_4Cl	15
$MgCl_2.6H_2O$	12
$CaCl_2.2H_2O$	18
$MgSO_4.7H_2O$	15
KH_2PO_4	1.6
H_3BO_3	0.185
$MnCl_2.4H_2O$	0.415
$FeCl_3.6H_2O$	0.08
$Na_2EDTA.2H_2O$	0.1
$NaHCO_3$	50
$ZnCl_2$	0.003
$CoCl_2.6H_2O$	0.0015
$Na_2MoO_4.2H_2O$	0.007
$CuCl_2.2H_2O$	1×10^{-5}

Table A7_4_1_3-3: Test organisms

Criteria	Details
Species	*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)
Strain	Obtained from an anexic strain of *P.subcapitata* (CCAP 278/4)
Source	Culture Collection of Algae and Protozoa (CCAP), SAMS Research Services Ltd., Oban, UK.
Laboratory culture	Yes (liquid slope)
Method of cultivation	Not specified. On receipt the slope was maintained under refridgeration in the dark.
Pre-treatment	Prior to testing, an aliquot of the slope culture was added to algal nutrient medium and incubated for at least 72 hours at 23 ±2°C to ensure growth was in the exponential phase.
Initial cell concentration	The quantity of algal culture inoculum added to each of the test vessels was sufficient to give an algal cell concentration of 1 x 10^4 cells/mL. The algal cell density in the stock cultures was measured using a Z2 Coulter Counter and confirmed using a haemocytometer. The required inoculum volume was then calculated. All flasks for a particular test were inoculated from the same stock culture

Table A7_4_1_3-4: Test system

Criteria	Details
Volume of culture flasks	250 mL
Culturing apparatus	Test vessels used in the definitive study were 250 mL Erlenmeyer conical flasks.
Light quality	Constant illumination (range = 9250 to 9730 over the duration of the study).
Procedure for suspending algae	The flasks were loose capped and incubated in cooled orbital incubators (103 cycles/min)
Number of vessels/ concentration	6 vessels for each series (control, solvent control and 100 µ ug/L test concentration) Additional test vessels containing algal control media/solvent control media with and without algae were incubated alongside the test vessels and pooled to achieve sufficient test media volume to perform the chemical analysis.
Test performed in closed vessels due to significant volatility of TS	No, flasks were loose capped only

Table A7_4_1_3-5: Test conditions

Criteria	Details
Test temperature	23.0-23.3 (mean = 23.0)
pH	See table A7_4_1_3-6
Aeration of dilution water	No
Light intensity	9250-9730 (lux)
Photoperiod	Constant illumination for duration of test (96 hours)

Table A7_4_1_3-6: Measured pH values

Nominal cypermethrin *cis:trans* 40:60 conc. (µg/L)	Measured pH values.				
	0-hour	96-hours (with algal cells)			96-hours (without algal cells)
Solvent control	7.4	10.2	10.3	10.2	8.0
		10.4	10.3	10.3	
Control	7.4	10.2	10.3	10.3	7.8
		10.3	10.3	10.3	
100	7.5	10.3	10.3	10.3	8.0
		10.2	10.3	10.3	

* Recorded using a digital temperature logger

Table A7_4_1_3-7: Measured test substance concentrations

Nominal cypermethrin *cis:trans* 40:60 conc. (µg/L)	Measured cypermethrin *cis:trans* 40:60 concentration (µg/L)			*Mean measured concentration (mg/L)	Mean as % of nominal
	0-hour	96-hour (with algae)	96-hour (without algae)		
Solvent control	-	-	0.0904	N/A	N/A
Control	-	-	-	N/A	N/A
100	94.6	11.5	21.3	33.0	33.0

* Mean value determined as the geometric mean of the 0 and 96-hour (with algae) measured concentrations (Geometric mean calculated as: 'Exp ((ln(94.6)+ln(11.5))/2)'. Measured values without algae are not included in the calculation).

N/A Not applicable

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

Figure A7_4_1_3-1: Growth curve

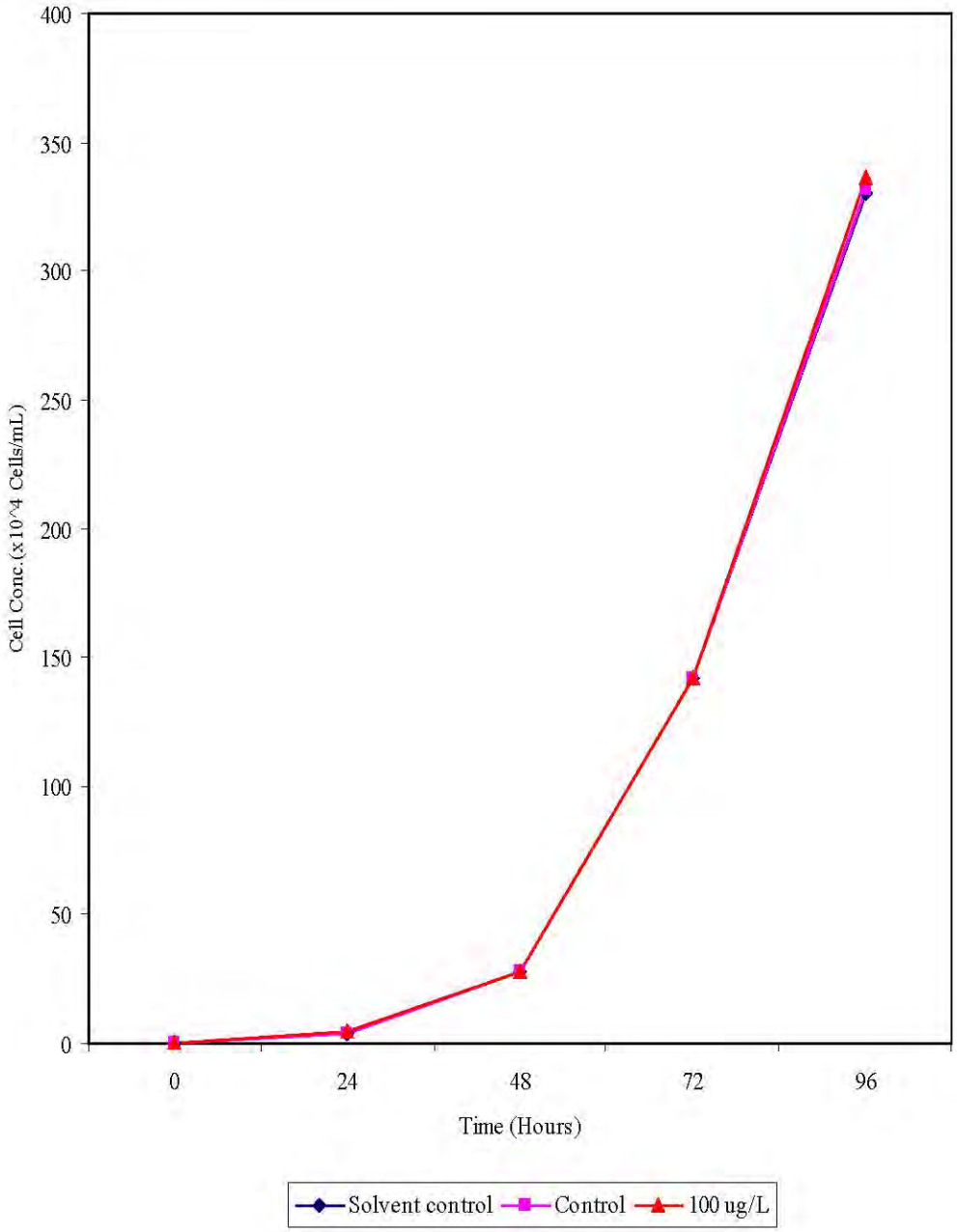


Table A7_4_1_3-8: Cell concentration data

Nominal cypermethrin *cis:trans* 40:60 conc. (µg/L)	Cell concentration (cells x 10^4/mL)				Mean cell concentration (cells x 10^4/mL)			
	24-h	48-h	72-h	96-h	24-h	48-h	72-h	96-h
Solvent control	3.86 4.18 4.28 4.61 3.87 3.73	27.4 28.4 27.8 30.5 27.4 26.5	140 147 138 156 140 129	331 363 334 371 320 259	4.09	28.0	142	330
Control	4.06 4.14 3.72 4.26 3.33 4.02	27.0 29.4 25.1 29.1 26.8 28.0	136 155 133 148 133 147	307 381 299 367 290 350	3.92	27.6	142	332
100	4.17 3.59 4.29 4.70 4.11 4.12	29.7 26.0 27.8 30.8 26.7 27.2	144 133 139 158 133 142	337 278 344 404 309 341	4.16	28.0	142	336

Nominal cypermethrin *cis:trans* 40:60 conc. (µg/L)	Mean area under growth curve (A x 10^6)				Average specific growth rate (µ x 10^{-2})			
	0 - 24 h	0 - 48 h	0 - 72 h	0-96 h	0 - 24 h	0 - 48 h	0-72 h	0-96 h
Solvent control	0.370	3.98	24.1	80.4	5.85	6.94	6.88	6.03
Control	0.351	3.89	24.0	80.7	5.68	6.91	6.88	6.04
100	0.380	4.00	24.1	81.1	5.93	6.94	6.88	6.05

Table A7_4_1_3-9: Inhibition (%) of growth

Nominal cypermethrin *cis:trans* 40:60 conc. (µg/L)	Reduction in average area under the growth curve relative to the control (%)				Reduction in average specific growth rates relative to the control (%)			
	0 - 24 h	0 - 48 h	0 - 72h	0 - 96h	0 - 24 h	0 - 48 h	0 - 72h	0 - 96h
Solvent control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Control	5	2	0	$^{\$}$0	3	0	$^{\$}$0	$^{\$}$0
100	$^{\$}$0	$^{\$}$0	$^{\$}$0	$^{\$}$0	$^{\$}$0	$^{\$}$0	0	$^{\$}$0

$^{\$}$ Negative values for reduction in algal growth relative to the solvent control are presented as zero reduction.

3. Tables for Applicant's Summary and Conclusion

3.1 Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**Yes**	
Concentration of test substance ≥80% of initial concentration during test		**Results based on geometric mean measured concentration**

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4 **Activated Sludge Respiration Inhibition**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Bealing, D. (2002); Cypermethrin – Determination of inhibition of respiration of activated sludge; Covance Laboratories Ltd., report no. 40/46 (CYP/T323), 18 June 2002 (unpublished) Dates of work: 25 March 2002 – 4 April 2002	
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 209 EC Commission Directive 87/302/EEC; Part C	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	x
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	a.s. batch number 2001060167 (test formulation AL 1604/8)	
3.1.2	Specification	Specification of a.s. as given in section 2	
3.1.3	Purity	chemical purity of a.s.: 96.5%	
3.1.4	Composition of Product	Study conducted on the a.s. in a surfactant due to low water solubility (see 3.2)	
3.1.5	Further relevant properties	Due to the low water solubility and immobility at room temperature a formulation containing 50% cypermethrin 40/60 in emulsifier surfactant was used in the study (see 3.2)	
3.1.6	Method of analysis	At the end of the 3 hour incubation, a portion of the first test mixture was transferred to fill a clean 250 mL (nominal volume) sample bottle containing a PTFE-coated stirrer bar. The DO probe was inserted in the sample bottle, taking care to avoid trapping air bubbles against the probe membrane. The probe was sealed against the bottle neck to ensure that the sample could not become re-oxygenated by contact with the atmosphere. The bottle was centred on a stirrer drive unit, the magnetic follower set spinning and the chart set to run. At intervals of 15 ± 1 minutes, the procedure was repeated for each subsequent sample following the sequence in which the incubations began. A fresh sample bottle was used for each test mixture and the probe was rinsed with reverse-osmosis water between samples. The stirrer speed was kept the same for all measurements to ensure that the DO meter response was not influenced artificially.	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4 Activated Sludge Respiration Inhibition

		Respiration rates were subsequently derived from the longest linear portion of each valid trace.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_4-1. Due to the low water solubility and immobility at room temperature a formulation containing 50% cypermethrin 40/60 in emulsifier surfactant was used in the study to ensure adequate aqueous dispersion of the a.s. required for uniform contact with the activated sludge flocs. The study design was extended to include formulation blanks (treatments containing the emulsifier alone) to identify a possible contribution made by the emulsifier to inhibition caused by the formulation.
3.3	**Reference substance**	Yes, dichlorophenol was used a positive control.
3.3.1	Method of analysis for reference substance	See section 3.1.6 above
3.4	**Testing procedure**	
3.4.1	Culture medium	100-fold OECD synthetic sewage concentrate composed of the following dissolved in reverse-osmosis water and made up to 2 litres (all weights ± 0.005 g): Bacteriological peptone (Oxoid), 32.00 g; 'Lab-Lemco' powder meat extract (Oxoid), 22.00 g; urea (BDH, AnalaR), 6.00 g; sodium chloride (BDH, AnalaR), 1.40 g; calcium chloride dihydrate (BDH, AnalaR), 0.80 g; magnesium sulphate heptahydrate (BDH, AnalaR), 0.40 g and potassium hydrogen phosphate (BDH, AnalaR), 5.60 g. A total of four litres of synthetic sewage concentrate was prepared, to provide sufficient concentrate for all maintenance feeding and testing purposes.
3.4.2	Inoculum / test organism	See table A7_4_1_4-2
3.4.3	Test system	The applied a.s. concentrations used were 50, 100, 150, 250 and 500 mg a.s./l. See table A7_4_1_4-3 for test conditions. All mixtures comprised 16 mL synthetic sewage diluted to 300 mL with either reverse-osmosis water alone (controls, test substance treatments and formulation blanks), or with reverse-osmosis water and the 3,5-DCP stock solution. The inocula used in this study had not been deliberately acclimatised or adapted to cypermethrin or the emulsifier before exposure under test conditions. The inocula were maintained by aeration at all times and were fed overnight with synthetic sewage at a rate of 50 mL/L during the intervals between preparation and final use.
3.4.4	Test conditions	See table A7_4_1_4-4. The inocula were maintained, the test mixtures incubated and the test measurements carried out in a facility where the temperature was set at a nominal 21°C. The pH and suspended solids content of the activated sludge were measured before use.
3.4.5	Duration of the test	3 hours
3.4.6	Test parameter	Respiration inhibition
3.4.7	Analytical parameter	Dissolved oxygen measurement
3.4.8	Sampling	DO measured after 3 hours incubation

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**

Annex Point IIA7.4 **Activated Sludge Respiration Inhibition**

3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	Formulation blanks, i.e. treatments containing the emulsifier, but without the a.i., were included to identify and, if necessary, correct for any inhibition caused by the emulsifier. A reference inhibitor, 3,5-dichlorophenol, was run in un-replicated preparations at concentrations of 5, 15 and 45 mg/L in both the range-finder and definitive tests. Four controls were also set-up: two at the start and two at the end of the test series, again for both the range-finder and definitive tests.
3.4.11	Statistics	A probit analysis program was run, using log-transformed concentration data, to generate EC_{20}, EC_{50} and EC_{80} values and the corresponding 95% confidence limits (CL) for cypermethrin.
		4 RESULTS
4.1	**Preliminary test**	Performed
4.1.1	Concentration	EC_{50} for cypermethrin estimated at 110 mg/L
4.1.2	Effect data	Significant inhibition seen at 10, 100 and 1000mg a.s./L. No significant inhibition seen in formulation blanks.
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	50, 100, 150, 250 and 500 mg a.s./L
4.2.2	Actual concentrations of test substance	Not reported
4.2.3	Growth curves	Not reported
4.2.4	Cell concentration data	Not reported
4.2.5	Concentration/ response curve	Not reported
4.2.6	Effect data	E_{50} = 163 mg/L (95 % c.l.: 118-230 mg/L) EC_{20} = 16 mg/L (95 % c.l.: 4-33 mg/L) EC_{80} = 1634 mg/L (95 % c.l.: 807-7906 mg/L) See able A7_4_1_4-5 Both the EC_{20} and EC_{80} estimates were extrapolated, beyond the lower and upper limits respectively of the definitive concentration range.
4.2.7	Other observed effects	None reported
4.3	**Results of controls**	The formulation blanks showed no significant inhibition when dosed at 482 mg Ethylan C12 AH/L, corresponding to the top formulation dose, confirming that inhibition was caused by cypermethrin alone.
4.4	**Test with reference**	Performed

Section A7.4.1.4	Inhibition to microbial activity (aquatic)
Annex Point IIA7.4	Activated Sludge Respiration Inhibition

	substance	
4.4.1	Concentrations	A reference inhibitor, 3,5-dichlorophenol, was run in un-replicated preparations at concentrations of 5, 15 and 45 mg/L
4.4.2	Results	See Table A7_4_1_4-5
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Samples of activated sludge (from a predominantly domestic sewage plant source) were exposed to various nominal concentrations of cypermethrin (purity 96.5%) as a 50% a.i. formulation, ranging from 1.0 to 1000 mg a.i./L in a range-finding experiment and from 50 to 500 mg a.i./L in a definitive test. Their respiration rates were measured after 3 hours contact time. The study comprised a series of test mixtures containing a fixed quantity of synthetic sewage to provide a uniform respiration substrate. The physical properties of cypermethrin hindered the formation of homogeneous aqueous dispersions required for uniform contact with the activated sludge flocs. The study was therefore conducted with a formulation comprising technical grade cypermethrin uniformly mixed with an emulsifier surfactant to obtain good dispersions.
5.2	**Results and discussion**	The formulation blanks showed no significant inhibition when dosed at 482 mg Ethylan C12 AH/L, corresponding to the top formulation dose, confirming that inhibition was caused by cypermethrin alone
5.2.1	EC_{20}	16 mg/L (95% CL: 4 to 33 mg/L)
5.2.2	EC_{50}	163 mg/L (95% CL: 118 to 230 mg/L)
5.2.3	EC_{80}	1634 mg/L (95% CL: 801 to 7906 mg/L)
5.3	**Conclusion**	No significant respiration inhibition occurred in formulation blanks that contained the emulsifier at the highest formulation concentration tested. The effective concentration of cypermethrin that caused a 50% reduction in respiration rate relative to untreated controls (EC_{50}), was 163 mg cypermethrin/L (95% confidence limits: 118 to 230 mg/L). Control respiration rates used to obtain the mean were within 15% of each other and the EC50 of the reference was between 5 and 30 mg/L showing that the study can be considered valid.
5.3.1	Reliability	1
5.3.2	Deficiencies	No Study was 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	November 2007
Materials and Methods	Deviation:

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4 **Activated Sludge Respiration Inhibition**

	Range-finder formulation blanks The doses of the Ethylan C12AH emulsifier applied to the formulation blanks of the range-finder were inadvertently not corrected for the emulsifier content of the formulated test substance. The concentrations applied were consequently 2.0, 20, 200 and 2000 mg/L in place of the intended doses of 0.96, 9.6, 96.4 and 964 mg/L. However, the emulsifier caused no significant inhibition, even at these unintentionally elevated doses, and neither the outcome nor the scientific integrity of the study were affected by this error.
Results and discussion	4.3 Respiration rate(mg O_2/L.h) Mean Control 72.0 75.1 72.7 79.0 76.8 Emulsifyer 77.9 80.1 82.3 Control respiration rates used to obtain the mean were within 15% of each other and the EC50 of the reference was between 5 and 30 mg/L showing that the study can be considered valid.
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_4_1_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Vehicle	Emulsifier surfactant (Ethylan C12AH)
Concentration of vehicle	48.2% w/w
Vehicle control performed	Yes, formulation blanks (treatments containing the emulsifier alone) to identify a possible contribution made by the emulsifier to inhibition caused by the formulation

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Not specified
Strain	Not specified
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	Burley Menston Sewage Treatment Works (Yorkshire Water, UK)
Laboratory culture	No, sample taken directly from sludge return lines.
Method of cultivation	Not specified
Preparation of inoculum for exposure	Sample aerated using a compressed air supply and fed overnight with synthetic sewage at 50ml/L.
Pre-treatment	The inocula used in this study were not deliberately acclimatised or adapted to the test substance or the emulsifier before exposure.
Initial cell concentration	5.66 g/L suspended solids

Table A7_4_1_4-3: Test system

Criteria	Details
Number of culture flasks/concentration	2 (definitive test)
Aeration device	Compressed air delivered through a Pasteur pipette
Measuring equipment	Dissolved Oxygen (DO) meter and probe
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

Criteria	Details
Test temperature	Nominally 21°C
pH	pH of the inoculum was 6.36 immediately prior to use. pH after 3 hours ranged from 7.66-8.18 in the test vessels.
Aeration of dilution water	Not specified, dechlorinated mains water was used for dilution of the inoculum.
Suspended solids concentration	Initially 5.66 g/L. The suspended solids content was re-determined within 24 hours of starting the definitive test and determined to be 4.46 g/L. The inoculum was diluted further to give a suspended solids content of 4±10% g/L.

Table A7_4_1_4-5: Inhibition results for cypermethrin and 3,5-DCP

	Cypermethrin	3,5-dichlorophenol
EC_{20}:	16 mg/L (95% CL: 4 to 33 mg/L)	5 mg/L
EC_{50}:	163 mg/L (95% CL: 118 to 230 mg/L)	9 mg/L
EC_{80}:	1634 mg/L (95% CL: 801 to 7906 mg/L)	19 mg/L

Section 7.4.2 **Annex Point IIA.VII.7.5**	**Bioconcentration**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [√]	**Technically not feasible** [ ] **Scientifically unjustified** [√]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	Based on the physico-chemical properties, cypermethrin can be considered non-polar. Log Kow values ranged from 5.3-5.6 which would initially indicate a high potential for bioaccumulation. However an experimental BCF of 373+/-45 in fish showed that cypermethrin actually has a low bioaccumulation potential (see DocIIIA7.4.3.3.1). The substance has a high Koc value which ranges from 80653 to 574360 (see DocIIIA7.2.3.1) and can therefore be considered non-mobile in nature. Cypermethrin would therefore adhere strongly to soil/sediment making it very difficult for organisms to uptake and accumulate it. In summary, the risk of secondary poisoning through the food chain from the aquatic environment is unlikely to be a problem, as the bioavailability of cypermethrin will be poor due to its rapid dissipation from water and strong adhesion to sediment. It can be concluded therefore that there is no concern for bioaccumulation in the aquatic compartment.	
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	November 2007	
Evaluation of applicant's justification	Applicant justification is acceptable	
Conclusion	Applicant justification is acceptable	
Remarks	The TGD provide a method to calculate the BCF based on the Log Kow. The RMS will add the calculation in his report. However, the experimental BCF will be used for the risk assessment.	
	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 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	Knight, B., Murphy, C.M. (2005); Cypermethrin cis:trans/40:60 Fathead Minnow, Early Life Stage test; Charles River Laboratories, study no. 805972, 3 February 2006 (unpublished) Dates of experimental work: 29 July 2005 – 18 December 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 210 (July 1992) OPPTS 850.1400	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3 METHOD	
3.1	**Test material**	As given in section 2 (non-radiolabelled cypermethrin) [^{14}C-cyclopropyl]-Cypermethrin-*cis*, specific activity 127.31 µCi/mg [^{14}C-cyclopropyl]-Cypermethrin-*trans*, specific activity 127.23 µCi/mg	
3.1.1	Lot/Batch number	25163S63 (non-radiolabelled cypermethrin)	
3.1.2	Specification	As given in section 2 (non-radiolabelled cypermethrin)	
3.1.3	Purity	93.05% w/w (non-radiolabelled cypermethrin)	
3.1.4	Composition of Product	Not applicable, test carried out on active substance	
3.1.5	Further relevant properties	Low water solubility, therefore test was a continuous flow design.	
3.1.6	Method of analysis	Determination of radioactivity in water samples determined by LSC.	

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A stock solution of [^{14}C]-Cypermethrin was prepared in the ratio of 40:60 cis:trans in hexane. This stock solution was maintained at *ca* -20°C and was used to prepare dose stock solutions throughout the study. To ensure an adequate supply of test item for the duration of the study, test concentrations of 1 and 0.32 µg/L were radio-diluted using the supplied non-radiolabelled technical Cypermethrin. The dose stock solutions of [^{14}C]-Cypermethrin were prepared for each test concentration by adding an aliquot (200 µL for test concentrations of 1, 0.32 and 0.1 µg/L, 64 µL for test concentration of 0.032 µg/L and 20 µL for test concentration of 0.01 µg/L) of the [^{14}C]-Cypermethrin stock solution into glass scintillation vials. The hexane was blown to dryness using nitrogen. For test concentrations of 1 and 0.32 µg/L nominal an aliquot (203 µL and 50 µL respectively) of non-radiolabelled technical Cypermethrin (prepared as a 1 mg/mL solution in hexane) was added. This was blown to dryness using nitrogen. Acetone (22.5 mL) was added to each vial and the vials ultrasonicated for *ca* 5-10 min to ensure dissolution. The stock solutions in acetone were stored at *ca* 4°C. Stock solution was delivered to the appropriate mixing vessel at a rate of 0.19 mL/h where it was mixed with dilution water and delivered at a rate of *ca* 15.6 mL/min to each replicate tank. Stock solutions were replenished at ≤96 h intervals throughout the test period as the test item was shown to be stable over 96 h.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	Test and culture water was reconstituted fresh water prepared by the test laboratory (see table A7_4_3_2-1 for recent analysis results)
3.4.2	Test organisms	See table A7_4_3_2-2
3.4.3	Test system	See table A7_4_3_2-3
3.4.4	Test conditions	See table A7_4_3_2-4
3.4.5	Duration of the test	28 days post hatch
3.4.6	Test parameter(s)	Numbers of live and dead embryos recorded daily, with any dead embryos being removed. Mortality, appearance, behaviour and abnormal effects were recorded if noted. Surviving fry counted at day 28 post-hatch and individually weighed and lengths recorded.
3.4.7	Examination / Sampling	Daily inspection of the embryos The temperature in one replicate control tank was measured continuously and in all tanks twice weekly. Dissolved oxygen and pH were measured in a sample of water taken from each tank once weekly. Conductivity, hardness and alkalinity of the dilution water were recorded once weekly.

Section 7.4.3.2 **Effects on reproduction and growth rate of fish**
Annex Point IIIA XIII 2.2

3.4.8	Monitoring of TS concentration	Yes On each day of the study, commencing at 48 h prior to the addition of the embryos, single aliquots (10 mL) of water were sampled from each replicate solvent control and test item tank and mixed with scintillation fluid (Zinsser Analytic) prior to determination of radioactive content by Liquid Scintillation Counting (LSC). These were subjected to 1 min analysis as a system check. On Days -2, -1, 0, 1, 3, 7, 10, 21, 28 and 31 duplicate samples (single samples on Day -2 and -1) were subjected to 5 min analysis (1 min. analysis for day -2). The samples taken on Days -1, 0, 1 and 3 for 5 min analysis were prepared using Aquasafe as the scintillant. This was subsequently shown to be unreliable and all further analysis was conducted using Quicksafe as the scintillant. In order to obtain reliable Day 0 analysis and generate measured test concentrations, extracts of the Day 0 samples were used. Radioactivity was quantified using a liquid scintillation analyser (Packard 2100 TR) with automatic quench correction by external standard-channels radio. A background count rate was determined prior to analysis and subtracted from each count rate with a limit of reliable determination of 30 dpm. A 1 L sample was removed from replicate test tank A at 1 µg/L and at 0.032 µg/L on study day -2. Similarly, 1 L samples were removed from each test and solvent control tank on study days 0, 14 and 31. Samples were extracted by partitioning with hexane (50ml) and allowing the layers to separate. The hexane layer was then passed through granular sodium sulphate into a collection vessel and the aqueous layer partitioned twice more with hexane and the resulting extracts combined. The extracted samples were analysed for the presence of the parent material by HPLC with UV detection at 280 nm (Spherisorb 5 µm silica 250 x 4.6 mm, flow rate 1 ml/min).
3.4.9	Statistics	2-tailed Fisher's Exact test was used to determine cumulative mortality and number of fry that hatched compared to the controls. Levine's test and ANOVA techniques were used for weight and length data.

4 RESULTS

4.1	**Range finding test**	Not performed, test concentrations decided based on published information on the toxicity of cypermethrin to fathead minnows.
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0 (solvent and non-solvent controls), 0.01, 0.032, 0.1, 0.32 and 1 µg/L (nominal concentrations)
4.2.2	Actual concentrations of test substance	See Table A7_4_3_2-5

Section 7.4.3.2 **Effects on reproduction and growth rate of fish**
Annex Point IIIA XIII 2.2

4.2.3 Effect data

Hatching success - results indicated a significantly lower hatching success at 0.032, 0.1 and 0.32 µg/L nominal, when compared to the solvent control. There was however no statistically significant difference in hatching success between all other groups, including 1 µg/L nominal and the solvent control. As a result it was not possible to statistically determine a NOEC with respect to hatching success. It is considered that this non-monotonic response does not indicate an effect of the test item but was rather a biological effect. On study day 2, a significant number of dead/fungus-covered embryos were removed from test concentrations of 0.1 and 0.32 µg/L which resulted in a lower hatching success at these test concentrations. An empirically derived NOEC would be regarded as 1 µg/L nominal (0.89 µg/L based on mean measured concentrations).

Fry survival - results indicated no significant difference in mortality between the 0.01 to 0.32 µg/L treated groups and the solvent control. The NOEC for fry survival is therefore concluded to be 0.32 µg/L nominal (0.30 µg/L mean measured), with the LOEC being 1 µg/L nominal (0.89 µg/L mean measured).

Fry growth (length) - Body length of surviving fry was found to be significantly greater in all treated groups when compared to the solvent control. This may be explained by the number of fry present in each tank. The solvent control replicates contained significantly more fry than all other tanks thus increasing competition for both food and space, resulting in slightly slower growth. The NOEC for deleterious effects on length was calculated as 0.32 µg/L nominal (0.30 µg/L mean measured) with the LOEC being 1 µg/L.

Fry growth (weight) - Body weight of surviving fry was found to be significantly greater in all treated groups when compared to the solvent control. This may be explained by the number of fry present in each tank. The solvent control replicates contained significantly more fry than all other tanks thus increasing competition for both food and space, resulting in slightly slower growth. The NOEC for deleterious effects on body weight was calculated as 0.32 µg/L nominal (0.30 µg/L mean measured) with the LOEC being 1 µg/L.

See Tables A7_4_3_2-6 to A7_4_3_2-10

4.2.4 Other effects

It was observed on Study Day 4 that all fry at 1 µg/L nominal appeared smaller and weaker than fry in all other tanks. They were observed to be swimming in spiralling movements with periods when they appeared to go into spasms. All fry at 1 µg/L nominal (0.89 µg/L mean measured) were dead by post-hatch Day 11.

It was noted on Study Day 8 that in each replicate tank (with the exception of replicate tank B at 0.32 µg/L nominal) there were numbers of smaller and weaker fry. From Study Day 21 to the end of the test these smaller and weaker fry began to undergo significant mortality. All mortality from Study Day 21 to the end on the test was of these smaller and weaker fry.

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	Hatching success in the solvent control group was 77% and fry survival 73%, showing the test was valid.
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effect of prolonged exposure to Cypermethrin in the cis:trans ratio of 40:60, on the early-life stages of the Fathead Minnow (*Pimephales promelas)* was assessed over embryo development, hatching and for 28 days post-hatch, in accordance with OECD (July 1992) Guideline 210 and OPPTS 850.1400 Fish Early-Life Stage Test. The test was conducted under continuous flow conditions, with embryos (less than 25 h old on exposure to the test solutions) and larvae/fry exposed to the following nominal concentrations of [^{14}C]-Cypermethrin; 0.01, 0.032, 0.1, 0.32 and 1 µg/L. Both solvent and non-solvent controls were included in the test. Duplicate tanks were tested at each concentration.
5.2	**Results and discussion**	
5.2.1	NOEC	Hatching success = 1 µg/L nominal (0.89 µg/L measured) Fry survival = 0.32 µg/L nominal (0.30 µg/L measured) Fry growth = 0.32 µg/L nominal (0.30 µg/L measured)
5.2.2	LOEC	Fry survival = 1 µg/L nominal (0.89 µg/L measured) Fry growth (length) = 1 µg/L nominal (0.89 µg/L measured) Fry growth (weight) = 1 µg/L nominal (0.89 µg/L measured)
5.3	**Conclusion**	See table A7_4_3_2-11
5.3.1	Other Conclusions	
5.3.2	Reliability	1
5.3.3	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	November 2007

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

Materials and Methods	Applicant's version is acceptable. 2.3 Deviations: In error, 20 embryos were added in replicate A of solvent test control instead of 0.01µg/L replicate B.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is adpoted
Reliability	1
Acceptability	Acceptable
Remarks	
	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_4_3_2-1: Dilution water

Criteria	Details
Source	Reconstituted fresh water prepared by the test laboratory.
Chloride	47 mg Cl/L
Sodium	5 mg Na/L
Potassium	<1 mg K/L
Magnesium	4 mg Mg/L
Hardness	80 mg $CaCO_3$
pH	6.66 as $CaCO_3$
Alkalinity	7 mg/L
Conductance	190 µS/cm at 20 °C
Holding water different from dilution water	No

Table A7_4_3_2-2: Test organisms

Criteria	Details
Species/strain	Fathead Minnow *(Pimephales promelas)*
Source	Charles River Laboratories
Wild caught	No
Age/size	25 hours
Pre-treatment	Embryos were randomly selected and added in small groups to the incubation chambers in order from the untreated control to the highest test concentration. This procedure of addition of embryos to the incubation chambers was to avoid possible contamination which may have occurred if embryos had been added randomly over the test concentrations.
Kind of food	Brine Shrimp *(Artemia salina)*
Amount of food	*Ad libitum*
Feeding frequency	At post hatch (day 0), then twice daily for the remainder of the test. Fry were not fed in the 24 hour period before termination of the test.

Table A7_4_3_2-3: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	Approx. 15.6 ml/min
Volume of test vessels	22 x 15 x 12cm (3L capacity) containing an 8cm glass egg incubation chamber
Number of animals/vessel	Between 30 and 76 embryos.
Number of vessels/ concentration	Two replicate test tanks at each concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2-4: Test conditions

Criteria	Details
Test temperature	23-27°C
Aeration of dilution water	A magnetic stirrer was placed in each tank to ensure adequate movement of surrounding water and to maintain suitable dissolved oxygen concentrations in the egg incubation chamber.
Photoperiod	Subdued lighting for the first 3 days after embryo introduction, then 16h light: 8 hour dark with 30 min dawn/dusk transition

Table A7_4_3_2-5: Measured concentration of cypermethrin during the test

Study Day	Nominal Concentration of Cypermethrin (µg/L)					
	0 (Solvent)	0.01	0.032	0.1	0.32	1
-2	0	0.006*	0.038	0.11	0.47	3.88
0	-	0.011	0.028	0.07	0.24	0.60
7	0	0.010*	0.026	0.11	0.33	0.97
10	0	0.006*	0.031	0.10	0.41	1.11
14	0	0.010*	0.034	0.11	0.30	NA
21	0	0.010*	0.034	0.10	0.22	NA
28	0	0.004*	0.030	0.10	0.35	NA
31	0	0.008*	0.030	0.11	0.28	NA
Overall Mean**	0	0.008*	0.030	0.10	0.30	0.89

NA = Not applicable as all fry died earlier.
* = Level of radioactivity below the limit of reliable determination.
** = Overall mean is mean of concentrations on Days 0-31.
\- = Extracted sample not analysed.

Table A7_4_3_2-6: Hatchability and survival data

Conc. µg/L		Initial no. of embryos	No.of Fry		Hatch (%)		Adjusted no. fry/tank	No. fry surviving at 28 d	Survival of Fry from release to 28 Days Post-Hatch (%)	
			Hatched	Released	Individual	Pooled			Individual	Pooled
0	A	55	35	32	64	68	35	19	54	68
	B	55	39	37	71		34	28	82	
0 Solvent	A	76*	60	54	79	77	50	34	68	73
	B	55	41	41	75		45	35	78	
0.01	A	55	40	39	73	68	29	23	79	74
	B	30	19	19	63		29	20	69	
0.032	A	42	26	26	62	60	26	18	69	74
	B	42	24	24	57		24	19	79	
0.1	A	43	19	19	44	38	19	17	89	80
	B	42	13	10	31		10	7	70	
0.32	A	42	18	15	43	38	15	12	80	80
	B	43	14	10	33		10	8	80	
1	A	53	27	23	51	68	23	0	0	0
	B	55	46	46	84		46	0	0	

* 20 embryos added in error

Table A7_4_3_2-7: Fry mortality (no. of dead fry)

Study Day	Nominal Concentration of Cypermethrin (µg/L)													
	0		0 Solvent		0.01		0.032		0.1		0.32		1	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
2	0	0	6*	0	0	0	0	0	0	0	0	0	0	0
3	3	2	0	0	1	0	0	0	0	3	3	4	4	0
4	1	0	2	0	0	0	0	0	0	1	2	1	1	0
5	1	0	2	0	0	0	0	0	1	1	0	0	8	9
6	1	1	0	2	3	2	3	1	0	0	1	0	9	35
7	0	0	0	0	0	1	0	1	1	0	0	0	3	1
8	0	0	0	0	0	0	0	0	0	0	0	1	1	1
9	0	0	0	0	0	0	0	0	0	0	0	0	0	-
10	0	0	0	0	0	0	0	0	0	0	0	0	0	-
11	0	0	0	0	0	0	0	0	0	0	0	0	1	-
12	0	0	0	0	0	0	0	0	0	0	0	0	-	-
13	0	0	0	0	0	0	0	0	0	0	0	0	-	-
14	1	0	0	0	0	0	0	0	0	0	0	0	-	-
15	0	0	0	0	0	0	0	0	0	0	0	0	-	-
16	0	0	0	0	0	0	0	0	0	0	0	0	-	-
17	0	0	0	0	0	0	0	0	0	0	0	0	-	-
18	0	0	0	0	0	0	0	0	0	0	0	0	-	-
19	1^	0	0	0	0	1^	0	0	0	0	0	0	-	-
20	0	0	0	0	0	0	0	0	0	0	0	0	-	-
21	0	0	0	0	0	0	0	0	0	1	0	0	-	-
22	1	1	0	0	0	0	1	0	0	0	0	0	-	-
23	1	0	1	3	0	0	0	0	0	0	0	0	-	-
24	0	0	5	3	0	1	2	1	0	0	0	0	-	-
25	1	0	4	0	4	0	0	0	0	0	0	0	-	-
26	0	1	0	0	0	0	0	0	0	0	0	0	-	-
27	2	0	0	0	0	0	0	0	0	0	0	0	-	-
28	1	2	0	0	0	0	0	0	0	0	0	0	-	-
29	1	1	0	0	0	0	0	0	0	0	0	0	-	-
30	0	0	0	0	0	1	0	0	0	0	0	0	-	-
31	0	0	0	0	0	0	0	0	0	0	0	0	-	-

* Dead larvae possibly due to movement created by magnetic stirrer beneath the egg chamber
^ Fry removed due to ill health
- All fry dead

Table A7_4_3_2-8: Fry growth (length in mm)

	Cypermethrin (µg/L)											
	0		0 solvent		0.01		0.032		0.1		0.32	
	A	B	A	B	A	B	A	B	A	B	A	B
Minimum	17.7	17.2	16.8	17.8	16.5	18.0	18.6	19.6*	16.3*	21.6*	18.8*	20.3
Maximum	22.3	22.3	21.0	20.7	21.9	22.3	22.1	22.9	22.4	23.3	22.8	23.7
Mean	20.2	19.8	18.6	19.1	19.4	19.8	20.3	20.9*	19.9*	22.1*	20.7*	22.4
Standard Deviation	1.10	0.99	1.04	0.75	1.42	1.13	1.16	0.98*	1.76*	0.92*	1.22*	1.20

* Excluding outliers

Table A7_4_3_2-9: Fry growth (weight in mg)

	Cypermethrin (µg/L)											
	0		0 solvent		0.01		0.032		0.1		0.32	
	A	B	A	B	A	B	A	B	A	B	A	B
Minimum	79.0	67.7	58.9	71.2	63.8	89.1	96.3	6.2	5.7	12.1	8.2	132.2
Maximum	163.4	143.7	123.1	132.7	158.7	175.2	158.1	171.5	165.2	213.6	175.5	212.4
Mean	122.8	110.9	89.9	95.6	102.8	123.9	122.6	119.6	105.7	131.5	111.1	174.3
Standard Deviation	20.68	17.65	15.51	13.97	22.83	23.82	21.11	52.16	56.41	83.18	62.55	28.26

Table A7_4_3_2-10: Summary of Effect data

	Parameter	NOEC	LOEC
Data based on nominal cypermethrin concentrations	Hatch	1 µg/L*	*
	Survival	0.32 µg/L	1 µg/L
	Length	0.32 µg/L^	1 µg/L
	Weight	0.32 µg/L^	1 µg/L
Data based on measured cypermethrin concentrations	Hatch	0.89 µg/L	*
	Survival	0.30 µg/L	0.89 µg/L
	Length	0.30 µg/L^	0.89 µg/L
	Weight	0.30 µg/L^	0.89 µg/L

* = No effect was noted on hatching success at 1 µg/L nominal (0.89 µg/L measured). Effect on hatching success was detected at 0.032, 0.10 and 0.32 µg/L nominal (0.0030, 0.10 and 0.30 µg/L measured)

^ = Length and weight of surviving fry was significantly greater at all test concentrations when compared to solvent control. NOEC values are for deleterious effects.

Table A7_4_3_2-11: Validity criteria for fish tests according to OECD Guidelines 210

	fulfilled	Not fulfilled
Hatching success of solvent control group >66%	Y	
Fry survival in solvent control >70%	Y	
Concentration of dissolved oxygen in all test vessels >60%	Y	
Test substance concentrations maintained within ± 20% of mean measured values	Y	

Section A7.4.2, A7.4.3.3.1

Annex Point IIA7.5, IIA8.2.3

Bioconcentration in aquatic organisms

Bioaccumulation in an appropriate species of fish

			Official use only
		1 REFERENCE	
1.1	**Reference**	Szeleczy, G. (1990); Draft report on flow-through test in rainbow trout to determine the bioaccumulation potential of cypermethrin; Toxicological Research Centre, Report no. 90-016 (CYP/T133), 9 July 1990 (unpublished). Dates of work: 2 March 1990 – 4 July 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, OECD guideline (1981) 305E	
2.2	**GLP**	Unclear from the final report (no copy of certificate however GLP is mentioned on page 8 of the study protocol)	X
2.3	**Deviations**	Yes. The volume of the aquaria allowed a fish weight:water volume ratio of c. 8 g/L which was around half of that specified in the guideline.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin technical	
3.1.1	Lot/Batch number	FSG 08611 J	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	94.2%	
3.1.4	Further relevant properties	Low water solubility (<5-10 ppb), log Pow of 4.47. Therefore the test substance was first dissolved in acetone before being introduced into the test system. See Table A7_4_3_3_1-1	X
3.1.5	Radiolabelling	Not applicable,	
3.1.6	Method of analysis	Samples of test chamber water and fish homogenate were analysed by Gas Chromatography (4mm I.D. glass column packed with 3% OV-101 on chromosorb W HP 100-200 mesh, nitrogen carrier gas flow rate 40ml/min) with Electron Capture Detection.	
3.2	**Reference substance**	No	
3.2.1	Method of analysis for reference substance	Not applicable	

Section A7.4.2, A7.4.3.3.1

Annex Point IIA7.5, IIA8.2.3

Bioconcentration in aquatic organisms

Bioaccumulation in an appropriate species of fish

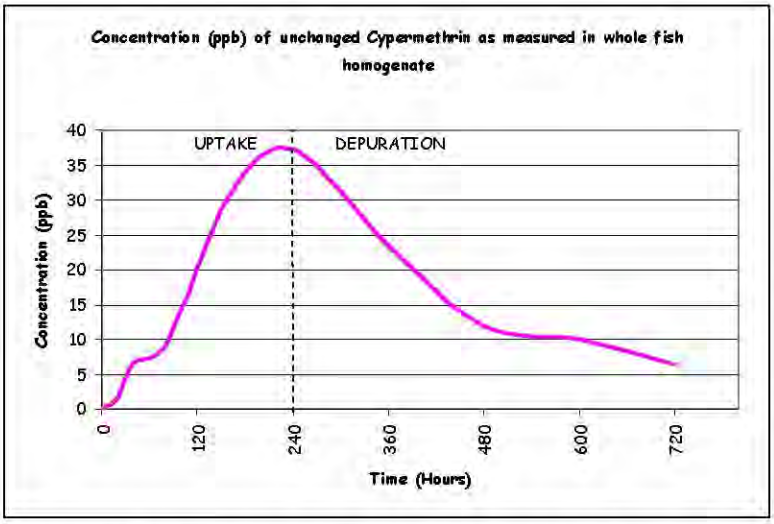
3.3	**Testing/estimation procedure**		
3.3.1	Dilution water	See Table A7_4_3_3_1-2	
3.3.2	Test organism	See Table A7_4_3_3_1-3	
3.3.3	Test system	See Table A7_4_3_3_1-4	
3.3.4	Test conditions	See Table A7_4_3_3_1-5	
3.3.5	Test duration	The uptake phase was set to 10 days. On day 10 the delivery of stock solution was terminated and the water in the test chamber removed and replaced with dilution water only and the flow rate restored. The depuration phase was set to 20 days.	X
3.3.6	Observations	Throughout the study fish were observed daily and any excess food or solid waste removed from the chamber.	
3.3.7	Sampling	Samples of test chamber water (c.500ml) were taken from two different locations in each test chamber. 300ml of each sample was then filtered through a Sep-Pack C18 cartridge fed by a water pump and the cartridges then flushed with acetone. The combined acetone/water sample was then evaporated until only a small amount of water remained in the test tube. 2ml of 16% NaCl and 3ml n-hexane were then added and the two phases shaken vigorously and the resulting sample analysed by GC-ECD. Each lot of stock test solution was also sampled. 1ml of fresh stock solution in acetone was evaporated to dryness and reconstituted in n-hexane for analysis by GC-ESD in the same way. Four or six fish samples (equivalent 100g fish) were also taken according to the schedule. Fish were removed from the tank, killed and stored frozen until analysis. 25g of fish homogenate were extracted with acetonitrile and analysed as described above. Details of the sampling schedule are given in Table A7_4_3_3_1-6.	
3.3.8	Estimation of bioconcentration	Two-compartment test model (water and fish) was used to describe the movement of test substance in and out of fish. The steady state bioconcentration factor was determined by dividing the concentration of test substance in the fish at steady state by the concentration in water. The update and depuration rates constants were determined graphically according to the ASTM Committee method.	
		4 RESULTS	
4.1	**Experimental data**		
4.1.1	Mortality/behaviour	The animals in the control group displayed normal behaviour during the study. The test group showed no signs of toxic effects, however 15 fish were found dead on day 14. This was not preceded by any apparent toxic symptoms and was the only incidence of mortality during the study.	
4.1.2	Lipid content	Not reported, results were based on whole fish homogenate.	

Section A7.4.2, A7.4.3.3.1

Annex Point IIA7.5, IIA8.2.3

Bioconcentration in aquatic organisms

Bioaccumulation in an appropriate species of fish

4.1.3	Concentrations of test material during test	See Table A7_4_3_3_1-7. Measurement of test chamber concentration showed that 0.1 ppb was attained with minute deviation. During the uptake phase the mean (±S.D.) concentration was found to be 0.09 (±0.007) ppb. The concentration of cypermethrin was below the limit of detection during the entire depuration phase. The concentration of cypermethrin in fish homogenate showed rapid increase up until the 160th hour of the study with smaller increases up to the 240th hour (end of the uptake phase). Therefore the determination of the BCF was based on concentration data from the end of the uptake phase which represented a quazi steady-state.  No cypermethrin was detected in either the water or in fish in the control aquaria.	X
4.1.4	Bioconcentration factor (BCF)	The BCF was based on concentration data from the end of the uptake phase (quazi steady-state) and was calculated to be 373.4 (±45.35) in relation to the whole body.	
4.1.5	Uptake and depuration rate constants	The depuration rate constant was calculated to be 0.00158 l/h. The goodness of fit of the straight line on the data points for the depuration phase indicated that the elimination process can be described by a two-compartment model.	
4.1.6	Depuration time	Cypermethrin showed a relatively high BCF and low depuration rate indicating that the elimination process in fish is relatively slow.	
4.1.7	Metabolites	Not determined	
4.1.8	Other Observations	None	
4.2	**Estimation of bioconcentration**	When tested at l0.1 ppm cypermethrin showed a relatively high bioconcentration factor and the low depuration rate.	

Section A7.4.2, A7.4.3.3.1	**Bioconcentration in aquatic organisms**
Annex Point IIA7.5, IIA8.2.3	**Bioaccumulation in an appropriate species of fish**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The bioconcentration of cypermethrin in fish was determined experimentally according to OECD guideline 305E. A flow-through system was employed with a test substance concentration of 0.1 ppb. The uptake phase lasted for 10 days and the depuration phase was 20 days. Unchanged test substance in both the aquaria water and whole fish homogenate was determined by GC-ECD.
5.2	**Results and discussion**	Despite of a log Pow value of 4.47, it took a relatively long period of time to reach steady-state, with the uptake phase lasting for 240 hours. The depuration rate constant was found to be 0.00158 l/h. and the BCF reached 373.4 (±45.35) by the end of the uptake phase.
5.3	**Conclusion**	The relatively high BCF and low depuration rate could offer an explanation for the high toxicity of cypermethrin in aquatic organisms.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes. The volume of the aquaria allowed a fish weight:water volume ratio of c. 8 g/L which was around half of that specified in the guideline. However this is not thought to have adversely affected the validity of the study.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	**November 2007**
Materials and Methods	**Applicant's version is acceptable.** **According to Bates 2002a, log Pow ranged from 5.3 to 5.6.** **The test duration of the uptake phase was only 10 days. In the actual test protocol, it is recommended that the test duration should be 28 days unless it is demonstrated that equilibrium has been reached earlier. Since this test was based on a former protocol, this demonstration is not available.**
Results and discussion	**Applicant's version is acceptable. Log pow ranged from 5.3 to 5.6**
Conclusion	**Applicant's versionis adopted.**

Section A7.4.2, A7.4.3.3.1 **Annex Point IIA7.5, IIA8.2.3**	**Bioconcentration in aquatic organisms** **Bioaccumulation in an appropriate species of fish**

Reliability	**2**
	2.2 GLP: Even if the GLP certificate is not provided, the study report is very well documented and seems to be performed with similar level of quality as for a full GLP study. However, the RMS was not able to find a proof that the laboratorium who had performed the test were GLP in 1990.
	2.3 This deviation is not considered to be of concern for the validity of the test.
Acceptability	**Acceptable:**
	Due to the consideration above regarding the test duration, the result of the test is considered as valid but with some reservations.
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*
Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_3_3_1-1: Preparation of TS

Criteria	Details
Vehicle	Acetone
Concentration in vehicle	Stock solution used for 24 hours was prepared daily from a 20 ppb in acetone
Vehicle control performed	Yes, a control aquaria received pure acetone
Concentration of TS in test chamber	0.1 ppb (0.0001 mg/L)
Other procedures	Stock solution was delivered to the dilution water by a peristaltic pump at a ratio of 1ml stock solution/L

Table A7_4_3_3_1-2: Dilution water

Criteria	Details
Source	Deep well source
Alkalinity	8.8 (mol)
Hardness	8.9 mg/L
pH	7.25
Oxygen content	6.3 mg/L (aquarium 8.7 mg/L)
Conductivity	562.3 uS

Table A7_4_3_3_1-3: Test Organism

Criteria	Details
Species/strain	Rainbow trout (Salmo irrideus Gairdneri)
Source	Balatoni Halgazdasag Odorogdi Pisztrang Telep
Wild caught	No
Age / size	4 weeks, mean bodyweight 3-5g
Kind of food	Tagger T888/1 pelleted fish food (supplied by TACO Tagger, Austria)
Amount of food	Corresponding to 1% of the total weight of fish
Feeding frequency	Once daily

Table A7_4_3_3_1-4: Test system

Criteria	Details
Test type	Flow through
Renewal of test solution	Full volume exchange rate 10/24 hours
Volume of test vessels	140L (filled to 130L)
Volume / animal	Fish weight to water volume of 8 g/L
Number of animals/vessel	Approx. 250 (1 kg) – dosed group Approx. 125 (0.5 kg) – control group
Number of vessels/concentration	2
Test performed in closed vessel due to significant volatility of TS	No

Table A7_4_3_3_1-5: Test conditions

Criteria	Details
Test temperature	16 ±1 °C
Dissolved oxygen	Determined prior to start of uptake phase, see table Table A7_4_3_3_1-2
pH	Determined prior to start of uptake phase, see table Table A7_4_3_3_1-2
Adjustment of pH	No
Aeration of dilution water	Yes
Intensity of irradiation	Not specified
Photoperiod	Not specified

Table A7_4_3_3_1-6: Sampling schedule for fish bioaccumulation study

Study timeline (hours)	Test group			Control group	
	Stock solution (1ml)	Aquaria water (500ml)	Fish (25g)	Aquaria water (500ml)	Fish (25g)
-24	1	2			
-16		2			
0 (start of uptake phase)	1	2			
20		2	4	2	2
24	1				
40		2	4		
48	1				
72	1				
80		2	4	2	2
96	1				
120	1				
144	1				
160		2	4	2	2
168	1				
192	1				
216	1				
240 (end of uptake phase)		2	6		
360		2	4		
480		2	4	2	2
600		2	4		
720 (end of depuration phase)		2	6		

Table A7_4_3_3_1-7: Results for fish bioaccumulation study

Study timeline (hours)	Cypermethrin concentration (ppb)	
	Test chamber water (mean value)	Whole fish homogenate (mean ±SD)
-48	0.089	-
-24	0.074	-
0	0.096	-
20	0.093	1.751 ±0.185 (n=4)
40	0.085	6.621 ±0.520 (n=3)
80	0.094	9.185 ±0.955 (n=3)
160	0.093	30.699 ±2.541 (n=5)
240	0.092	37.340 ±4.535 (n=5)
360	ND	23.314 ±3.152 (n=4)
480	ND	11.880 ±2.378 (n=3)
600	ND	10.026 ±0.697 (n=3)
720	ND	6.378 ±1.434 (n=6)

ND=Not Determined

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA XIII 2.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Dickhaus, S. (1990), 21-Days reproduction test with compound cypermethrin technical in Daphnia magna; Pharmatox Beratung und Forschung GmbH, report no. E.H./B.2-7-44-90 (CYP/T143), July 1990 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 202, part II (April 1984)	
2.2	**GLP**	Yes	X
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Cypermethrin Technical (cis:trans/40:60)	
3.1.1	Lot/Batch number	Sample no. 15/03/90	
3.1.2	Specification	As given in section 2	
		Deviating from specification given in section 2 as follows	
3.1.3	Purity	Not specified in study report	X
3.1.4	Composition of Product	Not applicable, technical a.s. was used in the study	
3.1.5	Further relevant properties	Cypermethrin is non-volatile therefore a semi-static design can be considered appropriate	
3.1.6	Method of analysis	Gas Chromatography (1m column packed with 3% OV-101 on Chromosorb W, HP 100-200 mesh, nitrogen carrier gas at flow rate 40 ml/min) with Electron Capture Detection (902A Chromopack).	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Due to low aqueous solubility of cypermethrin, the test substance was dissolved in acetone to give a 0.1% stock solution. This was then further diluted with water to give four different concentrations of 0.008, 0.040, 0.200 and 1.000 µg/L cypermethrin.	
		See Table A7_4_3_4-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA XIII 2.4

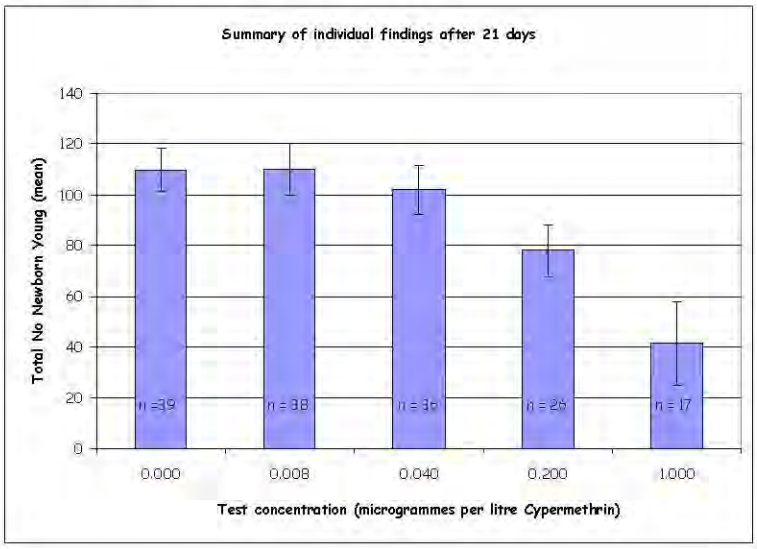
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_3_4-2. These parameters were measured daily and standard deviations did not go beyond 5%
3.4.2	Test organisms	See table A7_4_3_4-3
3.4.3	Handling of offspring	Live and dead offspring from the parental generation were counted and dead specimens removed either daily or at least 3 times per week (with an interval of 48-72 hours). Newborn young from the F1 generation were counted at least 3 times per week (with an interval of 48-72 hours) and a visual assessment of their condition recorded before the young were poured away. Only the parental animals were put into the renewal solution, animals from each F1 generation were poured away after counting and examination.
3.4.4	Test system	Semi-static study design as described in table A7_4_3_4-4
3.4.5	Test conditions	See table A7_4_3_4-5
3.4.6	Duration of the test	21 days
3.4.7	Test parameter	Number of young born Number of dead and live daphnia from parental generation
3.4.8	Examination / Sampling	Live and dead offspring from the parental generation were counted and dead specimens removed either daily or at least 3 times per week (with an interval of 48-72 hours). Newborn young from the F1 generation were counted at least 3 times per week (with an interval of 48-72 hours) and a visual assessment of their condition recorded before the young were poured away. The presence of eggs on the bottom of the vessel was also recorded.
3.4.9	Monitoring of TS concentration	Yes. 2 Litres of each test concentration were produced separately and the concentration checked on day 1, 7, 14 and 21.
3.4.10	Statistics	The EC_{50} was calculated by probit analysis with Gauss' Integral

4 RESULTS

4.1	**Range finding test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	See Table A7_4_3_4-6
4.2.2	Actual concentrations of test substance	See Table A7_4_3_4-6

Section 7.4.3.4
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

4.2.3	Effect data	For reproduction: LOEC = 0.2 µg/L NOEC = 0.05 µg/L EC_{100} = 1.0 (± 0.2) µg/L EC_{50} = 0.35 (0.22-0.48) µg/L The cumulative mortality (LC_{50}) for parental animals was estimated at 0.9 (± 0.3) µg/L See Tables A7_4_3_4-6 and A7_4_3_4-7
4.2.4	Concentration/ response curve	
4.2.5	Other effects	Average oxygen in the test system remained at 8.0 mg/L throughout the study
4.3	**Results of controls**	See Tables A7_4_3_4-6 and A7_4_3_4-7
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Cypermethrin was tested to determine affects on the reduction in reproduction according to OECD guideline no. 202, part II over a period of 21 days with the invertebrate species *Daphnia magna*. A semi-static study design was used with test solution renewals performed every 48 hours. Animals of each F1 generation were counted and examined. Four different test concentrations of 0.008, 0.040, 0.200 and 1.000 µg/L cypermethrin were tested along with a water control.
5.2	**Results and discussion**	Results indicated cypermethrin had a medium potential to reduce the reproduction of Daphnia magna.
5.2.1	NOEC	NOEC = 0.05 µg/L
5.2.2	LOEC	LOEC = 0.2 µg/L
5.2.3	EC_{50} (EC_x)	EC_{50} = 0.35 (0.22-0.48) µg/L

Section 7.4.3.4 **Annex Point IIIA XIII 2.4**	**Effects on reproduction and growth rate with an invertebrate species**

5.3	**Conclusion**	The study can be considered valid for the assessment as the species used was Daphnia magna and the test duration was 21 days. Concentration of the test substance in the system was confirmed by analysis of duplicate test concentrations over the 21 day time period.
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	**November 2007**
Materials and Methods	**Applicant's version is acceptable.** **2.2 GLP: A declaration is provided claiming that the test has been performed according to GLP. However, not certificate of GLP compliance is present in the report. More over, after some research, the RMS was not able to find a proof that the laboratorium who had performed the test were GLP in 1990. Nevertheless, a quality assurance statement is present in the document.** **3.1.3: Purity no specified and no certificate of analisys is provided in the report. Even if technical grade are usually hight, this deviation is of concern since we do not know what heve been tested. A sample number is still provided.**
Results and discussion	**Applicant's version is adopted.**
Conclusion	**Applicant's version is adopted.**
Reliability	**2 (according to the materials and methods remarks)**
Acceptability	**Acceptable**
Remarks	
	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_4_3_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes, acetone
Concentration of vehicle	Not specified. A stock solution of 0.1% cypermethrin in acetone was prepared 3 times per week.
Vehicle control performed	No
Other procedures	

Table A7_4_3_4-2: Dilution water

Criteria	Details
Source	Not specified
Hardness	5.4 °d
pH	7.1
Oxygen content	8.0 mg/L
Holding water different from dilution water	No

Table A7_4_3_4-3: Test organisms

Criteria	Details
Species / strain	Daphnia magna (Strauss)
Source	Institut für Wasser-, Boden- und Lufthygiene des Bundesgesundheitsamtes, 100 Berlin 33.
Age	< 24 hours old at beginning of test
Breeding method	Not specified
Kind of food	Log-phase unicellular green algae
Amount of food	1ml suspension in each vessel
Feeding frequency	At least daily
Pre-treatment	Not specified
Feeding of animals during test	Yes, at least daily

Table A7_4_3_4-4: Test system

Criteria	Details
Test type	Semi-static
Renewal of test solution	3 times per week (Mon, Wed, Fri). Glassware being emptied and food residues removed. Two sets of glassware were used alternately, each one being rinsed with distilled water after renewal.
Volume of test vessels	250 ml glass containers filled with 200ml of test solution
Volume/animal	At least 40ml / animal
Number of animals/vessel	5
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-5: Test conditions

Criteria	Details
Test temperature	18-22°C (±1°C)
Dissolved oxygen	Measured daily to ensure standard deviation from the mean value did not exceed 5% . Average oxygen content remained at 8.0 mg/L
pH	Measured daily and recorded for in-house QA check (individual values not reported)
Adjustment of pH	No
Aeration of dilution water	Test solutions aerated prior to introduction of test substance and Daphnia
Quality/Intensity of irradiation	Not specified
Photoperiod	8 hours darkness and 16 hours light

Table A7_4_3_4-6 Analysis of test substance concentration

Group	No. animals	Cypermethrin concentration (µg/L)				
		Initial	Day 1	Day 7	Day 14	Day 21
I	40	0.008	0.008	0.008	0.008	0.008
II	40	0.040	0.036	0.038	0.034	0.035
III	40	0.200	0.181	0.190	0.178	0.184
IV	40	1.000	0.946	0.940	0.926	0.925

Table A7_4_3_4-7 Summary of individual findings after 21 days

Test concentration (µg/L cypermethrin)	Day first litters appeared	Total no. of newborn young (mean)	Reduction in reproduction (%)	No. of mortalities in P generation	Mortality rate in P generation (%)
0.000 (control)	7	109.8 ± 8.26	-	1	2.5
0.008	7	110.0 ± 10.27	-	2	5.0
0.040	7	101.9 ± 9.53	7.2	4	10.0
0.200	9	78.1 ± 10.40	28.9	14	35.0
1.000	11	41.4 ± 16.47	62.6	23	57.5

Table A7_4_3_4-8 Total number of newborn young produced during the test

Replicate number	Nominal Cypermethrin concentration (µg/L)				
	0	0.008	0.040	0.200	1.000
1	108	123	100	81	28
2	94	109	96	92	30
3	102	107	102	77	38
4	114	110	101	87	46
5	110	123	93	68	32
6	115	115	116	63	76
7	118	96	116	87	51
8	117	97	91	70	28
Mean	109.8	110.0	101.9	78.1	41.4

Section IIIA.7.4.3.5.1 **Annex Point IIIA XIII 3.4**	**Effects on sediment dwelling organisms**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [√]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [√]**	
Detailed justification:	Cypermethrin has been shown to have a relatively short half-life in aquatic systems, rapidly binding to organic matter. In the outdoor pond study (section A 7.4.3.5), measured concentrations of cypermethrin in water samples taken from selected enclosures gave estimated water column DT_{50} values of 22.3 hours and 20.9 hours for enclosures treated with 0.05 and 1.0 µg/L cypermethrin respectively. Cypermethrin concentration in the sediment was <LOD (0.41 µg.kg) in enclosures treated with 0.05 µg/L and ranged from 1.88 µg/kg (day 4) to a maximum of 6.77 µg/kg (day 16, 2 days after the second application) in the ponds treated with 1.0 µg/L. This suggests that cypermethrin was rapidly degraded in the water column before reaching the sediment. The water-sediment study (section 7.1.2.2.2) also showed that cypermethrin degrades rapidly. Degradation of cypermethrin was very rapid in both systems with DT_{50} values between 3.5 and 9.8 days in sediment. Dissipation from the water phase was more rapid with DT_{50} values of 0.5 days in both systems. In addition, high organic carbon content (Koc) values were reported in the adsorption-desorption study in each of the four soils (Koc ≥202418, ≥574360, ≥80653, ≥152388) and one sediment (Koc ≥527972). The high Koc values and relatively low DT_{50} for cypermethrin shows that the risk to sediment dwelling organisms should be relatively low based on limited exposure, with cypermethrin degrading rapidly in the water column and binding strongly to sediment particles. In addition, a spiked sediment study is unlikely to give meaningful results, since the test material would most likely to rapidly degrade and bind strongly to the sediment itself before any effects could be seen. Finally, it should be noted that cypermethrin is listed on Annex I of Directive 91/414/EC for plant protection use, whereby the risk of overspray is much higher and an increased volumes of this active substance are used. During the review process the risk to sediment dwelling organisms was not considered to be of significance.	
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	**November 2007**	
Evaluation of applicant's justification	**Applicant's justification is acceptable in a first step. However, if emission to the sediment compartment is found, the test will be required.**	

Section IIIA.7.4.3.5.1 **Annex Point IIIA XIII 3.4**	**Effects on sediment dwelling organisms**
Conclusion	**Acceptable**
Remarks	References to directive 91/414 are non significant since application methods and rate are not comparable and since the aim of the application is not comparable. Semi continuous exposure of the environment is usually expected following biocide use whilst single exposure is likely to occur from a PPP use.
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.4.3.5 Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk

Annex Point IIIA XIII 3.4

Mesocosm study

Official use only

1 REFERENCE

1.1	**Reference**	Schnoder, F. (2003); Evaluation of direct and indirect effects of Cyperkill 10 on aquatic organisms on outdoor enclosures (multi-site study); Covance Laboratories Ltd, study no. 0040/045 (CYP/T331), 20 June 2003 (unpublished). Dates of work: 14 May 2002 – 10 January 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**	This study was conducted to support the agricultural use of cypermethrin, taking into account the recommendations of various workshops on higher tier risk assessments for aquatic organisms (e.g. HARAP, CLASSIC).
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 METHOD

3.1	**Test material**	According to current guidance the formulated product should be tested rather than the active substance. Cyperkill 10 EC, containing 100g/l cypermethrin 40:60 cis:trans, the emulsifiable concentrate (EC) formulation has been tested.
3.1.1	Composition of Product	Cyperkill 10 EC, containing 100g/l cypermethrin cis:trans 40:60
3.2	**Administration of the test substance**	In order to simulate the agricultural application the effects of two applications of Cyperkill 10 EC, with a minimum 14-day spray interval, were investigated. Nominal test concentrations were 0.0016, 0.005, 0.016, 0.05, 0.2 and 1.0 µg cypermethrin/L. The test item was applied below the surface, directly into the water column.
3.3	**Testing procedure**	
3.3.1	Test organisms	Natural aquatic ecosystems (pond study). Biological parameters included abundance of Zooplankton, Macrozoobenthos, emerging Insects, Phytoplankton, Periphyton and Macrophytes.
3.3.2	Screening test	Prior to initiating the pond study, a bioscreening test with a control and four concentrations of cypermethrin (0.02, 0.06, 0.2 and 0.6 µg/l) was conducted. The test organisms were collected in untreated control ponds of the mesocosm facility. 10 organisms from each species were introduced in the beakers and observed after 3, 24 and 48 hours.

Section A7.4.3.5
Annex Point IIIA XIII 3.4

Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk

Mesocosm study

3.3.3	Test System	The effects of an EC formulation 'Cyperkill 10EC' containing 100 g/L cypermethrin (nominal) on naturalised ecosystems were determined using outdoor stainless steel enclosures containing freshwater assemblages of pelagic and benthic organisms. Twenty-two enclosures, each containing approximately 1060 L water (depth approximately 1.1 m.) and a sediment layer of *ca.* 15 cm, were allowed to equilibrate for >6 months before application. The enclosures contained various macrophytes (*Potamogeton crispus, Myriophyllum spicatum, Chara intermedia*). The macrophytes covered a limited surface area up to 5-6 weeks after the last application. It can be concluded that they did not disturb the mesocosm behaviour at the start of the study (around application time, first weeks recovery period). Two applications of the test substance were made, 14 days apart, with the application being made below the water surface into the water column. Nominal test concentrations were 0.0016, 0.005, 0.016, 0.05, 0.2 and 1.0 µg cypermethrin/L with three replicate enclosures used for each of the four lowest test concentrations and two replicate enclosures used for each of the two highest test concentrations. Six untreated enclosures were used as controls.
3.3.4	Analytical methods	Water samples were analysed for cypermethrin using gas chromatography with electron capture detection (GC/ECD) and gas chromatography with mass spectroscopy (GC/MS), for all enclosures following each application. In addition, water and sediment samples from selected enclosures at 1.0, 0.05 and 0.005 µg cypermethrin/L were analysed to determine the dissipation of cypermethrin. All enclosures were monitored for physical and chemical parameters of the water at appropriate weekly or bi-weekly intervals. Analytical method performance was measured by performing procedural recovery experiments at appropriate concentrations, in water and sediment, with each batch of samples analyzed. Mean recovery in pond water: 100%, each batch procedural recovery in the range 70-110% except one batch (117%) Mean recovery in sediment: 101%, each batch procedural recovery in the range 70-110% except 2 batches (111 and 112%)
3.3.5	Examination / Observation	Biological parameters, including abundance of Zooplankton, Macrozoobenthos, emerging Insects, Phytoplankton, Periphyton and Macrophytes, were monitored at weekly or bi-weekly intervals for a total of 111 days after the first application. Sampling for each of these parameters was carried out as follows: **Zooplankton.** Depth-integrated samples were taken using a Zieris-tube, which consists of a graduated 1 m long plastic tube with an inner diameter of 72 mm. The tube was immersed into the water column and then the bottom of the tube closed mechanically. Two water samples (approximately 3 L) were taken per enclosure and the samples pooled and filtered through a 63 µm mesh sieve to retain the zooplankton. The zooplankton samples were fixed immediately after sampling using a formalin solution.

Section A7.4.3.5
Annex Point IIIA XIII 3.4

Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk

Mesocosm study

Chaoborus. Samples were collected using a stainless steel sieve (mesh width 200 µm) fixed to a steel rod. The sieve was placed at a depth of approximately 90 cm at two different positions and raised vertically through the water column to the surface. Organisms were then counted alive and returned to their respective enclosure. As Chaoborus was known to be a sensitive species and in order to ensure sufficient numbers for statistical analysis a Zieris-tube and pebble basket (used to collect zooplankton and macrozoobenthos) were also used to collect this species.

Emergent insects. Samples were collected using emergence traps. Each trap was approximately 35 cm in diameter and therefore covered not more than 15% of the surface area of each enclosure. The traps consisted of a nylon mesh enclosing a buoyant circular stainless-steel structure. At the apex of the structure, a 250 mL plastic bottle containing about 100 mL ethylene glycol was used to retain the emergent insects, which were preserved in ethanol after collection.

Macrozoobenthos. Samples were collected using specially designed pebble-baskets, which consisted of an open stainless-steel basket (200 x 200 x 75 mm) containing pebbles (15-35 mm in diameter) and seven stainless-steel plates (100 x 100 mm; with and without perforation) placed directly above the pebbles to enlarge the attraction for the organisms and the variability of the substrate.

Phytoplankton. Samples were collected using a depth-integrated sampler to a depth of 80 cm (44 mm inner diameter). Sample size was approximately 500 mL.

Periphyton. Samples were collected using microscope slides, used as artificial substrates and exposed for at least two weeks at a depth between 40 cm and 60 cm below the water surface.

Macrophytes. Visual observations of macrophyte growth were made at intervals during the study and recorded by mapping.

3.3.6 Statistical analysis

Abundance data were analyzed for 5 main categories of test organisms : zooplankton, emergent insects macrozoobenthos, phytoplankton and periphyton and for 2 additional data types : Chaoborus (combined sampling techniques), and blue-green algae.

Principal Response Curves were produced form each main data type using CANOCO version 4.02 software. NOEC and EC50 values were calculated using SAS version 6.12 software.

No Observed Adverse Effect Concentration is the concentration where complete recovery was observed 8 weeks after the first application.

Section A7.4.3.5
Annex Point IIIA XIII 3.4

Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk

Mesocosm study

4 RESULTS

4.1 Water

In all except two enclosures, measured cypermethrin concentrations in the test enclosure water samples where the nominal concentrations were above the limit of quantification (0.01 µg/L), taken 2 hours after each treatment, ranged from 103% to 140% of nominal. The two enclosures where concentrations were outside of this range were the test enclosure dosed at 0.016 µg/L where the measured concentration was 150% of nominal after the first application and the test enclosure dosed at 1.0 µg/L where the measured concentration was 74% of nominal after the second application. These results indicate that treated enclosures were dosed correctly and the target exposure concentrations were achieved. No cypermethrin was detected in control enclosures.

Measured concentrations of cypermethrin in the water samples from enclosures monitored at 0.05 µg/L and 1.0 µg/L declined rapidly over time. The estimated water column DT_{50} values were 22.3 hours and 20.9 hours for nominal concentrations 0.05 µg/L and 1.0 µg/L respectively.

4.2 Sediment

Cypermethrin concentrations in sediment were analysed in selected enclosures at 0.05 µg/L and 1.0 µg/L. Measured concentrations (as dry weight equivalents) for total cypermethrin in sediment samples at 0.05 µg/L were below the limit of detection (LOD) of 0.41 µg/kg during the whole course of the study. Cypermethrin concentrations in sediment at 1.0 µg/L ranged from 1.88 µg/kg on day 4 to a peak of 6.77 µg/kg on day 16 (2 days after the second application). While there was some variability in the measured concentrations (samples were below the LOQ of 0.79 µg/kg on days 2, 18 and 42), which may reflect heterogeneity in the sediment sampling, measured sediment concentrations declined to 1.42 µg/kg (2 x the LOQ) by day 84 (last sediment sampling date).

4.3 Biological Parameters

Measurements on the various biological parameters were as follows:

Zooplankton. No species was eliminated from any enclosure at any treatment level during the study. All species, with the exception of *Daphnia longispina,* showed a complete and rapid recovery within 8 weeks after the last application. The variation in the population of *Daphnia longispina* was not considered to be treatment related, but rather a change in the population structure due to seasonal and successional reasons, indicated both by the use of a further laboratory bioassay over the test concentrations used in the study (see point 4.4) and also from knowledge of the species from other similar studies. An overall NOEC <0.05 µg/L was determined for several of the zooplankton species. Those species with an overall NOEC lower than 0.05 µg/L were all taxa belonging to the Cladocera (overall NOEC 0.016 µg/L), all taxa belonging to the Copepoda (overall NOEC 0.005 µg/L) and the taxa *Keratella* (overall NOEC 0.016 µg/L) and *Synchaeta* (overall NOEC <0.0016 µg/L) of the class Rotatoria. An overall NOEAEC (No Observed Ecologically Adverse Effect Concentration) of 0.05 µg/L was recommended due to the rapid recovery of species.

Section A7.4.3.5
Annex Point IIIA XIII 3.4

Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk

Mesocosm study

Chaoborus. The overall NOEC was <0.0016 µg/L, calculated from days 3 to 42. NOEC values of 0.2 µg/L, calculated from day 49 to day 63, and ≥1.0 µg/L from day 70 until the end of the study confirmed that there was recovery occurring with time. As Chaoborus was not eliminated from any enclosure a NOEAEC of 0.05 µg/L was recommended.

Emergent insects. An overall NOEC <0.05 µg/L was established. The family Baetidae and the genus Chaoborus corresponded to taxa affected within the Macrozoobenthos group. Complete and full recovery was observed within 8 weeks after the last application and justified the proposed NOEAEC of 0.05 µg/L.

Macrozoobenthos. The macrozoobenthos community was abundant and diverse throughout the study. Abundance for the whole group of macrozoobenthos was not affected at the three lower levels, but a decrease in abundance at the three upper levels was observed after the first application. Population recovery at 0.05 µg/L and 0.2 µg/L occurred by day 14, with recovery at 1.0 µg/L occurring by day 70. An overall NOEC <0.05 µg/L was determined for several of the species. Those species with an overall NOEC lower than 0.05 µg/L were Chironomidae and the Baetidae with an overall NOEC of 0.005 µg/L and Planorbidae with an overall NOEC of 0.016 µg/L. Diptera had an overall NOEC of 0.05 µg/L. A NOEAEC of 0.05 µg/L was justified for the Macrozoobenthos community because effects were only transient and, therefore, considered of minor ecological relevance. No species was eliminated from any enclosure at any treatment level.

Phytoplankton. No apparent treatment-related effects in the abundance of the colour groups, measured by DF-Kinetic Photometry were observed during the study. Transient treatment-related reductions of the total Phytoplankton and within the class Cryptophyceae counted by microscope were observed at all treatment levels. The taxa with an overall NOEC <0.05µg/L were total Phytoplankton and all taxa belonging to the class Cryptophyceae. These had an overall NOEC of <0.0016 µg/L. The phylum Chrysophyta, the class Bacillariophyceae, the family Synuraceae, the family Scenedesmaceae and the family Euglenophyceae were characterised by an overall NOEC of 0.005 µg/L whereas for the genus Tetrachlorella, the family Chlorellaceae and the class Cyanophyceae an overall NOEC of 0.016 µg/L was determined. A NOEAEC of 0.05 µg/L was proposed as, for all taxa, full recovery of populations was observed within 8 weeks after application.

Periphyton. An overall NOEC <0.05µg/L was determined only for one taxon. Abundances of the colour groups "diatoms" and "blue-green algae", measured by DF-Kinetic, showed a positive deviation from the controls. The diatoms showed an indirect treatment-related increase at the five upper treatment levels at day 14 only. The blue-green algae (Cyanophyceae) were affected at the five highest treatment levels (0.005 µg/L to 1.0 µg/L) at day 14 and at all treatment levels at day 18. Full recovery of populations was observed by day 29. However it is notable

Section A7.4.3.5
Annex Point IIIA XIII 3.4

Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk

Mesocosm study

that these indirect effects were not observed for the blue-green algae counted by microscope. The class Chrysophyceae and the family Chroococcaceae were affected at 0.016 µg/L to 1.0 µg/L and at 0.2 µg/L to 1.0 µg/L respectively, at day 4, however both taxa showed a complete and rapid recovery by day 14. Based on the full and rapid recovery the proposed NOEAEC was 0.05 µg/L.

Macrophytes. Maximum macrophyte growth was observed between day 86 to day 111 (August to September) in both controls and treated enclosures. No apparent treatment-related effects were observed. The overall NOEC was ≥1.0 µg/L.

See tables 7_4_3_5-1 to 7_4_3_5-5

4.4 Further remarks

On study day 86 (72 days after second application) a bioassay was conducted to establish whether *Daphnia longispina* would survive in test enclosures. Water samples were collected in the different test enclosures. A mixed population of D longispina was used for the bioassay. 10 organisms were introduced in the respective test vessels at 0.016 , 0.05 and 0.2 µg/l and 6 organisms at 1.0 µg/l. Biological observations were made at 3, 24 and 48 hours after introduction.

Mortality in the control and in the treatments were in the range 10-17%. No dose response was observed indicating that on day 86 D longispina would survive in all test enclosures.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The effect of the agricultural use of Cyperkill 10, EC (containing 100 g/l cypermethrin) on naturalized ecosystems was investigated in an outdoor pond study.

The enclosures were treated twice on 14 and 28 May 2002 with 0, 0.0016, 0.005, 0.016, 0.05, 0.2 and 1.0 µg a.s./l. An artificial pond 6X 6 m in area was subdivided in 22 pond-enclosures with stainless boxes (0.97 m^2) pushed down into the sediment. There were 6 replicates for the control. There were 3 replicates for the treatment groups 0.0016 to 0.05, 2 replicates for treatment groups 0.2 and 1 µg a.s./l

Each pond contained 5 cm clay layer with a 10-15 cm overlying layer natural sediment. The water depth of the pond was approximately 1.1 m.

Biological samples were collected both before and after treatment with last sampling on day 111. Abundance data were analyzed for 5 main categories of test organisms: zooplankton, emergent insects macrozoobenthos, phytoplankton and periphyton and for 2 additional data types : Chaoborus (combined sampling techniques), and blue-green algae.

Principal Response Curves, NOEC and EC50 were produced for each data category.

5.2 Results and discussion

Measured mean a.s. concentrations in water two hours after each application, for those enclosures where the nominal concentration was

Section A7.4.3.5
Annex Point IIIA XIII 3.4

Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk

Mesocosm study

above the LOQ of the analytical methods, ranged form 93% to 129 % of the nominal. This indicated that the target exposure concentration was achieved at each dosing concentration

Measured a.s. concentrations in sediment samples above the limit of detection (0.41 µg/kg dry sediment) ranged from 0.490 to 6.770 µg/kg sediment in one enclosure treated at 1 µg/l initial concentration (weekly samplings from day 2 to 84). No cypermethrin concentrations above the limit of detection were detected in any of the other control and treated enclosure sediment samples.

Zooplankton :

The lowest overall NOEC community determined by Principal Response Curve analysis was 0.0 5 µg a.s./l. The Principal Response Curve analysis indicated a recovery from day 56 onwards at 0.2 µg a.s./l. at the 1.0 µg a.s./l the PRC analysis indicated no recovery by the end of the study. A general change in the population structure of Daphnia longispina was not considered treatment-related but rather due to seasonal and successional reasons. Results of the bioassay conducted at day 86 showed mortality in the control and in the treatments were in the range 10-17%. No dose response was observed indicating that on day 86 D longispina would survive in all test enclosures.

Emergent insects :

Statistical analysis resulted in NOEC of 1 µg/l for almost all taxonomic groupings on all sampling occasions. These NOEC were considered unreliable due to low abundances in both control and treatment enclosures. The Principal Response Curve analysis indicated no significant treatment-related deviation from the control up to day 35. Although the deviation from the control increased from day 42 onwards a dose response was not apparent.

Phytoplankton :

No apparent treatment related effects were observed by the delayed fluorescence technique. Transient treatment related effects were observed by microscope counting. The lowest overall NOEC community was 0.005 µg a.s./l/.

The Principal Response Curve analysis indicated a deviation from the control at 0.016, 0.05, 0.2 and 1.0 µg a.s./l on day 28. At day 56 and 70, the deviation appears not to be dose-related (NOAEC = 1 µg a.s./l).

Macrophytes :

No apparent treatment-related effects were observed. The NOEC and NOAEC were 1.0 µg a.s./l.

5.3 Conclusion

The treated enclosures were shown to have been dosed correctly and the target exposure concentrations were achieved.

Although several overall NOEC values were lower than 0.05 µg/L, this concentration was considered to cause no ecologically adverse effects because all observed effects on the different communities (Zooplankton, Macrozoobenthos, Emergent insects, Phytoplankton and Periphyton) during the study were only transient and, therefore, considered of minor

Section A7.4.3.5
Annex Point IIIA XIII 3.4

Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk

Mesocosm study

ecological relevance. There were no long-term effects on population growth or reproduction. Given the rapid dissipation of the test item, the biological recovery indicated that the chemical was not biologically available in the sediment and, therefore, had no effect on the emergent insects.

The NOEAEC for all species is 0.05 µg/L based on:

Chaoborus (sampling techniques of larvae, adult emergence): From day 42 onwards recovery to control level was apparent at 0.05 µg a.s./L

The emergence of Baetidae from day 70 onwards at 0.05 µg a.s./L was equivalent or higher than in the control enclosures. Macrozoobenthos (Baetidae) recovery was observed at day 56-63 at 0.05 µg a.s./L

Complete recovery of Cladocera, Synchaeta, Keratella quadrata was observed at 0.05 µg a.s./L after 8 weeks

5.3.1	Reliability	1
5.3.2	Deficiencies	None

This study was 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 2008
Materials and Methods	Adopted
Results and discussion	Applicant's version is adopted
Conclusion	Applicant's version is adopted
Reliability	1
Acceptability	(acceptable)
Remarks	
	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*

Section A7.4.3.5
Annex Point IIIA XIII 3.4

Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk

Mesocosm study

Remarks

Table 7_4_3_5-1: Summary of NOEC values < 1.0 µg a.s./L, including an overall NOEC/NOAEC, for Zooplankton

Day of study	Total Zooplankton	Order Cladocera	Family Daphniidae	Daphnia longispina	Genus Chydorus	Sub-class Copepoda	Family Cyclopidae	nauplii	Class Rotatoria	Genus Polyarthra	Genus Synchaeta	Family Synchaetidae	Species Keratella quadrata	Genus Chaoborus
1						0.005		0.005						
3	0.016	0.016	0.016	0.016		0.005	0.016	0.005						<0.0016
7	0.2	0.016	0.016	0.016		0.005	0.005	0.005						0.005
14	0.2					0.05	0.016	0.05	0.2					0.005
15						0.05		0.05						N/A
17	0.2					0.05		0.05						<0.0016
21	0.2					0.05		0.05	0.2				0.2	<0.0016
28						0.05	0.05	0.2						<0.0016
35	0.05					0.05	0.2	0.05	(<0.0016)		0.0016	0.2	0.0016	<0.0016
42		0.05				0.05		0.05			<0.0016	0.2	0.016	<0.0016
49						0.2	0.2	0.2						0.2
56						0.2	0.2							0.2
63		(0.005)			0.016	0.2	0.2							0.2
70							0.2						0.2	
77													<0.0016	
84				See bioassay										
98							0.2							
Overall NOEC	0.016	0.016	0.016	N/A	0.05	0.005	0.005	0.005	0.2	0.2	<0.0016	0.2	0.016*	<0.0016
NOAEC	1.0	**0.05**	**0.05**		**0.05**	1.0	0.2	1.0			**0.05**		**0.05**	0.2

(data in brackets not reliable)

Table 7_4_3_5-2 : Summary of all NOEC values < 1.0 µg a.s./L (NOEC = 1 µg/L in the empty boxes) for Emergent insects

Day of study	Class Insecta	Order Diptera (*)	Family Chironomidae (*)	Sub-family Chironomimae (*)	Genus Chironomus (*)	Sub-family Orthrodiinae (*)	Genus cricotopus (Isocladius) (*)	Genus Corynoneura (*)	Genus Chaoborus (*)	Order Coleoptera	Family Baetidae
3+7	0.2										
14											
17+21											
28	< 0.0016										
35											
42	0.2										
49	0.2										
56											
63					0.2						< 0.0016
70											
77											
84											
91										(0.2)	
98											
105											
Overall NOEC	1.0	0.2	0.2	1.0	0.2	1.0	1.0	1.0	<0.0016	1.0	0.016
NOAEC	1.0								**0.05 ~~(0.2)~~**	1.0	**0.05 ~~(0.2)~~**

Data between brackets : not relevant

Table 7_4_3_5-3: Summary table of all NOEC values < 1.0 µg a.s./L (NOEC = 1 µg/L in the empty boxes) for Arthropoda

Day of study	Family Planorbidae (Gastropodae)	Family Baetidae (Ephemeroptera)	Family Chironomidae (Diptera)
4	0.016		
8	0.0016	<0.0016	0.005
13			
18	0.2		0.2
22			0.2
29			(<0.0016)
36	0.2	0.0016	0.05
43		0.005	0.2
50		0.005	0.2
57		0.005	0.2
64		0.05	
71		0.2	
78		0.2	
85		0.2	
Overall NOEC	0.016	0.005	0.005
NOAEC	1.0	**0.05**	0.2 ~~(1.0)~~

Data between brackets : not relevant

Table 7_4_3_5-4: Summary table of all NOEC values < 1.0 µg a.s./L for Phytoplankton

Day of study	Total phytoplankton	Phylum Chrysophyta	Class Bacillariophyceae	Family Nitzschiaceae	Class Chrysophyceae	Family Synuraceae	Family Chromulinaceae	Class Chlorpphyceae	Family Chlorelallaceae	Genus Monoraphidium
3			0.2	0.2						
13	<0.0016		0.2				0.2		0.2	
17	<0.0016									
28	0.016	0.0016	0.005	0.2		0.005				
56	0.005	0.005	0.016	0.2						
70	0.005									
Overall NOEC	<0.0016	0.0016	0.005	0.2	1.0	0.005	1.0	1.0	0.016	1.0
NOAEC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Day of study	Family Chlorococcaceae	Family Scenedesmaceae	Genus Tetrachlorella	Genus Chlamydomonas	Class Cyanophyceae	Class Euglenophyceae	Class Cryptophyceae	Family Cryptomonadaceae	Genus Cryptomonas	Genus Chromonas
3										
13		0.05					<0.0016	<0.0016		<0.0016
17							0.0016	0.0016		0.0016
28		0.005	0.2	0.2	0.016					
56		0.016	<0.0016		0.005	0.005	<0.0016	<0.0016	<0.0016	
70					0.005					
Overall NOEC	1.0	0.005	0.016	0.2	1.0	0.005	<0.0016	<0.0016	<0.0016	<0.0016
NOAEC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Table 7_4_3_5-5: Summary table of all NOEC values < 1.0 µg a.s./L for Periphyton

Day of study	Total Periphyton	Class Bacillariophycaed	Family Nitzschiaceae	Class Chlorophycea	Genus Chlorella	Family Chlamydomonaceae	Family Dictyosphaericaceae	Family Tetrasporaceae	Family Coleochaetaceae	Class Chrysophyceae	Class Euglenophyceae	Class Cyanophyceae	Class Chroococcaceae	Class Oscillatoriaceae
4										0.005			0.05	
14														
18														
29														
Overall NOEC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.005	1.0	1.0	0.05	1.0
NOAEC	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

Official use only

		1 REFERENCE
1.1	**Reference**	Servajean, E. (2005), Laboratory assessment of the side-effects of cypermethrin, technical grade, on the mineralization of nitrogen; Phytosafe s.a.r.l., Report no. 04-99-058-ES, 17 October 2005 (unpublished). Dates of work: 21 February 2005 – 26 April 2005
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD guideline 216 as adopted in January 2000.
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	SL25163S63
3.1.2	Specification	As given in section 2
3.1.3	Purity	93.05%
3.1.4	Composition of Product	Not applicable, test conducted with a.s.
3.1.5	Further relevant properties	Not applicable
3.1.6	Method of analysis	The treatment solutions used in the final test were checked for cypermethrin content on the day of the treatment applications. The method was based on GC/FID analysis by external calibration.
3.2	**Reference substance**	Yes, Fumical, 510 g/L metam-sodium
3.2.1	Method of analysis for reference substance	The concentration of Fumical was calculated on the basis of a 1000 L/ha application rate, a uniform incorporation of the substance to a depth of 5 cm, and a soil bulk density of 1.5 : 1.3 mL/kg dry soil.
3.3	**Testing procedure**	
3.3.1	Soil sample / inoculum / test organism	See Table A7_5_1_1-1 for details of the soil collection and pre-treatment.
3.3.2	Test system	See Table A7_5_1_1-2

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

3.3.3	Application of TS	Details of the treatment solutions are reported in Table A7_5_1_1-3 for the range-finding test and the final test respectively. In both cases, the most concentrated treatment solution was prepared by diluting the pure test substance in acetone, and the subsequent treatment concentrations by serial dilution in acetone. In every case, 3.0 mL of the treatment solution was carefully mixed with 10 g of pure quartz sand, and the sand was allowed to dry for 1 hour in a well ventilated area. Aliquots of sand were then firmly mixed with the soil portion.
3.3.4	Test conditions	See Table A7_5_1_1-4 The test was carried out in the dark. A thermo recorder was used as a permanent control of the temperature conditions in the test room. Range-finding test: 19.0 to 21.5 °C Final test: 19.0 to 19.5°C Weight deviation between two successive sampling periods served for the calculation of the evaporation during the incubation period. Extreme values were calculated to correspond to 40%, at least, of the soil total water holding capacity (WHC). The soil moisture was adjusted to 50%-WHC with demineralised water just before sampling.
3.3.5	Test parameter	Inhibition of microbial nitrogen transformation
3.3.6	Analytical parameter	Nitrate production
3.3.7	Duration of the test	28 days
3.3.8	Sampling	Each soil and replicate was analysed for nitrate formation within 6 hours of addition of the product, and then on days 7 and 28 of the test, at least. An accurately weighed amount of soil corresponding to 10.0 g dry soil was sampled from each treatment and replicate. The nitrates were assessed according to the standardised method n° 300.1 of EPA Revision 1.0 dated April 27, 1999. The nitrates were extracted by shaking the sample with 40 mL of a 50 mg/L ethylenediamine solution, mixed with 0.1% v/v H_2SO_4. The mixtures were shaken for 60 minutes, and then centrifuged at 5000 rpm for 10 min. The extract solutions were then diluted 1/10 v/v in the ethylenediamine solution and stored in amber glass vials until analysis. When necessary, the samples were stored at 4-8°C until analysis, but not for more than 2 days. The supernatants were analysed for nitrate concentration by ionic chromatography previously calibrated for nitrate concentrations ranging between 0.125 and 12.547 mg/L.

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

3.3.9	Monitoring of TS concentration	The treatment solutions used in the final test were checked for cypermethrin content on the day of the treatment applications. The method is based on GC/FID analysis by external calibration. Soon after the treatment application, duplicate samples of the 96 mg/kg treated soil were extracted with acetone so that even distribution of the treatment in soil was confirmed. Percent recovery of the nominal treatment concentration was calculated.
3.3.10	Controls	**Water control:** The water control received 10 g of untreated sand. **Solvent control:** The solvent control received 10 g of sand previously mixed with 3.0 mL of acetone. **Toxic standard:** The concentration of Fumical was calculated on the basis of a 1000 L/ha application rate, a uniform incorporation of the substance to a depth of 5 cm, and a soil bulk density of 1.5 : 1.3 mL/kg dry soil.
3.3.11	Statistics	F-variance analysis at 5% confidence level
		4 RESULTS
4.1	**Range finding test**	Performed
4.1.1	Concentration	0.1, 1.0, 9.4, 93.9, 939.2 mg/kg cypermethrin
4.1.2	Effect data	124.3, 127.7, 121.8, 76.2, 99.5 % nitrate compared to the water control on day 28
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	8.9, 16.1, 28.9, 52.0, 93.6 mg a.s. /kg dry soil. The concentration of the treatment solutions were confirmed by HPLC analysis. Percent deviation compared to the nominal value was 5.5 %
4.2.2	Actual concentrations of test substance	Soon after the treatment application, duplicate samples of the most concentrated soil were extracted with acetone so that even distribution of the treatment in soil was confirmed. Percent recovery of the nominal treatment concentration was calculated and was found to be as high as 9.6% and thus slightly higher than the threshold value of 7%. The explanation was that the accuracy of the quantification might have been reduced as an additional concentration step was needed prior to the analysis of the extracts for such low soil treatment concentrations.
4.2.3	Growth curves	Not applicable, only the top dose showed a significant difference in the production of nitrate when compared to the water control.
4.2.4	Cell concentration data	Not applicable
4.2.5	Concentration/ response curve	Not applicable, only the top dose showed a significant difference in the production of nitrate when compared to the water control.

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

4.2.6	Effect data	See Table A7_5_1_1-5 The nitrate concentration was the same level at test initiation for every treatment and replicate. On day 7, the nitrate concentration was reduced by 1 to 2 mg/kg in the water control, and in the solvent control as well. That was also the case for the 8.9 mg/kg cypermethrin treated soil. In all other cases (including the Fumical treated group) the decrease was much more accentuated than that in the water control soil. However the deviation within a same treatment group was high, and F-variance analysis at 5% confidence level concluded that the deviation to the water control group was not significant, except for the 93.6 mg/kg cypermethrin treated group. On day 28 of the incubation period the production of nitrate ranged between 98.1 % and 105.8 % that of the water control group for treatment groups ranging between 8.9 and 52.0 mg/kg dry soil. In the 93.6 mg/kg group, the production of nitrate was only 78.9 % that of the water control group and F-variance analysis at 5%-confidence level concluded that the deviation was significant. The conclusions were then as follows: - LOEC = 93.6 mg/kg dry soil - NOEC = 52.0 mg/kg dry soil In the 93.6 mg/kg treated soil the nitrate content compared to the water control was reduced by 21 %. The EC_{50} value was not determined.
4.2.7	Other observed effects	See above point
4.3	**Results of controls**	See Table A7_5_1_1-5
4.4	**Test with reference substance**	See Table A7_5_1_1-5 Fumical is classically a 'soft' toxic reference, intermediate values should differ when compared to the water control. This was met in this study even though the deviation within the Fumical treated group did not show a statistically significant deviation. On day 28 of the incubation period the nitrate concentration should be almost restored. This is to verify that the biomass dynamics is restored from a 28 day-exposure period with a substance which has no long-term effect.

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was aimed at the determination of the treatment concentration susceptible to reduce the formation of nitrate as compared to the control. The final test was performed using three replicate bulk samples of treated soil for each of five treatment concentrations between 8.9 and 93.6 mg/kg so that the NOEC (No Observed Effect Concentration) was encircled.

After 0, 7 and 28 days of incubation, samples were extracted with an aqueous solvent, and the quantities of nitrate in the extracts were determined.

The quantities of nitrate formed in treated samples were compared to that of a water control, of a solvent control and of a toxic reference. Percent deviation compared to the water control was calculated with respect to each test concentration, respectively.

F-variance analysis at 5% confidence level was used to evaluate significant deviation to the water control.

5.2 Results and discussion

On day 7, the nitrate concentration was reduced by 1 to 2 mg/kg in the water control, and in the solvent control as well. That was also the case for the 8.9 mg/kg cypermethrin treated soil. In all other cases (including the Fumical treated group) the decrease was much more accentuated than that in the water control soil. However the deviation within a same treatment group was high, and F-variance analysis at 5% confidence level concluded that the deviation to the water control group was not significant, except for the 93.6 mg/kg cypermethrin treated group.

On day 28 of the incubation period the production of nitrate ranged between 98.1 % and 105.8 % that of the water control group for treatment concentrations ranging between 8.9 and 52.0 mg/kg dry soil.

In the 93.6 mg/kg, the production of nitrate was only 78.9 % that of the water control group and F-variance analysis at 5%-confidence level concluded that the deviation was significant.

5.2.1 NOEC 52.0 mg/kg dry soil

5.2.2 EC_{10} Not determined

5.2.3 EC_{50} Not determined

5.3 Conclusion

Variation of the nitrate concentration in the replicate water control samples was less than ±15% of the mean value, therefore the validity criteria was fulfilled. Results from this study using multiple concentrations of cypermethrin showed that at day 28 only the top dose (93.6 mg a.s./kg soil) showed a significant deviation in the production of nitrate when compared to the water control. Therefore the NOEC was considered to be 52.0 mg/kg dry soil. The EC50 was not determined.

5.3.1 Reliability 1

5.3.2 Deficiencies No

Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

	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 2008
Materials and Methods	Applicant version is acceptable
Results and discussion	Applicant version is acceptable
Conclusion	Applicant's version is accepted.
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_5_1_1-1: Microbial sample / Inoculum

Criteria	Details
Nature	soil sample
Sampling site 1:	
Geographical reference on the sampling site	Mr Castagnède, parcelle Guilhem (40110 Arengosse, France).
Data on the history of the site	Plant cover = wheat, under agro biological practice. No treatment with pesticide or organic fertilizer had been applied for at least 6 months.
Use pattern	Agricultural biological practice.
Depth of sampling [cm]	20
Sampling site 2:	
Geographical reference on the sampling site	Mr Dupouy, parcelle Parille (40500 Coudures, France).
Data on the history of the site	Plant cover = triticales, under agro biological practice. No treatment with pesticide or organic fertilizer had been applied for at least 6 months.
Use pattern	Agricultural biological practice.
Depth of sampling [cm]	20
Sand / Lime / Clay content [% dry weight]	56.0/43.0/1.0 (NF X31-107)
pH *(in water)*	6.70 (ISO 10390)
Organic carbon content [% dry weight]	1.77 (NF X31-109)
Nitrogen content [% dry weight]	0.18 (ISO 11261)
Cation exchange capacity [meq./100g]	14.4 (NF X31-130)
Initial microbial biomass	1.56 % total organic carbon (ISO 14240)
Reference of methods	(As given above)
Collection / storage of samples	The natural soil was collected on February 02, 2005. According to ISO 10381-6. Sampling was avoided during or immediately following long period of drought or water logging. The soil samples were transported using containers and under temperature conditions which guarantee that the initial soil properties are not significantly altered.
Preparation of inoculum for exposure	The soils were previously air dried in order to allow sieving. The vegetation, soil animals, stones and large soil clumps were removed by sieving to a particle size less than or equal to 2 mm. They were then mixed 50/50 w/w dry weight, and the resulting soil was sampled for further analysis. The stock soil was stored outdoors in plastic containers, which allowed free access of air.
Pre-treatment	Bulk samples of soil were used for each treatment and replicate. The soil was divided into distinct portions corresponding to 1000.0 g dry soil, and moistened to 45% of its total water holding capacity with demineralised water. The soil fractions were pre-incubated for three days, in the dark, at 20°C. The duration of the pre-incubation period was 3 days for the range-finding test, and 6 days for the final test. At the end of the pre-incubation period the soil fractions were amended with 5.0 g powdered lucerne-grass-green meal, milled to pass a 0.5 mm sieve.

Table A7_5_1_1-2: Test System

Criteria	Details
Culturing apparatus	Appropriate containers with sufficient headspace
Number of vessels / concentration	3
Aeration device	None specified
Measuring equipment	Soil extracts were analysed for nitrate concentration by ionic chromatography (Ionic chromatograph Metrohm Compact IC 761) previously calibrated for nitrate concentrations ranging from 0.125-12.547 mg/L
Test performed in closed vessels	No

Table A7_5_1_1-3: Application of test substance

Treatment solutions	Dilution preparation			Subsequent treatment concentration
	Nature	Amount	Acetone	mg a.i./kg dry soil
Range-finding test				
Solution 5	Cypermethrin	3.3645 g	to 10 mL	939.20
Solution 4	Solution 5	1.0 mL	to 10 mL	93.92
Solution 3	Solution 4	1.0 mL	to 10 mL	9.39
Solution 2	Solution 3	1.0 mL	to 10 mL	0.94
Solution 1	Solution 2	1.0 mL	to 10 mL	0.09
Final test				
Solution 5	Cypermethrin	0.838.2 g	to 25 mL	93.59
Solution 4	Solution 5	13.9 mL	to 25 mL	52.04
Solution 3	Solution 4	13.9 mL	to 25 mL	28.93
Solution 2	Solution 3	13.9 mL	to 25 mL	16.09
Solution 1	Solution 2	13.9 mL	to 25 mL	8.94

Table A7_5_1_1-4: Test conditions

Criteria	Details
Organic substrate	5.0 g powdered lucerne-grass-green meal, milled to pass a 0.5 mm sieve
Incubation temperature	Range-finding test: 19.0 to 21.5 °C Final test: 19.0 to 19.5°C
Soil moisture	Weight deviation between two successive sampling periods served for the calculation of the evaporation during the incubation period. Extreme values were calculated to correspond to 40%, at least, of the soil total water holding capacity (WHC). The soil moisture was adjusted to 50%-WHC with demineralised water just before sampling.
Method of soil incubation	Bulk samples of soil were used for each treatment and replicate.
Aeration	No

Table A7_5_1_1-5: Concentration of nitrate (N-NO_3 mg/kg dry weight) on days 0, 7 and 28 of the incubation period

Day (D)	D(0)			D(7)			D(28)		
Water control	18.3	18.3	18.3	14.1	14.4	17.2	62.0	59.5	59.7
Mean ± S.D.	**16.6 ± 8.6%**			**15.2 ± 11.3%**			**60.4 ± 2.3%**		
Solvent control	16.1	16.1	16.1	14.4	16.7	14.7	64.4	66.9	59.6
Mean ± S.D.	**16.6 ± 2.6%**			**15.3 ± 8.0%**			**63.6 ± 5.8%**		
Cypermethrin 8.9 mg/kg	16.8	16.8	16.8	15.0	15.9	16.7	61.9	62.6	65.9
Mean ± S.D.	**17.2 ± 2.2%**			**15.9 ± 5.3%**			**63.5 ± 3.4%**		
Cypermethrin 16.1 mg/kg	16.8	16.8	16.8	14.1	10.8	11.9	55.7	58.9	64.0
Mean ± S.D.	**16.6 ± 2.1%**			**12.3 ± 13.7%**			**59.6 ± 7.0%**		
Cypermethrin 28.9 mg/kg	16.4	16.4	16.4	9.7	12.7	15.2	55.0	61.6	63.1
Mean ± S.D.	**16.7 ± 3.1%**			**12.5 ± 21.7%**			**59.9 ± 7.2%**		
Cypermethrin 52.0 mg/kg	16.9	16.9	16.9	11.3	15.8	13.9	59.6	60.7	63.1
Mean ± S.D.	**16.7 ± 0.7%**			**13.6 ± 16.5%**			**61.1 ± 2.9%**		
Cypermethrin 93.6 mg/kg	16.9	16.9	16.9	10.5	12.0	10.9	48.9	52.7	51.7
Mean ± S.D.	**16.6 ± 1.7%**			**11.1 ± 7.2%**			**51.1 ± 3.9%**		
Fumical 1.3 mL/kg	16.5	16.5	16.5	11.6	15.7	16.1	59.4	60.0	65.2
Mean ± S.D.	**16.5 ± 1.4%**			**14.5 ± 17.1%**			**61.5 ± 5.2%**		

Section A7.5.1.2 (01) Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	Inglesfield, C. (1984); Toxicity of the pyrethroid insecticides cypermethrin and WF 85871 to the earthworm, *Eisenia foetida* Savigny; Bull. Environ. Contam. Toxicol. (1984) 33:568-570 (CYP/T61) (published).	
1.2	**Data protection**	No	
1.2.1	Data owner	Published article	
1.2.2			
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, method used is comparable to OECD guideline 207.	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed, however the test laboratory was a well-recognised facility at the time the study was conducted.	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Cypermethrin	
3.1.1	Lot/Batch number	Not specified in published report	
3.1.2	Specification	Not specified in published report	
3.1.3	Purity	Technical grade active substance, purity was not specified in the published report.	X
3.1.4	Composition of Product	Not applicable, test was performed with the active substance	
3.1.5	Further relevant properties	Due to the low water solubility of cypermethrin, the test article was tested as technical grade material dissolved in acetone.	
3.1.6	Method of analysis	Not applicable, results are based on nominal concentrations of test substance applied to the soil substrate at study initiation.	
3.2	**Reference substance**	Yes, Chloracetamide was used as the reference substance.	
3.2.1	Method of analysis for reference substance	Not applicable, results are based on nominal concentrations of reference substance applied to the soil substrate at study initiation.	
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	Cypermethrin was dissolved in acetone before being introduced onto the soil substrate (see table A7_5_1_2-1)	

Section A7.5.1.2 (01) Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

3.3.2	Application of the test substance	For the test substance and acetone control 5ml of test solution was sprayed onto the soil substrate using a Brinkman TLC hand-held sprayer and mixed to ensure a uniform distribution. The solvent was then evaporated off and deionised water mixed into the medium giving a weigt of 750g of soil with a moisture content of 20% (dry weight).
3.3.3	Test organisms	See table A7_5_1_2-2
3.3.4	Test system	See table A7_5_1_2-3
3.3.5	Test conditions	Ssee table A7_5_1_2-4
3.3.6	Test duration	14 days
3.3.7	Test parameter	Mortality
3.3.8	Examination	After 7 days any dead worms were removed and discarded. After a further 7 days (day 14 of the study) the number of live worms in each dish was recorded.
3.3.9	Monitoring of test substance concentration	No
3.3.10	Statistics	Graphical interpolation was used to calculate the LC_{50} for the reference substance.
		4 RESULTS
4.1	**Filter paper test**	Not performed
4.2	**Soil test**	
4.2.1	Initial concentrations of test substance	0.1, 1.0, 10 and 100 mg cypermethrin /kg soil (dry weight basis).
4.2.2	Effect data (Mortality)	See table A7_5_1_2-5 and table A7_5_1_2-6
4.2.3	Concentration / effect curve	Not included in published article
4.2.4	Other effects	None reported
4.3	**Results of controls**	
4.3.1	Mortality	See table A7_5_1_2-7
4.3.2	Number/ percentage of earthworms showing adverse effects	Not applicable, only mortalities were recorded.
4.3.3	Nature of adverse effects	Not applicable
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	0.1, 1.0, 10 and 100 mg/kg soil

Section A7.5.1.2 (01) Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

4.4.2	Results	See table A7_5_1_2-7
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The method used was accepted for inclusion into OECD and EEC guidelines. Worms (Eisenia foetida Savigny) were maintained for 14 days in a soil-like medium mixed with four different concentrations of test substance (0.1, 1.0, 10 and 100 mg/kg soil). A reference substance (chloracetamide) was also tested at the same concentrations along with a solvent and water control. 40 worms were used per dose level and the number of surviving worms recorded at day 14.
5.2	**Results and discussion**	Cypermethrin did not cause significant mortality at any concentration level.
5.2.1	LC_0	
5.2.2	LC_{50}	100 mg/kg soil
5.2.3	LC_{100}	
5.3	**Conclusion**	The test was considered valid as there were no mortalities in the solvent control and only 1 worm was found dead in the water control (2.5% mortality).
5.3.1	Other Conclusions	
5.3.2	Reliability	2
5.3.3	Deficiencies	Yes. As this is a published article, the report is somewhat limited particularly with respect to the raw data. However the work was conducted by Shell Research Ltd, a well recognised facility at that time, and the method used was accepted for inclusion in the OECD and EEC guidelines. A summarised table of results is presented which clearly show that cypermethrin had no toxic effects. Therefore the study is considered to be sufficiently robust to cover this particular data point and any further testing would be unnecessary. In addition this study was accepted in the Cypermethrin monograph published under the Plant protection Products 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 2008
Materials and Methods	Material and methods is similar to the OECD guideline 207 The technical grade of the active used for the test is not known.
Results and discussion	The LC_{50} dosen't seem to have been reach since the mortality of the earthworms stay at a similar level from the lowest to the highest concentration.

Section A7.5.1.2 (01) Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

Conclusion	LC_0: LC_{50}:>100mg/kg_{soil} LC_{100}: *Other conclusions:* The results shows that cypermethrin doesn't have acute toxicity up to 100mg/kg soil
Reliability	3 Since this is only a publication with poor details and since the LC_{50} range seems not te be reached.in the study
Acceptability	Acceptable but only indicative
Remarks	

	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_5_1_2-1: Preparation of TS solution

Criteria	Details
Dispersion	No
Vehicle	Acetone
Concentration of vehicle	Not specified
Vehicle control performed	Yes, four replicates were prepared as acetone controls.
Other procedures	None

Table A7_5_1_1-2: Test organisms

Criteria	Details
Species/strain	Eisenia foetida Savigny
Source of the initial stock	Not specified in published article
Culturing techniques	Not specified in published article.
Age/weight	2-3 months old (sexually mature), each weighing between 300-600mg
Pre-treatment	Worms were acclimitised for several days at 20 (±1)°C in a medium of sand, clay, peat and horse manure.

Table A7_5_1_1-3: Test system

Criteria	Details
Artificial soil test substrate	Industrial sand, kaolinite clay (English Chiina clay GTY powder) and sphagnum peat ('Shamrock' Irish moss peat) were mixed together in a ratio of 7:2:1 respectively. The pH was adjusted to 6.5 by the addition of calcium carbonate. The moisture content of the soil was 20% (dry weight basis).
Test mixture	0.1, 1.0, 10 and 100 mg/kg soil (dry weight basis).
Size, volume and material of test container	Glass crystalline dishes (150mm diameter x 75mm height)
Amount of artificial soil (kg)/ container	750 g of treated soil / container
Nominal levels of test concentrations	Both the test article cypermethrin and the reference substance were tested at 0.1, 1.0, 10 and 100 mg/kg soil (dry weight basis).
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Continuous light source (unspecified in published article)
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7_5_1_2-4: Test conditions

Criteria	Details
Test temperature	20 (± 1) °C
Moisture content	20% (re-adjusted after 7 days)
pH	6.5
Adjustment of pH	No, adjusted at at soil preparation only
Light intensity / photoperiod	Not specified in published article
Relevant degradation products	Not determined

Table A7_5_1_2-5: Mortality data – Test substance

Test Substance Concentration (nominal) [mg/kg artificial soil]	Mortality			
	Number		Percentage	
	7 d	14 d	7 d	14 d
0.1	ND	2	ND	5.0
1.0	ND	3	ND	7.5
10	ND	2	ND	5.0
100	ND	2	ND	5.0

ND=Not determined/Not Reported

Table A7_5_1_2-6: Effect data – Test Substance

	14 d [mg/kg soil][1]	95 % c.l.
LC_{50}	>100	

[1] Based on nominal (n) concentration

Table A7_5_1_2-7: Mortality data – Reference Substance and Controls

Chloracetamide Concentration (nominal) [mg/kg artificial soil]	Mortality			
	Number		Percentage	
	7 d	14 d	7 d	14 d
0.1	ND	0	ND	0
1.0	ND	0	ND	0
10	ND	0	ND	0
100	ND	40	ND	100
Acetone control	ND	0	ND	0
Water control	ND	1	ND	2.5

Table A7_5_1_2-6: Effect data – Reference Substance

	14 d [mg/kg soil][1]	95 % c.l.
LC_{50}	30	

[1] Based on nominal (n) concentration

Table A7_5_1_2-8: Validity criteria for acute earthworm test according to OECD 207

	fulfilled	Not fulfilled
Mortality of control animals < 10%	**Yes**	

Section A7.5.1.2 (02) Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

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		1 REFERENCE
1.1	**Reference**	Dickhaus, S. (1989); Ecotoxicological investigation of Cyperkill 10 in the earthworm; Pharmatox Beratung und Forschung GmbH, Germany; report no. EH/B.1-7-96-89 (CYP/T127), July 1989 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Chimac-Agriphar s.a.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	OECD Guideline for Toxicology of Chemicals (1981) BBA-Guideline (1982) Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, FDA (1959)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 METHOD
3.1	**Test material**	Cypermethrin 100 g/L (Cyperkill 10EC)
3.1.1	Lot/Batch number	FSG 08510 I
3.1.2	Specification	Not applicable
3.1.3	Purity	Not applicable
3.1.4	Composition of Product	Emulsifiable Concentrate containing 100 g/L cypermethrin cis:trans/40:60
3.1.5	Further relevant properties	Not applicable
3.1.6	Method of analysis	Not applicable, results are based on nominal concentrations of test substance applied to the soil substrate at study initiation.
3.2	**Reference substance**	Not used
3.2.1	Method of analysis for reference substance	Not applicable.
3.3	**Testing procedure**	
3.3.1	Preparation of the test substance	Formulated product was incorporated directly into the test substrate.
3.3.2	Application of the test substance	Test substance was incorporated directly into the test substrate.
3.3.3	Test organisms	See table A7_5_1_2-1
3.3.4	Test system	See table A7_5_1_2-2

Section A7.5.1.2 (02) Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

3.3.5	Test conditions	Ssee table A7_5_1_2-3
3.3.6	Test duration	14 days
3.3.7	Test parameter	Mortality
3.3.8	Examination	At 7 and 14 days. Substrate spread onto glass plate and worms counted. Animals declared dead if they did not respond to mechanical stimulation at the anterior end. Any dead worms were removed after the 7 day assessment. Bodyweights (10 animals per group) was also recorded after 7 and 14 days.
3.3.9	Monitoring of test substance concentration	No
3.3.10	Statistics	Graphical interpolation was used to calculate the LC_{50} for the reference substance.

4 RESULTS

4.1	**Filter paper test**	Not performed
4.2	**Soil test**	
4.2.1	Initial concentrations of test substance	0, 20, 100, 250, 500 1000 mg /kg soil (dry weight basis).
4.2.2	Effect data (Mortality)	See table A7_5_1_2-4 and table A7_5_1_2-6
4.2.3	Other effects	Dose-related difference in weight gain compared to control group.
4.3	**Results of controls**	
4.3.1	Mortality	See table A7_5_1_2-4 (negative control only)
4.3.2	Number/ percentage of earthworms showing adverse effects	Not applicable, only mortalities were recorded.
4.3.3	Nature of adverse effects	Not applicable
4.4	**Test with reference substance**	Not Performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The method used was in accordance with OECD guidelines. Test material was an EC formulation containing 100 g/L cypermethrin. Worms (Eisenia foetida Savigny) were maintained for 14 days in an artificial substrate mixed with five different concentrations of formulated product (20, 100, 250, 500, 1000 mg/kg soil) plus an untreated control (substrate only). 40 worms were used per dose level and the number of surviving worms recorded at days 7 and 14.

Section A7.5.1.2 (02) Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

5.2	**Results and discussion**	
5.2.1	LC_0	100 mg/kg
5.2.2	LC_{50}	575 mg/kg soil
5.2.3	LC_{100}	1000 mg/kg soil
5.3	**Conclusion**	The test is considered valid as there were no mortalities in the untreated control.
5.3.1	Other Conclusions	
5.3.2	Reliability	1
5.3.3	Deficiencies	No
		Results of this study are included in the Cypermethrin monograph published under the Plant Protection Products 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 2008
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is adopted.
Conclusion	LC_0: 100mg/kg_{soil} LC_{50}:575mg/ kg_{soil} LC_{100}:1000mg/ kg_{soil}
Reliability	1
Acceptability	Acceptable
Remarks	No GLP certificate, only a declaration of compliance.
	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_5_1_2-1: Test organism

Criteria	Details
Species/strain	Eisenia foetida Savigny
Source of the initial stock	Not specified in report
Culturing techniques	Not specified in report
Age/weight	Minimum 2 months old, 300-600 mg live weight
Pre-treatment	Not specified in report

Table A7_5_1_1-2: Test system

Criteria	Details
Artificial soil test substrate	Quartz sand containing 10% Sphagnum-peat soil (pH 5.5-6.0, air-dried and finely crushed), 20% Kaolin, ca. 1% CaCo3 powder (for pH adjustment). Particle size 0.05-0.2 mm, water content approx. 35% (dry weight basis).
Test mixture	Stock solution of 100 g/L cypermethrin (formulated product)
Size, volume and material of test container	Glass dishes with perforated lid
Amount of artificial soil (kg)/ container	500 g of dry substrate / container
Nominal levels of test concentrations	0, 20, 100, 250, 500, 1000 mg/kg soil (dry weight basis)
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Continuous light source (700 lux)
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7_5_1_2-3: Test conditions

Criteria	Details
Test temperature	20 (± 2) °C
Moisture content	35%
pH	5.5-6.0
Adjustment of pH	No, adjusted at at soil preparation only
Light intensity / photoperiod	24 hour illumination
Relevant degradation products	Not determined

Table A7_5_1_2-4: Mortality data

Test Substance Concentration (nominal) [mg/kg artificial soil]	Mortality			
	Number		Percentage	
	7 d	14 d	7 d	14 d
0	0	0	0	0
20	0	0	0	0
100	0	0	0	0
250	1	2.5	1	2.5
500	6	15	6	15
1000	40	100	40	100

Table A7_5_1_5: Development of bodyweight (g)

Test Substance Concentration (nominal)[mg/kg artificial soil]	Initial weight (mean of 10 worms/group)	Final weight (14 days)	Weight gain (mg/animal)
0	3.5	5.4	1900
20	3.6	5.6	2000
100	3.4	4.8	1400
250	3.6	4.2	600
500	3.7	4.1	400
1000	3.6	-	-

Table A7_5_1_6: Effect data – Test Substance

	14 d [mg/kg soil][1]	95 % c.l.
LC_{50}	575 (400-750)	

[1] Based on nominal (n) concentration

Table A7_5_1_2-7: Validity criteria for acute earthworm test according to OECD 207

	fulfilled	Not fulfilled
Mortality of control animals < 10%	Yes	

Section IIIA.7.5.1.3 **Annex Point IIIA XIII 3.4**	**Terrestrial plant toxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [√]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	H.Mills (2002); Cypermethrin – literature search: phytotoxic effects on non-target flora; Covance Laboratories Ltd; study no. COV_MCC/001 (company ref. CYP/T322), unpublished. There are no preliminary studies on non-target plants available for cypermethrin. This active substance has a well-established use in agriculture where it has been used for over 15 years as a broad-spectrum insecticide on a wide range of agricultural and horticultural crops. Indeed cypermethrin is now listed on Annex I of directive 91/414/EC having shown acceptable levels of safety. Rather than perform further studies on this well-known molecule the above literature search was carried out. Although there is much published data on cypermethrin, there were very few references to phytotoxic effects. This in itself indicates that the use of cypermethrin does not cause any concern in terms of toxicity to plant species. From the numerous field trials submitted as part of the agrochemical dossier, cypermethrin demonstrated no phytotoxic effects when applied to a wide variety of crops (cabbage, beans, winter wheat, winter oilseed rape, spring oilseed rape, winter and spring barley) at application rates of 12.5-25.0 g a.s./ha. In addition to the company's field trial data, public domain data confirms that cypermethrin does not cause phytotoxicity on younger plants (cereals, oilseed rape, soybean, tomato) : WHO report N°58, The Pesticide Manual. In the absence of any phytotoxic effects resulting from the use of cypermethrin in agriculture, it is felt that toxicity test on terrestrial plants are not required to support its safe use as a biocidal product where exposure to plant species will be much lower in comparison. This literature search was evaluated and accepted under Directive 91/414/EC.	
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 2008	
Evaluation of applicant's justification	Applicant justification is accepted in a first tier. However since the soil compartment is expected to be at risk, a new test might be requested later on.	

Section IIIA.7.5.1.3 **Annex Point IIIA XIII 3.4**	**Terrestrial plant toxicity**
Conclusion	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 A7.5.2.1 Earthworm reproduction study
Annex Point IIIA XIII 3.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	Servajean, E. (2011); Earthworm reproduction test with Cypermethrin; Phytosafe s.a.r.l., France, report no. 11-99-064-ES, 30th November 2011 (unpublished). Dates of experimental work: 22nd August 2011 – 14 November 2011	
1.2	**Data protection**	Yes	
1.2.1	Data owner	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**	OECD 222 (April 2004)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Definitive test: the adult worms were fed on day 0 instead of day 1. Definitive test: additional assessments were performed out from the un-populated units for cypermethrin concentrations on days 7, 13, and 21. Definitive test: the soil moisture was 30-40% of the soil total water holding capcity instead of 40-60% In the opinion of the Study Director, the above changes did not adversely affect the quality and integrity of the study.	
		3 METHOD	
3.1	**Test material**	Cypermethrin cis:trans/40:60	
3.1.1	Lot/Batch number	SL25163S63	
3.1.2	Specification	As given in section 2.	
3.1.3	Purity	93.56% w/w	
3.1.4	Composition of Product	Not applicable, test was carried out on active substance.	
3.1.5	Further relevant properties	Even distribution of test substance in the soil was focused as a critical point in the study.	
3.1.6	Method of analysis	Test item concentration in soil extract determined by HPLC	
3.2	**Reference substance**	Carbendazim	
3.2.1	Method of analysis for reference substance	Not applicable	
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	Stock solutions of Cypermethrin were prepared in acetone.	

Section A7.5.2.1 Earthworm reproduction study
Annex Point IIIA XIII 3.2

3.3.2	Application of the test substance	Concentrations were calculated so that test item treatments were achieved by adding 2ml stock solution to 10g of sand. Acetone was then allowed to evaporate and the coated sand was then mixed with soil.
3.3.3	Test organisms	See table A7.5.2.1-1
3.3.4	Test system	See table A7.5.2.1-2
3.3.5	Test conditions	See table A7.5.2.1-3
3.3.6	Test duration	Range finding test (acute toxicity): 14 days Definitive test (reproduction test): 8 weeks
3.3.7	Test parameter	Mortality, number of juveniles
3.3.8	Examination	In the definitive study, number of survivors on day 28 of the study was reported. At the end of the exposure period (day 28), survivors were washed and mean weight calculated as total weight reported to the number of survivors. Eight weeks after test initiation the juveniles were hand sorted from soil and the number per container reported.
3.3.9	Monitoring of test substance concentration	Yes, this was focused as a critical point in the study. Samples corresponding to 10g dry soil were sampled on days 0, 7, 13, 21 and 28 from the unpopulated units. Test item treatments were checked once again at the end of the test from each test item treatment and replicates (populated units).
3.3.10	Statistics	F-variance analysis at 5% confidence level to judge significant deviation of the number of juveniles compared to control.

4 RESULTS

4.1	**Range-finding test**	
4.1.1	Initial concentrations of test substance	0.1, 1.0, 10.1, 100.5 and 1005.3 mg/kg dry soil (range-finder)
4.1.2	Effect data	See Tables A7.5.2.1-4 and A7.5.2.1-5 In the Range-finding test, NOEC (biomass was considered to be 100.5 mg/kg dry soil. The definitive test was therefore performed using 100 mg/kg as the highest test treatment.
4.2	**Definitive test**	

Section A7.5.2.1 Earthworm reproduction study
Annex Point IIIA XIII 3.2

4.2.1	Concentration of test substance	Initial concentrations 1.6, 3.0, 5.2, 9.6, 17.2, 30.8, 55.6 and 100.0 mg/kg dry soil (definitive test). The test item concentrations were not checked for the three lowest test item treatments because the nominal values were below the quantification level for HPLC determination of cypermethrin. The measured concentrations for cypermethrin concentrations in the un-populated units represented more than 80 % of the nominal values over the first 28 days of the test (See Table 5). Thus the test item treatments were not further adjusted. At the end of the test, the measured concentrations represented 62-77% of the nominal values. See Tables A7.5.2.1-6 and A7.5.2.1-7
4.2.2	Number/ percentage of earthworms showing adverse effects	See Tables A7.5.2.1-8 and A7.5.2.1-9 No mortality was observed for the water controls, solvent controls or any of the test item treatments.
4.2.3	Nature of adverse effects	<u>Biomass Changes</u> For both the water controls and the solvent, the final biomass represented more than twice the initial biomass. That was also the case for every test item treatments up to and including 30.8 mg/kg: F-variance analysis at the 5% confidence level showed that mean gain of biomass was similar to that of the water controls. At both 55.6 and 100.0 mg/kg mean gain of biomass was significantly reduced (5% confidence level). For the reference item treatments, the gain of biomass was considered as similar to the controls at 1 mg/kg dry soil, but significantly reduced at 2 and 5 mg/kg dry soil. <u>Production of juveniles</u> In the control group, the mean number of juveniles was 99.5 per unit with a relative standard deviation of 15.5%. The solvent controls gave similar values: mean reproductive performance was 98.5 juveniles per unit with a relative standard deviation of 18.7 %. The water controls and the solvent controls were thus pooled to improve the sensitivity of the statistical analysis. Mean value for juveniles/unit was 99.0 with a relative standard deviation of 8.4 %. F-variance analysis at a 5% confidence level showed that the reproductive performance was similar to the controls for every test item treatment up to and including 5.2 mg/kg. For every higher test item treatment, the production of juveniles was significantly reduced (5% confidence level). NOEC (reproduction) = 5.2 mg/kg dry soil

Section A7.5.2.1 Earthworm reproduction study
Annex Point IIIA XIII 3.2

4.3	**Test with reference substance**	EC_{50} value for Carbendazim was between 1.0 and 5.0 mg/kg dry soil and complied with Phytosafe historical data. The function of the test system was thus confirmed.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	This study aimed to determine the effect of Cypermethrin on the reproduction of earthworms, when the specimens are maintained under laboratory conditions on an artificial soil substrate previously amended with the test item at different concentrations. A range-finding test was first performed as an acute toxicity test, using one replicate unit of 10 worms for each of five test item treatments between 0.1 and 1000 mg/kg dry soil. The results served for the determination of the No Observed Effect Concentration (NOEC) based on the biomass deviation throughout the test period. The definitive test was first performed as a limit test at 0 and 1005 mg/kg dry soil. The definitive test was thus reproduced as a full definitive test using four replicate units each containing 10 worms for each of eight test item treatments between 1.6 and 100 mg/kg dry soil, and 8 replicate units for each the water control and the solvent control, as recommended in the guideline for a combines ECx and NOEC approach. The adults were maintained in the artificial soil substrate for 4 weeks. Then, the observations consisted in percent mortality and mean weight of the survivals. The adults were discarded and the test units were maintained in the climatic chamber for 4 additional weeks. At the end of the period, the number of juveniles was assessed. One additional set of units were performed and conducted as the test system but without earthworm (abiotic units). They were used for the assessment of the test item concentration during the adult exposure period. The test item treatments were checked at the end of the test out from both the abiotic units and the biotic units.
5.2	**Results and discussion**	The assessment of cypermethrin throughout the test period in the test item treated soils was performed for the five highest test item treatments. The results showed that the concentrations remained > 80% of the nominal value over the adult exposure period. At the end of test the measured concentrations still represented 62-77% of the nominal.
5.2.1	NOEC mortality	≥ 100.0 mg/kg dry soil
5.2.2	NOEC biomass	30.8 mg/kg dry soil
5.2.3	NOEC reproduction	5.2 mg/kg dry soil
5.2.4	LOEC reproduction	9.6 mg/kg dry soil
5.2.5	EC_{50} reproduction	25.2 mg/kg dry soil (95%-confidence interval = 10.6 – 59.7 mg/kg) See table A7.5.2.1-10

Section A7.5.2.1 Earthworm reproduction study
Annex Point IIIA XIII 3.2

5.3	**Conclusion**	The test is considered valid, NEOC reproduction was considered to be 5.2 mg/kg
5.3.1	Other Conclusions	The tests were considered as valid as the results fulfilled the following conditions: - Control mortality < 10%, - Production of juveniles in the control ≥ 30 per unit, - Coefficient of variation of reproduction in the control ≤ 30 %. Additionally, the EC_{50} value for Carbendazim was between 1.0 and 5.0 mg/kg dry soil and complied with Phytosafe historical data. The function of the test system was thus confirmed.
5.3.2	Reliability	1
5.3.3	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	*December 2011*
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is adopted.
Conclusion	*NOECmortality:100 mg/kg* *NOECbiomass:30.8 mg/kg* *NOEC reproduction:5.2 mg/kg*
Reliability	*1*
Acceptability	Acceptable
Remarks	
	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 A7.5.2.1-1: Test organism

Criteria	Details
Species/strain	Eisenia fetida
Source of the initial stock	Phytosafe stock
Culturing techniques	Not specified in report
Age/weight	Same generation, minimum 2 months old and younger than 1 year. Individual weight 300-600 mg (acute test) or 250-600 mg (repro test).
Pre-treatment	Test specimens weighed individually in the breeding chamber. Batches of 10 worms were separated, prior to addition to each test unit.

Table A7.5.2.1-2: Test system

Criteria	Details
Artificial soil test substrate	Sphagnum-peat soil (grounded and sieved): 10% Kaolin clay: 20% Quartz sand: 70%
Test mixture	Prepared in acetone and added to 10g sand. Acetone evaporated and coated sand mixed with soil substrate
Size, volume and material of test container	1.5-2 Litre glass containers
Amount of artificial soil/ container	500g dry substrate / container
Nominal levels of test concentrations	1.6, 3.0, 5.2, 9.6, 17.2, 30.8, 55.6 and 100.0 mg/kg dry soil (definitive test)
Number of replicates/concentration	4 (test item), 8 (water/solvent controls)
Number of earthworms/test concentration	10
Number of earthworms/container	10
Light source	16 hours light (400-800 lux)/8 hours dark
Test performed in closed vessels due to significant volatility of test substrate	No, test units covered with mesh to avoid worms escaping

Table A7.5.2.1-3: Test conditions

Criteria	Details
Test temperature	20 (± 2) °C
Moisture content	40-60% of water holding capacity of artificial soil
pH	6.0±0.5
Adjustment of pH	No, adjusted at soil preparation only and re-tested at the end of the test period
Light intensity / photoperiod	16 hours light (400-800 lux)/8 hours dark
Relevant degradation products	Not determined

Table A7.5.2.1-4: Range-finding test – Induced Mortality

Concentration	Observed population			Percent mortality	
	D(0)	D(7)	D(14)	D(7)	D(14)
Water control	10	10	10	0	0
Solvent control	10	10	10	0	0
Cypermethrin					
0.1 mg/kg	10	10	10	0	0
1.0 mg/kg	10	10	10	0	0
10.1 mg/kg	10	10	10	0	0
100.5 mg/kg	10	10	10	0	0
1005.3 mg/kg	10	10	10	0	0

Table A7.5.2.1-5: Range-finding test – Biomass changes

Concentration	Initial	Final	% Deviation
Water control	520.7	538.5	3.4 %
Solvent control	484.7	514.2	6.1 %
Cypermethrin			
0.1 mg/kg	501.6	504.3	0.5 %
1.0 mg/kg	497.6	508.2	2.1 %
10.1 mg/kg	408.4	494.2	21.0 %
100.5 mg/kg	415.9	427.5	2.8 %
1005.3 mg/kg	416.7	386.8	-7.2 %

Table A7.5.2.1-6: Definitive test – Measured concentrations of cypermethrin in the un-populated units

Period of assessment		Nominal values, mg/kg				
		9.6	17.2	30.8	55.6	100.0
Day 0	Measured mg/kg	9.04	16.59	29.46	51.64	91.26
	% recovery	94.2 %	96.4 %	95.7 %	92.9 %	91.3 %
Day 7	Measured mg/kg	10.06	16.62	31.93	57.90	99.81
	% recovery	104.8 %	96.6 %	103.7 %	104.2 %	99.8 %
Day 13	Measured mg/kg	9.36	16.34	27.47	51.01	100.62
	% recovery	97.5 %	95.0 %	89.2 %	91.8 %	100.6 %
Day 21	Measured mg/kg	9.64	17.09	29.06	54.28	99.46
	% recovery	100.4 %	99.3 %	94.4 %	97.6 %	99.5 %
Day 28	Measured mg/kg	10.10	17.55	31.21	59.08	108.09
	% recovery	105.2 %	102.0 %	101.3 %	106.3 %	108.1 %

Table A7.5.2.1-7: Definitive test – Measured concentrations of cypermethrin in the populated units at the end of the test

	Nominal values, mg/kg				
	9.6	17.2	30.8	55.6	100.0
Replicate 1	6.23	11.20	20.87	36.99	78.79
Replicate 2	6.36	11.00	21.06	38.09	73.13
Replicate 3	5.74	10.96	20.38	36.73	75.17
Replicate 4	5.56	11.00	20.22	39.21	81.60
Mean mg/kg	5.97	11.04	20.63	37.76	77.17
Mean % recovery	62.2 %	64.2 %	67.0 %	67.9 %	77.2 %

Table A7.5.2.1-8: Definitive test – Biomass changes (mean biomass deviation over the 4 week exposure period for the adults)

Treatment	Body weight deviation
Water control	132.5 %
Solvent control	115.7 %
Cypermethrin	
1.6 mg/kg	132.3 %
3.0 mg/kg	128.4 %
5.2 mg/kg	121.8 %
9.6 mg/kg	118.7 %
17.2 mg/kg	123.6 %
30.8 mg/kg	125.0 %
55.6 mg/kg	74.7 %
100.0 mg/kg	16.6 %
Carbendazim	
1.0 mg/kg	116.0 %
2.0 mg/kg	62.0 %
5.0 mg/kg	-8.5 %

Table A7.5.2.1-9: Definitive test – Number of Juveniles at the end of the test period

Concentration	Replicates								Mean
	1	2	3	4	5	6	7	8	± S.D.
Water control	92	115	114	79	87	86	119	104	99.5 ± 15.4
Solvent control	87	91	87	104	132	71	113	103	98.5 ± 18.7
Cypermethrin									
1.6 mg/kg	79	82	99	150					102.5 ± 32.9
3.0 mg/kg	92	81	78	82					83.3 ± 6.1
5.2 mg/kg	97	95	101	87					95.0 ± 5.9
9.6 mg/kg	71	84	72	83					77.5 ± 7.0
17.2 mg/kg	68	88	52	71					69.8 ± 14.8
30.8 mg/kg	51	63	74	39					56.8 ± 15.1
55.6 mg/kg	29	12	17	8					16.5 ± 9.1
100.0 mg/kg	0	0	1	0					0.3 ± 0.5