Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA 7.4 and IIIA 7.3

5.3	**Conclusion**	The abiotic control indicated absence of chemical oxygen demand. The respiration rates of the controls varied by less than 15 %. The EC_{50} of the reference substance was within the acceptable range of 5 to 30 mg/l. Thus, the test fulfils all validity criteria. Establishment of a dose-response relationship is not appropriate.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	April 2012
Materials and Methods	The Applicant's version is considered to be acceptable with the following amendment: Page 17 of Doc IVA: Percent Inhibition = [1- Rs/Rc] x 100 OECD 209 equation : Percent Inhibition = [1- 2Rs/Rc] x 100
Results and discussion	The Applicant's version is considered to be acceptable
Conclusion	The Applicant's version is considered to be acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
COMMENTS FROM ...	
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.1.4- 1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	Yes
Vehicle	No
Concentration of vehicle	Not appropriate
Vehicle control performed	Not appropriate
Other procedures	For test concentrations < 100 mg/l, a stock solution of the test substance was prepared in dichloromethane, and appropriate volumes of the stock solution were dispensed into the test vessels, and the solvent was allowed to evaporate overnight. After evaporation of the solvent, the appropriate volumes of water, synthetic sewage feed and microbial inoculum were added to the test vessels. For the 100 and 1000 mg/l treatments, an appropriate amount of test substance was weighed onto glass coverslips. The glass coverslips, with the test substance were added directly into the test flasks.

Table A7.4.1.4- 2: Inoculum / test organisms.

Criteria	Details
Nature	Activated sludge
Species	Mixed species population
Strain	Not applicable
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	Taunusstein-Bleidenstadt (Germany)
Laboratoy culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Threefold washing with tap water and centrifugation; resuspension in the laboratory's well water and aeration
Pre-treatment	No
Initial cell concentration	3.6–4.4 g/l suspended solids (inoculum)

Table A7.4.1.4-3: Test system.

Criteria	Details
Culturing apparatus	Glass bottles, 290–300 mL volume
Number of culture flasks/concentration	1
Aeration device	Compressed air from central system
Measuring equipment	Oximeter: WTW model 530 with Tri Oximatic EO 200 electrode pH-meter: WTW portable pH-325
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.4-4: Test conditions.

Criteria	Details
Test temperature	20 ± 2°C
pH	7.45
Aeration of dilution water	Yes
Suspended solids concentration	1.6 g/l

Section A7.4.2 **Bioconcentration in aquatic/terrestrial organisms**
Annex Point IIA7.5

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.2/01:** Sendor T (2005) Estimation of the bioconcentration factor (BCF) of Alphacypermethrin. EBRC Consulting GmbH, Hannover, Germany, Report no. BAS-051114-01, November 14, 2005 (unpublished), BASF DocID: 2005/1034074.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Not applicable
2.2	**GLP**	No
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	Not applicable
3.1.2	Specification	Not applicable
3.1.3	Purity	Not applicable
3.1.4	Further relevant properties	Not applicable
3.1.5	Radiolabelling	Not applicable
3.1.6	Method of analysis	Not applicable
3.2	**Reference substance**	Not applicable
3.2.1	Method of analysis for reference substance	Not applicable
3.3	**Testing/estimation procedure**	

Section A7.4.2 **Bioconcentration in aquatic/terrestrial organisms**
Annex Point IIA7.5

3.3.1	Test system/ performance	Not applicable
3.3.2	Estimation of bioconcentration	On the basis of log P_{ow}, as specified in the TGD on risk assessment. The experimentally determined log P_{ow} value is reported in reference A3.9/01. log P_{ow} = 5.5

4 RESULTS

4.1	**Experimental data**	
4.1.1	Mortality/behaviour	Not applicable
4.1.2	Lipid content	Not applicable
4.1.3	Concentrations of test material during test	Not applicable
4.1.4	Bioconcentration factor (BCF	Not applicable
4.1.5	Uptake and depuration rate constants	Not applicable
4.1.6	Depuration time	Not applicable
4.1.7	Metabolites	Not applicable
4.1.8	Other Observations	Not applicable
4.2	**Estimation of bioconcentration**	log BCF = 3.975

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Estimation of the bioconcentration factor (BCF) based on log P_{ow}, as specified by the TGD on risk assessment.
5.2	**Results and discussion**	Based on the experimentally determined partition coefficient (5.5), the bioconcentration factor was estimated at log BCF = 3.975. BCF = 9440

Section A7.4.2 Bioconcentration in aquatic/terrestrial organisms

Annex Point IIA7.5

5.3	**Conclusion**	Since the estimation was performed using an officially recommended method, based on measured values determined by fully valid experimental procedures, this calculation is considered valid without restrictions. Nevertheless, it is explicitly noted here that an experimental bioaccumulation study in fish according to a draft OECD test guideline is also available (ref. A7.4.3.3.1/01), that should be preferred over the current calculation.
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	March 2009
Materials and Methods	The Applicant's version is considered to be acceptable
Results and discussion	The Applicant's version is considered to be acceptable
Conclusion	The Applicant's version is considered to be acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.3.1 Prolonged toxicity to an appropriate species of fish

Annex Point IIIA 13.2.1

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [X]	
Detailed justification:	The TNsG on data requirements state with respect to this dossier section: "Usually this test is not required, as it does not add information as needed in the risk assessment. The existing test guidelines are not sufficient." Furthermore, a study that may be considered to cover the endpoint "Effects on reproduction and growth rate on an appropriate species of fish" is available (see section A7.4.3.2). Since that study represents a higher-tier investigation, a study specifically allocated to the current dossier section is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	March 2009	
Evaluation of applicant's justification	Applicant's justification are considered to be acceptable	
Conclusion	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.4.3.2 **Effects on reproduction and growth rate of fish**
Annex Point IIIA 13.2.2

Official use only

1 REFERENCE

1.1	**Reference**	**Cross-reference to A7.4.1.1/01:** [redacted] (1983) WL85871 and cypermethrin: a comparative study of their toxicity to the fathead minnow *Pimephales promelas* (Rafinesque). [redacted] Report no. SBGR.82.298, March 02, 1983, BASF RDI No.: AL-512-002 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	None
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**	No The study was conducted prior to adoption of OECD guidelines. However, performance of the study was consistent to OECD 210 in all important aspects.
2.2	**GLP**	No GLP was not mandatory when the study was conducted.
2.3	**Deviations**	Yes In relation to the currently valid OECD guideline 210, the following deviations are noted: Only 4 test concentrations were employed instead of 5.

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	OCD/7
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	≥ 94.4% w/w
3.1.4	Composition of product	Not applicable
3.1.5	Further relevant properties	Water solubility approx. 5.80 µg/l

X

Section A7.4.3.2 Effects on reproduction and growth rate of fish

Annex Point IIIA 13.2.2

3.1.6	Method of analysis	GC-ECD following extraction from water samples with hexane. LoD = 0.02 µg/l
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Yes In view of the low water solubility, stock solutions were equilibrated using test substance coated carrier material in order to ensure constant concentrations (see Table A7.4.3.2- 1 for details).
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not appropriate
3.4	**Testing procedure**	(Non-entry field)
3.4.1	Dilution water	Details are given in Table A7.4.3.2- 2.
3.4.2	Test organisms	Fathead minnow, as described in Table A7.4.3.2- 3.
3.4.3	Handling of embryos and larvae (OECD 210)	Eggs were obtained from 4 or 5 batches of breeding fish. Eggs were removed from the spawning surfaces (plastic piping) by gentle rubbing. The embryos from the different batches were combined and examined, any infertile or unhealthy eggs were discarded. Groups of 30 eggs were then impartially distributed to each of the twenty test vessels. Until hatching the eggs were held in mesh-bottomed glass cups through which the influent water to each test vessel passed. The eggs, and subsequently the larvae, were inspected daily, counted and any dead removed. Once hatching had commenced hatched larvae were released from the cup and allowed to swim freely in the test vessel.
3.4.4	Test system	See Table A7.4.3.2- 4.
3.4.5	Test conditions	As given in Table A7.4.3.2- 5.
3.4.6	Duration of the test	34 days
3.4.7	Test parameters	Pre-hatch mortality (day 3) Post-hatch mortality (day 6) Overall mortality (day 34) Wet weight at test termination (day 34)
3.4.8	Examination/ Sampling	Daily
3.4.9	Monitoring of TS concentration	Yes Controls: Days–2, 2, 10, 31 C1 (0.03 µg/l): Days 5, 10, 20, 31 C2 (0.1 µg/l): Days –2, 2, 5, 10, 13, 20, 31 C3 (0.3 µg/l): Days –2, 2, 5, 10, 13, 20, 31 C4 (1.0 µg/l): Days –2, 2, 5, 20
3.4.10	Statistics	χ^2-test on pre-hatch, post-hatch and overall mortality. Analysis of variance on larval weight.

Section A7.4.3.2 **Effects on reproduction and growth rate of fish**
Annex Point IIIA 13.2.2

4 RESULTS

4.1	**Range finding test**	Not performed. Nevertheless, information from the acute toxicity test conducted in the same species and documented in the same report was considered in selecting the concentrations.
4.1.1	Concentration	
4.1.2	Number/percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal [µg/l]: Control 0.03 0.1 0.3 1.0 First measurement [µg/l]: < LoD 0.02 0.09 0.2 0.73
4.2.2	Actual concentrations of test substance	Nominal [µg/l]: Control 0.03 0.1 0.3 1.0 Overall means [µg/l]: < LoD 0.03 0.09 0.19 0.74 As detailed in Table A7.4.3.2- 6, the variation of concentrations was sometimes larger than 20% of the mean. From the analytical results, there was evidence of racemisation of the active substance, resulting in a proportion of 30–50% of an enantiomer coded as WL86711, comprising the 1R-cis-R plus 1S-cis-S pair of isomers. The above figures refer to the sum of Alphacypermethrin and WL86711.

Section A7.4.3.2 Effects on reproduction and growth rate of fish

Annex Point IIIA 13.2.2

4.2.3	Effect data	*Time to start of hatching:* max. 3 days in controls and all treatment levels. *End of hatching:* max. until day 6 in controls and all treatment levels. *Numbers of larvae hatching each day:* not reported. *Length of surviving animals:* not reported. *Weight of surviving animals:* Significantly increased weight in C1; however, this enhancement of growth is not considered an adverse effect; details are provided in Table A7.4.3.2- 7. *Numbers of deformed larvae:* not reported. *Numbers of fish exhibiting abnormal behaviour:* not reported. Detailed mortality data are presented in Table A7.4.3.2- 8. The resulting effect concentrations are given as follows (measured concentrations): LOEC pre-hatch mortality: > 0.74 µg/l post-hatch mortality: 0.19 µg/l overall mortality: 0.09 µg/l NOEC pre-hatch mortality: 0.74 µg/l post-hatch mortality: 0.09 µg/l overall mortality: 0.03 µg/l
4.2.4	Concentration / response curve	A graph of the concentration-mortality curve is presented in Figure A7.4.3.2- 1.
4.2.5	Other effects	None reported.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	See Table A7.4.3.2- 8.
4.3.2	Nature of adverse effects	Mortality
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	
4.4.2	Results	

Section A7.4.3.2 **Effects on reproduction and growth rate of fish**
Annex Point IIIA 13.2.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The effects of Alphacypermethrin to the presumably most susceptible stages in the life cycle of fish have been investigated in an early life stage test. The study was performed prior to the issuing of agreed test guidelines, but it was based on a draft of OECD guideline 210. Thus, the study is consistent to OECD guideline 210 in all important aspects.

Compared to the currently valid OECD 210 (1992), the following deviations were noted:

- Only 4 instead of 5 prescribed test concentrations were employed; however, this is considered not to affect the validity of the test since a NOEC could nevertheless be determined;
- Temperature in the test vessels is insufficiently documented;
- The numbers of larvae hatching each day were not reported;
- The length of surviving animals was not reported;
- The numbers of deformed larvae was not reported;
- The numbers of fish exhibiting abnormal behaviour was not reported.

5.2 Results and discussion

Material-specific properties, particularly the low water solubility, have been appropriately considered in the test design (see Table A7.4.3.2- 1). Analytical monitoring of the test substance ensured that actual exposure concentrations are accounted for. The observed racemisation of the active substance is an inherent property that cannot be avoided and reflects the likely behaviour of Alphacypermethrin in natural aquatic systems. Furthermore, the comparative investigation of cypermethrin and Alphacypermethrin in the test report at hand demonstrates basically equivalent toxicity to fish, with respect to both acute and chronic effects. Since both Alphacypermethrin and the evolving pair of enantiomers (WL86711, i.e. the 1R-cis-R and 1S-cis-S isomers) are elements of Cypermethrin, it may safely be concluded that the observed racemisation does not alter the conclusions regarding toxic effects in any way.

By day 6 significant treatment related mortalities had occurred in the fish exposed to Alphacypermethrin at C2, C3 and C4. Subsequent to day 12, few mortalities occurred in any of the treated or control fish in the early life stage tests. This indicates that the toxic effects observed were acutely toxic effects on sensitive life stages, rather than chronic toxic effects resulting from prolonged exposure.

5.2.1 NOEC

Pre-hatch mortality:	0.74 µg/l
Post-hatch mortality:	0.09 µg/l
Overall mortality:	0.03 µg/l

(Measured concentrations)

5.2.2 LOEC

Pre-hatch mortality:	> 0.74 µg/l
Post-hatch mortality:	0.19 µg/l
Overall mortality:	0.09 µg/l

Section A7.4.3.2 Effects on reproduction and growth rate of fish

Annex Point IIIA 13.2.2

5.3	**Conclusion**	Test substance concentrations varied by more than 20% of the mean. Thus, one single validity criterion is formally not fulfilled. However, it needs to be considered that constant maintenance of such low concentrations as required in this test is intrinsically very difficult. Great care has been taken to ensure constancy of exposure concentrations and the observed variation should be regarded as evidence that such low levels are extremely difficult to handle. Since deviations of > 20% were relatively infrequent, it is suggested to consider the test nevertheless as valid.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes The test design involving only 4 instead of 5 test concentrations may be considered as only a minor deficiency, since a NOEC could nevertheless be established. The non-reporting of the daily numbers of hatched larvae, the length of surviving fish, the numbers of deformed larvae, and the numbers of fish exhibiting abnormal behaviour, as well the insufficient reporting of temperature variation, are considered as reporting deficiencies of minor importance. This reflects the general level of standards at the time of study performance rather than actual deficiencies. It is believed that the non-reporting of malformations or abnormal behaviour is an indication of their absence in this test since otherwise they would have been mentioned. Thus, it is concluded that the noted deficiencies do not affect the reliability of the study, which is therefore assessed as valid with restrictions.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	February 2009
Materials and Methods	The Applicant's version is acceptable with the following comment: Section 3.1.5: more details on vapour pressure should have been useful
Results and discussion	The Applicant's version is considered to be acceptable
Conclusion	The Applicant's version is considered to be acceptable
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.3.2- 1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	Stock solutions were prepared by passing filtered (8 µm) dechlorinated tap water up through a glass column containing 250 g of sieved pumice (particle size >2 mm) onto which 0.25 g of test substance had been adsorbed. The test substance was adsorbed onto the pumice by dissolving it in 250 ml of Analar acetone, pouring the acetone onto the pumice, and then evaporating the acetone by passage of air. After preparation of the columns, diluent water was pumped through these at approximately 50 mL min^{-1} for at least 2 weeks prior to their use in tests to allow them to stabilise.

Table A7.4.3.2- 2: Dilution water.

Criteria	Details
Source	Tap water, filtered (8 µm), dechlorinated
Salinity	253–275 mg/l
Hardness	259–300 mg/l
pH	7.3–7.8
Oxygen content	8.3–9.1 mg/l
Conductance	500–580 µS/cm
Holding water different from dilution water	No

Table A7.4.3.2- 3: Test organisms.

Criteria	Details
Species/strain	*Pimephales promelas*
Source	Not reported
Wild caught	Not reported
Age/size	Start of test: Eggs < 24 h old End of test: 62–79 mg (mean wet weight)
Kind of food	*Artemia salina* nauplii, newly hatche (< 24 h old) up to day 2 post-hatching, 24–48 h old afterwards
Amount of food	Not reported
Feeding frequency	Twice daily
Post-hatch transfer time	< 24 h
Feeding of animals during test	Yes, see above
Treatment for disease within 2 weeks preceding test	Not applicable

Table A7.4.3.2- 4: Test system.

Criteria	Details
Test type	Flow-through
Renewal of test solution	Flow-rate = 20 mL/min
Volume of test vessels	1 l (test solution volume)
Volume/animal	1/30 l per egg (initially)
Number of animals/vessel	30 eggs per vessel
Number of vessels/concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.2- 5: Test conditions.

Criteria	Details
Test temperature	22.1–25.0°C
Dissolved oxygen	7.5–9.6 mg/l
pH	7.6–8.1
Adjustment of pH	No
Aeration of dilution water	Yes Light aeration in the mixing chamber
Quality/Intensity of irradiation	Not reported
Photoperiod	18:6 h (L:D)

Table A7.4.3.2- 6: Analytical verification of Alphacypermethrin (including WL86711) concentrations: individual measurements and their deviation from the mean.

	C2		C3		C4	
	Individual measurement	**Percent deviation**	**Individual measurement**	**Percent deviation**	**Individual measurement**	**Percent deviation**
	0.07	–18.2%	0.18	–7.4%	0.68	–7.5%
	0.09	5.2%	0.2	2.9%	0.53	–27.9%
	0.08	–6.5%	0.17	–12.6%	0.79	7.5%
	0.08	–6.5%	0.12	–38.3%	0.78	6.1%
	0.06	–29.9%	0.18	–7.4%	0.4	–45.6%
	0.15	75.3%	0.27	38.9%	0.81	10.2%
	0.11	28.6%	0.21	8.0%	0.89	21.1%
	0.08	–6.5%	0.2	2.9%	1.0	36.1%
	0.08	–6.5%	0.17	–12.6%		
	0.08	–6.5%	0.2	2.9%		
	0.1	16.9%	0.08	–58.9%		
	0.11	28.6%	0.25	28.6%		
	0.08	–6.5%	0.23	18.3%		
	0.1	16.9%	0.21	8.0%		
	0.04	–53.2%	0.19	–2.3%		
	0.06	–29.9%	0.17	–12.6%		
	0.08	–6.5%	0.23	18.3%		
	0.09	5.2%	0.24	23.4%		
Mean	0.086		0.194		0.735	

Table A7.4.3.2- 7: Mean wet weights of fish at the end of exposure (day 34) in the early life stage test.

	Treatment level				
	Control	**C1**	**C2**	**C3**	**C4**
Mean wet weight (SD) [mg]	62 (22)	79 (28)**	68 (30)	63 (16)	Not determined (100% mortality)

**: significantly different ($p < 0.01$) to the control

Table A7.4.3.2- 8: Pre-hatch, post-hatch and overall mortality in the early life stage test.

	Mean mortality [%]				
	Control	**C1**	**C2**	**C3**	**C4**
Pre-hatch (day 3)	18	20	29	25	22
Post-hatch (day 6)	22	22	33	38**	37*
Overall (day 34)	47	52	62*	79***	100^{+}

*: significantly different ($p < 0.05$) to the control, χ^2-test
**: significantly different ($p < 0.01$) to the control, χ^2-test
***: significantly different ($p < 0.001$) to the control, χ^2-test
+: not included in statistical analysis since 100% mortality is unequivocally significant

Table A7.4.3.2- 9: Validity criteria for fish early life stage test according to OECD guideline 210.

	Fulfilled	**Not fulfilled**
Concentration of dissolved oxygen > 60% saturation throughout the test	X	
Difference of water temperature < 1.5°C between test chambers or successive days at any time during test; temperature within range for specific test species	Not traceable due to insufficient documentation of temperature data	
Overall survival of fertilized eggs in controls ≥ 66%, specified for *P. promelas*	X	
Test substance concentrations maintained within ± 20% of mean measured values		X
No effect on survival nor any other adverse effect found in solvent control	Not applicable	

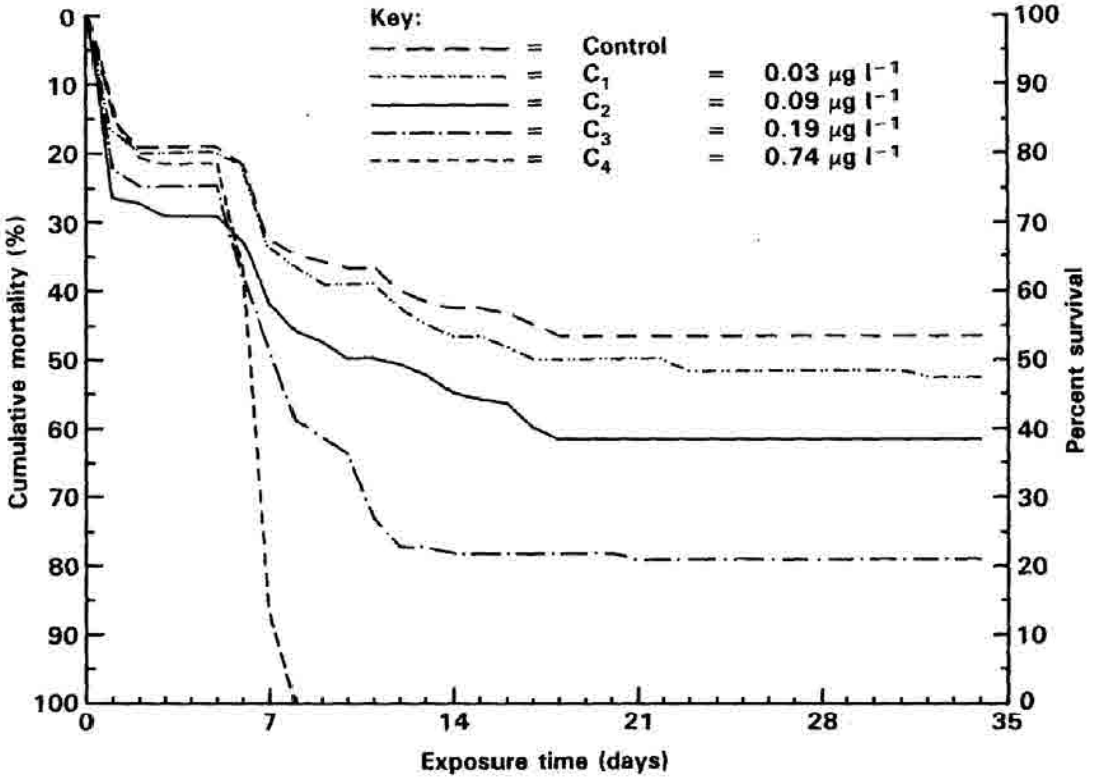


Figure A7.4.3.2- 1: Cumulative mortality of *P. promelas* exposed to Alphacypermethrin in the early life-stage test.

Section A7.4.3.3.1 Bioaccumulation in an appropriate species of fish

Annex Point IIIA 13.2.3

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.3.3.1/01:** [redacted] (1997) Bioconcentration study of alpha-cypermethrin with carp. [redacted], Report no. 6B332G, June 06, 1997 (unpublished), BASF-RDI-no. AL-519-004.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	No	
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 305C (1981)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes The feeding frequency was lower that recommended by OECD 305C, 1981 (see Table A7.4.3.3.1- 1). However, this is considered a favourable deviation since contamination with organic matter is minimised.	

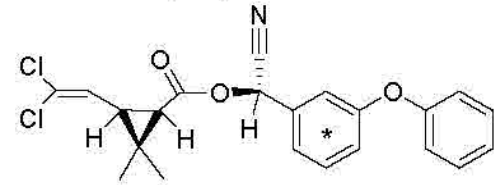
3 MATERIALS AND METHODS

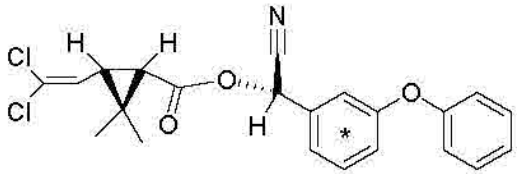
3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	AC10194-61	
3.1.2	Specification	As given in Section A2.	
3.1.3	Purity	96.1%	
3.1.4	Further relevant properties	Alphacypermethrin is only poorly soluble in water (4.59 µg/l at pH 7 and 7.87 µg/l at pH 9 according to OECD 105 test, ref. A3.5/01). In the current study, the test concentrations were below water solubility. Degradation by hydrolysis and phototransformation was compensated by utilising a flow-through system. The toxicity to fish was determined in the current study by pre-test prescribed by guideline OECD 305C. Result: LC_{50} = 12.0 µg/l.	X

Section A7.4.3.3.1 Bioaccumulation in an appropriate species of fish

Annex Point IIIA 13.2.3

3.1.5	Radiolabelling	Yes Batch no.: AC10727-40A Specific activity: 60.8 µCi/mg Chemical purity: > 99% Radiochemical purity: 99.6%





3.1.6	Method of analysis	Liquid scintillation counting (LSC). <u>Sample preparation (water samples):</u> Direct submission to LSC with the high concentration; Extraction with ethyl acetate, evaporation, re-dissolution in methanol, then submission to LSC with the low concentration. <u>Sample preparation (fish samples):</u> Homogenisation, drying, oxidisation and CO_2 absorption, submission to LSC.
3.2	**Reference substance**	None
3.2.1	Method of analysis for reference substance	

Section A7.4.3.3.1 Bioaccumulation in an appropriate species of fish

Annex Point IIIA 13.2.3

3.3	**Testing/estimation procedure**		
3.3.1	Test system/ performance	Flow-through test using the common carp. The test organism is described in Table A7.4.3.3.1- 1. Details on the test system are given in Table A7.4.3.3.1- 2. The test conditions are specified in Table A7.4.3.3.1- 3. Test substance concentrations (nominal): 0.1 µg/l (high) 0.01 µg/l (low) Duration of uptake phase: 10 weeks Duration of depuration phase: 2 weeks Sampling of fish: at 2, 4, 6, 8 and 10 weeks (uptake phase) at 10 weeks (tissue distribution) at 4, 7 and 14 days (depuration phase) For concentration measurements, two fish were removed at each sampling date. Tissue distribution was investigated on one fish per concentration level. Fish were dissected for examination of tissue distribution into viscera, skin, fillet, and head. Samples for analytical verification of test substance concentration in water were taken twice a week (Table A7.4.3.3.1- 4); temperature and dissolved oxygen were measured concurrently. DOC in the test solution and photoperiod were not reported (not required according to OECD 305C, 1981).	X
3.3.2	Estimation of bioconcentration	The intrinsic potential for bioaccumulation has been estimated separately (see section A7.4.2). The result is briefly summarised as follows: Intrinsic BCF = 9440	

4 RESULTS

4.1	**Experimental data**		
4.1.1	Mortality/ behaviour	No mortality occurred. Aberrant behaviour was not observed.	
4.1.2	Lipid content	See Table A7.4.3.3.1- 1.	
4.1.3	Concentrations of test material during test	Alphacypermethrin concentrations in fish over time in relation to exposure level are presented in Table A7.4.3.3.1- 5. Concentrations in fish during the depuration phase are given in Table A7.4.3.3.1- 6. Tissue distribution is shown in Table A7.4.3.3.1- 7.	

Section A7.4.3.3.1 **Bioaccumulation in an appropriate species of fish**
Annex Point IIIA 13.2.3

4.1.4	Bioconcentration factor (BCF)	An overview of BCF values calculated for all sampling dates in the uptake phase is given in Table A7.4.3.3.1- 5. Alphacypermethrin concentrations in fish declined subsequent to reaching a maximum after 6 and 8 weeks at the low and high exposure level, respectively. Considering the concentration curve over time, it is concluded that a steady had been reached after a period of approximately 4 weeks. Based on the concentration maximum in fish at 6 weeks (low level), the steady-state bioconcentration factor may be estimated as: $BCF_{ss} = 910$
4.1.5	Uptake and depuration rate constants	Uptake and depuration rate constants were not reported (not required according to OECD 305C, 1981).
4.1.6	Depuration time	The depuration half-lives were estimated at $t_{1/2} = 8.6$ d (high level) and $t_{1/2} = 6.9$ d (low level).
4.1.7	Metabolites	Not identified.
4.1.8	Other observations	Deviations from prescribed procedures or any other events that may have affected the reliability of the test were not reported.
4.2	**Estimation of bioconcentration**	See 3.3.2 above.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The bioconcentration factor of alphacypermethrin in fish was determined experimentally using the common carp (*Cyprinus carpio*) exposed to high and low levels of the test substance in a flow-through system. The test was conducted following OECD guideline 305C (1981). Whereas this guideline deviates from the more recent version OECD 305 (1996) in several respects, particularly with regard to the level of detail of data analysis (uptake and depuration rate constants), all important aspects are nevertheless covered. The guideline referred to in the study is fully appropriate with respect to the physical-chemical properties of the test substance. OECD guideline 305C (1981) recommends feeding of the fish three times daily. In the current study, fish were only fed once daily. However, this is not considered a deficiency. Rather, it may be regarded as a favourable feeding regime since contamination with organic matter is minimised. The body weight data demonstrate that fish grew satisfactorily, indicating that feeding was conducted at a sufficient frequency.

Section A7.4.3.3.1 Bioaccumulation in an appropriate species of fish

Annex Point IIIA 13.2.3

5.2	**Results and discussion**	Experimental studies Alphacypermethrin is poorly soluble in water, has a high log P_{ow} and a low vapour pressure. The selected test method is fully appropriate for such a substance. Test item concentrations were analytically confirmed to have been satisfactorily maintained at nominal over the entire test period. There was no mortality in any of the tested and control groups, nor were there any other signs of adverse effects. BCF_{ss} = 910 Uptake rate constant: not determined (not required according to OECD 305C, 1981). Alphacypermethrin was predominantly dist Depuration rate constant / half-life: DT_{50} = 6.9–8.6 d
5.3	**Conclusion**	Validity criteria are not given in OECD 305C (1981). However, when the validity criteria of OECD 305 (1996) are applied, the test can be considered fully valid:
		Temperature variation < ± 2°C: fulfilled O_2 concentration > 60% saturation: fulfilled TS concentration ± 20%: fulfilled (average) Mortality < 10%: fulfilled Absence of other adverse effects: fulfilled
5.3.1	Reliability	2
5.3.2	Deficiencies	No The study was performed according to a meanwhile outdated test guideline that deviates from the current version. Thus, the conduct of the studies partially deviates from current standards. However, since the study design is consistent with OECD 305 (1996) in all important aspects and since the validity criteria were fulfilled, the test is considered to be valid with restrictions.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

EVALUATION BY RAPPORTEUR MEMBER STATE (*)

Date

August 2011

Materials and Methods

The Applicant's version is acceptable with the following amendments:
Section 3.1.4
Solubility 4,59 µg/l at pH 4; 5,80 µg/l at pH 7
The toxicity to fish was determined in the current study by pre-test prescribed by guideline OECD 305C. Result: LC_{50} (48h) = 12 µg/l
Section 3.3.1
Table A7.4.3.3.1-1:

- Body length longer than recommended by OECD 305, 1996 (5.0 ± 3 cm);
- No data on age and sex of fishes;
- Acclimation period shorter ('over 1 week', see p.19) than recommended by OECD 305, 1996 (2 weeks).
- Duration of uptake phase: 28 or 60 days (in this study: 73 days).
- Duration of depuration phase: ½ uptake phase (in this study: 14 days).
- Sampling of fish: only 3 sampling for depuration phase (4 needed in OECD 305, 1996).

pH, photoperiod, uptake and depuration rate constants needed in OECD 305, 1996.

Results and discussion

The Applicant's version is acceptable with the following comment:
The results of bioaccumulation should also be expressed in relation to lipid content.

Conclusion

The Applicant's version is considered to be acceptable.

Reliability

2

Acceptability

Acceptable

Remarks

BE CA considers the performed test as appropriated and fulfilling all important and necessary scientific aspects to determine a bioaccumulation factor in fish. BE CA is well aware that the performed test was completed with a meanwhile outdated version (OECD 305, 1981) of the reference guideline and thus partially deviates from the current standards. Deviations and deficiencies listed above are considered as minor:

- Body length is longer than recommended, however all tested fishes are within the same range of length;
- Data on age and sex of fishes: additional data to be updated to current requirements;
- Shorter acclimation period ('over 1 week', see p.19) : minor deviation;
- Uptake phase is longer but this however can be considered as positive as it left enough time to observe a steady-state plateau at both tested exposure levels;
- Duration of the depuration phase is shorter than recommended however half-lives of the test substance at both exposure levels were shown to be shorter than 2 weeks;
- Sampling of fish: to be adapted to current requirements;
- pH, photoperiod, uptake and depuration rate constants: additional data to be updated to current requirements.

BE CA thus validate the study.

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.3.3.1- 1: Test organisms.

Criteria	Details
Species/strain	*Cyprinus carpio*
Source	Sankyo Suisan Co., Tokyo, Japan
Wild caught	No
Age/size	Body length: 9–13 cm
Fat content	5.0% (range: 4.4–6.0%, n=4)
Kind of food	Minipet, Kyorin C. Ltd.
Amount of food	2% of body weight (during acclimation period)
Feeding frequency	Once daily, except holidays
Pre-treatment	Acclimation period: 1 week
Feeding of animals during test	Yes Minipet feed, 2.5% of body weight, once daily except holidays

Table A7.4.3.3.1- 2: Test system.

Criteria	Details
Test type	Flow-through
Renewal of test solution	Turnover rate = 9 volumes/d
Volume of test vessels	45 l water volume
Volume/animal	Test fish: 2.05 l/individual Control: 3.75 l/individual
Number of animals/vessel	Test vessels: 22 Control vessels: 12
Number of vessels/concentration	1
Solubilising agents / co-solvents	Polyoxyethylene hydrogenated castor oil: 1.0 µg/l (high concentration) 0.1 µg/l (low concentration) 1.0 µg/l (control) Acetonitrile: 2.5 ppm v/v (high concentration) 0.25 ppm v/v (low concentration) 2.5 ppm v/v (control)

Table A7.4.3.3.1- 3: Test conditions.

Criteria	Details
Test temperature	25 ± 2°C
Dissolved oxygen	> 5.6 ppm (i.e. > 67.8%, based on the saturation concentration at 25°C of 8.26 ppm)
pH	Not reported
Adjustment of pH	No
Aeration of dilution water	Yes Continuous
Intensity of irradiation	Not reported
Photoperiod	Not reported

Table A7.4.3.3.1- 4: Analytically verified concentrations of alphacypermethrin in the test water.

Sampling date	Exposure period		High concentration	Low concentration
	Week	Day	[µg/l]	[µg/l]
11/08/96	0	0	0.119	0.0120
11/12/96		4	0.108	0.0064
11/15/96	1	7	0.090	0.0084
11/19/96		11	0.102	0.0083
11/22/96	2	14	0.124	0.0075
11/26/96		18	0.101	0.0084
11/29/96	3	21	0.087	0.0131
12/03/96		25	0.133	0.0078
12/06/96	4	28	0.125	0.0076
12/10/96		32	0.116	0.0138
12/13/96	5	35	0.117	0.0167
12/17/96		39	0.132	0.0128
12/20/96	6	42	0.127	0.0148
12/24/96		46	0.130	0.0123
12/27/96	7	49	0.099	0.0138
12/31/96		53	0.130	0.0150
01/03/97	8	56	0.101	0.0084
01/07/97		60	0.121	0.0155
01/10/97	9	63	0.090	0.0110
01/14/97		67	0.075	0.0092
01/17/97	10	70	0.116	0.0154
01/20/97		73	0.087	0.0094
Average [µg/l]			0.110	0.0113
S.D. [µg/l]			0.0173	0.00321
C.V. [%]			15.7	28.5

Table A7.4.3.3.1- 5: Measured concentrations of alphacypermethrin in fish in the uptake phase and resulting bioconcentration factors.

Conc. level	Week	Fish no.	Body weight [g]	C_{fish} [µg/kg]	C_{water} [µg/l]	BCF
High						
	2	1	24.39	31.2	0.109	286
		2	23.96	32.0	0.109	294
	4	1	29.27	51.3	0.110	466
		2	27.37	32.3	0.110	294
	6	1	27.18	46.3	0.114	406
		2	26.24	48.6	0.114	426
	8	1	34.68	52.2	0.114	458
		2	30.86	66.0	0.114	579
	10	1	35.08	43.4	0.112	388
		2	34.93	33.9	0.112	303
Low						
	2	1	25.14	1.32	0.0085	155
		2	23.16	1.17	0.0085	138
	4	1	27.98	4.99	0.0088	567
		2	28.11	4.17	0.0088	474
	6	1	29.87	9.57	0.0106	903
		2	30.21	9.65	0.0106	910
	8	1	39.92	4.85	0.0110	441
		2	33.08	4.64	0.0110	422
	10	1	41.46	5.23	0.0113	463
		2	37.55	3.44	0.0113	304

Table A7.4.3.3.1- 6: Measured concentrations of alphacypermethrin in fish in the depuration phase.

Conc. level	Day	Fish no.	Body weight [g]	C_{fish} [µg/kg]	Residual rate [%]
High					
	4	1	40.62	25.5	54.5
		2	38.63	37.6	80.3
	7	1	45.83	17.9	38.2
		2	33.48	19.4	41.5
	14	1	38.24	15.5	33.1
		2	38.49	13.3	28.4
Low					
	4	1	35.27	1.97	33.8
		2	37.00	2.22	38.1
	7	1	33.92	2.18	37.5
		2	34.65	2.38	40.9
	14	1	39.00	1.19	20.4
		2	39.43	0.95	16.3

Table A7.4.3.3.1- 7: Concentrations of alphacypermethrin and bioconcentration factors (BCF) in tissues after 10 weeks of exposure.

Conc. level	Tissue	Tissue weight [g]	C_{tissue} [µg/kg]	C_{water} [µg/l]	BCF
High					
	Viscera	3.82	338.5	0.110	3077
	Skin	5.28	78.0	0.110	709
	Fillet	15.27	32.7	0.110	297
	Head	8.90	93.5	0.110	850
Low					
	Viscera	3.67	29.03	0.0113	2569
	Skin	4.47	7.22	0.0113	639
	Fillet	17.02	2.65	0.0113	235
	Head	10.64	6.14	0.0113	543

Section A7.4.3.3.2 Bioaccumulation in an appropriate invertebrate species

Annex Point IIIA 13.2.3

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [X]	**Other justification** [ ]	
Detailed justification:	The bioconcentration potential has been estimated based on log P_{ow} (Section A7.4.2). Furthermore, actual bioconcentration was tested in an appropriate fish species (Section A7.3.3.1). An experimental study on invertebrates would only be appropriate if direct release to marine or brackish water was likely, as outlined in the TNG on data requirements. However, according to the envisaged use pattern (domestic hygiene), direct release of the active substance to surface waters is very unlikely. Thus, direct release to marine or brackish water is not expected and a bioaccumulation study in invertebrates is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Evaluation of applicant's justification	Applicant's justification are considered to be acceptable
Conclusion	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.4.3.4 **Effects on reproduction and growth rate with an invertebrate species**
Annex Point IIIA 13.2.4

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.3.4/01:** Garforth B (1982) WL 85871 and cypermethrin: chronic toxicity to *Daphnia magna*. Shell Research Ltd, SRC, Sittingbourne, UK, Report no. SBGR.82.119, July 26, 1982 (unpublished), BASF RDI No.: AL-523-001.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	No	
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**	No The study was conducted prior to issuing of OECD guideline 202 II (1984). However, the experiment was consistent to OECD guideline 202 II in all important aspects.	
2.2	**GLP**	No GLP was not compulsory in the EU at the time the study was conducted.	
2.3	**Deviations**	Yes See discussion under 5.3 below.	

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.	
3.1.1	Lot/Batch number	OCD/7	
3.1.2	Specification	As given in Section A2.	
3.1.3	Purity	94.9–96.2% (w/w)	X
3.1.4	Composition of product	Not applicable.	
3.1.5	Further relevant properties	Water solubility approx. 5.80 µg/l. Thus, the test concentrations were below maximum water solubility.	X
3.1.6	Method of analysis	GC-ECD following extraction from water samples with hexane. A selective clean-up procedure for alphacypermethrin was used, removing any contributions from WL 86711, the product of racemisation (also see section A7.4.3.2).	

Section A7.4.3.4 Effects on reproduction and growth rate with an invertebrate species

Annex Point IIIA 13.2.4

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Yes In view of the low water solubility, stock solutions of alphacypermethrin in acetone were used (see Table A7.4.3.4- 1 for details).	X
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	Temperature, pH and oxygen were measured daily. The variation of these parameters, together with other water parameters, is given in Table A7.4.3.4-2 and Table A7.4.3.4-5.	
3.4.2	Test organisms	*Daphnia magna*, as described in Table A7.4.3.4-3.	
3.4.3	Handling of offspring	The live adults in each beaker were transferred by pipette to a beaker of fresh test solution, leaving any offspring and dead adults behind. Dead adults and dead young were counted and discarded. Live young were removed by sieving, killed, and placed in a small dish with a little water. They were then taken out a few at a time, using a Pasteur pipette, counted by eye and discarded.	
3.4.4	Test system	Please refer to Table A7.4.3.4-4.	
3.4.5	Test conditions	Test conditions are specified in Table A7.4.3.4-5.	
3.4.6	Duration of the test	21 days	
3.4.7	Test parameters	Offspring production (absolute and relative to live adults) Adult mortality First day of reproduction Growth (body length of adults)	
3.4.8	Examination/ Sampling	Daily Mortality, offspring production and day of first reproduction were assessed as already described in 3.4.3 above. Adult body length: On the final day of the experiment the remaining live adults in each beaker were removed and preserved in ethanol. Eleven days later the length of each *Daphnia* was measured (from the apex of the helmet to the base of the spine) using a microscope and graticule.	

Section A7.4.3.4 **Effects on reproduction and growth rate with an invertebrate species**
Annex Point IIIA 13.2.4

3.4.9	Monitoring of TS concentration	Yes (i) An initial experiment to investigate the partitioning of the test substance between water, particulate matter (algae), and glass surfaces was carried out utilising an additional beaker (without *Daphnia*): The water was removed from the beaker. Vessel walls were washed with acetone. Particulate matter and water were separated by centrifugation. These three fractions were analysed separately using the analytical method described under 3.1.6 above. (ii) Main experiment: On days 1, 6, 12, and 18, extra replicates were prepared from excess test solution (without Daphnia) and samples drawn from the freshly prepared (0.5 h) and 24 h old solution (0.3 and 0.1 µg/l concentrations only), respectively. In these analyses only dissolved alphacypermethrin, i.e. from water separated from suspended solids was measured.
3.4.10	Statistics	ANOVA, with post-hoc Newman-Keuls test on the following parameters: - cumulative mortality by day 21 - cumulative number of young by day 21 - cumulative number of young per live adult by day 21 - first day of reproduction - mean length of live adults at day 21.

4 RESULTS

4.1	**Range finding test**	Not performed
4.1.1	Concentration	
4.1.2	Number/percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	

Section A7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA 13.2.4

4.2 Results test substance

4.2.1 Initial concentrations of test substance

The experiment on partitioning between water, suspended solids and glass revealed the following distribution for fresh test solution:

	Water	Solids	Glass	Total recovery
% of nominal	40	18	3	61

In the recovery experiments performed during the in-life phase on days 1, 6, 12, and 18, the initial concentration of alphacypermethrin on average amounted to 30% of nominal. Details are given in Table A7.4.3.4- 6.

It is noted here that due to the selective clean-up procedure the product of racemisation (WL 86711) was not recovered for analysis. Thus, the total amount of alphacypermethrin including its racemisation product is likely to be considerably underestimated. Furthermore, since only the dissolved fraction was considered in the main recovery experiments (see 3.1.6 above), actual exposure including that due to alphacypermethrin adsorbed to algae and other particles may likewise be underestimated.

4.2.2 Actual concentrations of test substance

The experiment on partitioning between water, suspended solids and glass revealed the following distribution for 24 h old test solution:

	Water	Solids	Glass	Total recovery
% of nominal	7	14	4	25

In the recovery experiments performed during the in-life phase on days 1, 6, 12, and 18, the concentration of alphacypermethrin after 24 h on average amounted to 20% of nominal. Details are given in Table A7.4.3.4- 6.

With respect to recovery of the racemisation product and of alphacypermethrin adsorbed to particles, the same remarks as given under 4.2.1 above apply.

4.2.3 Effect data

Details are given in Table A7.4.3.4- 7.

NOEC (reproduction) = 0.03 µg/l (nominal)

NOEC (mortality of parents) = 0.1 µg/l (nominal)

4.2.4 Concentration / response curve

Not available

4.2.5 Other effects

Adult length at test termination and first day of reproduction were affected at a nominal concentration (LOEC) of 0.1 µg/l; see Table A7.4.3.4- 7 for details.

4.3 Results of controls

Please refer to Table A7.4.3.4- 7.

4.4 Test with reference substance

Not performed

4.4.1 Concentrations

4.4.2 Results

Section A7.4.3.4
Annex Point IIIA 13.2.4

Effects on reproduction and growth rate with an invertebrate species

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effects of alphacypermethrin on reproduction and growth rate of *Daphnia magna* were investigated in a semi-static test (renewal of test solution every 24 h) over 21 days. The study was conducted prior to issuing of OECD guideline 202 II (1984) but was nevertheless consistent to the test guideline in all important aspects.
5.2	**Results and discussion**	Due to its adsorptive properties and its relatively rapid racemisation to a more diverse mixture of cypermethrin isomers denoted as WL 86711 (also see section A7.4.3.2), the test substance per se could not be maintained within 80% of nominal. However, due to the selective clean-up procedure WL 86711 was not recovered for analysis. Thus, the total amount of alphacypermethrin including its racemisation product is likely to be considerably underestimated. Furthermore, the analyses were conducted close to the limit of detection, which is why the analytical results are partly inaccurate (see variation of data in Table A7.4.3.4- 6). In view of the uncertainty associated with the verification of test substance concentrations and the fact that test solutions were renewed every 24 h, it is considered appropriate to relate the effects to nominal concentrations.
5.2.1	NOEC	0.03 µg/l
5.2.2	LOEC	0.1 µg/l
5.2.3	EC_{50} (EC_x)	Not applicable
5.3	**Conclusion**	The test was conducted prior to issuing of an adequate OECD test guideline. Thus, validity criteria may not strictly apply. Moreover, the validity criteria differ between OECD 202, part II (1984) and OECD 211 (1998). In the current test, pH varied by max. 0.6 units, which is fully compliant with 211 (1998) but not with the 0.3 units criterion given OECD 202 (1984) which seems, however, overly restrictive. According to the more recent OECD 211, the variation in pH values is fully acceptable. Test concentrations, however, could not be maintained within 80% of nominal, due to the specific properties of the test substance as discussed above. In view of the careful conduct of the study, including frequent renewal of the test solution, the test should be considered as acceptable. It is also noted here that the study was accepted under 91/414.
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and Methods	The Applicant's version is acceptable with the following comments: Section 3.1.3 Purity : 94.9-96.5% Section 3.1.5 Water solubility : 5.80 µg/l at pH 7 Section 3.2 Table A7.4.3.4- 1: The vehicle control was performed with 0.025% of acetone whereas the concentration of vehicle in samples is 0.1%.
Results and discussion	The Applicant's version is considered to be acceptable
Conclusion	The Applicant's version is considered to be acceptable
Reliability	2
Acceptability	Acceptable
Remarks	Vehicle control should have been performed with 0.1% v/v acetone
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.3.4- 1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	Yes Organic solvent: acetone
Concentration of vehicle	0.1% v/v
Vehicle control performed	Yes 0.025% (v/v) acetone
Other procedures	None

Table A7.4.3.4-2: Dilution water.

Criteria	Details
Source	Tap water from local water supply
Salinity	Not applicable
Hardness	259–300 mg/L $CaCO_3$
pH	7.3–7.8
Ca / Mg ratio	106 mg/l : 2.2 mg/l
Na / K ratio	11 mg/l : 1.3 mg/l
Oxygen content	8.8–11.8 mg/L
Conductance	580 µS/cm
TOC	Not reported
Holding water different from dilution water	No

Table A7.4.3.4-3: Test organisms.

Criteria	Details
Strain / Clone	In-house cultivation of IRChA (France) strain
Source	See above
Age	< 24 h
Breeding method	Water quality, light and feeding regime as in the experiment
Kind of food	Mixture of *Chlorella vulgaris*, fish food, beef extract and glucose
Amount of food	$0.1–1.0 \times 10^9$ cells/mL
Feeding frequency	Not reported
Pre-treatment	None
Feeding of animals during test	Yes, daily

Table A7.4.3.4-4: Test system.

Criteria	Details
Test type	Semi-static (renewal of test solution every 24 h)
Renewal of test solution	Daily Adult *Daphnia* were transferred into the fresh test solutions using a pipette
Volume of test vessels	500 mL (volume of solution)
Volume/animal	50 mL
Number of animals/vessel	10
Number of vessels/concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.4-5: Test conditions.

Criteria	Details
Test temperature	18.5–20.2°C
Dissolved oxygen	8.8–11.8 mg/L
pH	8.1–8.7
Adjustment of pH	No
Aeration of dilution water	No
Quality/intensity of irradiation	White-type fluorescent tubes, 20 W, 1 m above the test vessels
Photoperiod	16:8 h (L:D)

Table A7.4.3.4- 6: Actual test substance concentrations during the experiment.

Nominal concentration [µg/l]	Age of test solution	Day of determination				Mean	Mean % of nominal
		1	6	12	18		
0.3							
	Fresh (0.5 h)	0.07	n.a.	n.a.	0.10	0.09	30
	24 h	0.16	n.a.	n.a.	0.05	0.11	37
0.1							
	Fresh (0.5 h)	0.04	0.03	0.02	0.02	0.03	30
	24 h	0.06	< 0.01	0.01	< 0.01	0.02	20

Table A7.4.3.4- 7: Effect data from the *Daphnia* reproduction test.

	Concentration [µg/l], nominal						
	Control	Solvent control	0.003	0.01	0.03	0.1	0.3
No. of offspring per adult	152	118	123	133	129	58*	–
Adult deaths	0.5	0.5	0.8	0.3	0.5	1.3	10*
1st day of reproduction	10	9.0	10.3	8.8	9.3	12.5*	–
Mean adult length [mm]	4.63	4.66	4.73	4.63	4.55	3.53*	–

*) significantly different from control (p = 0.05)

Table A7.4.3.4-8: Validity criteria for invertebrate reproduction test according to OECD Guideline 202, part II (1984), or OECD guideline 211 (1998) if explicitly stated.

	Fulfilled	**Not fulfilled**
Mortality of parent animals in the controls < 20% at test termination	X	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60 (in the controls, OECD 211, 1998)	X	
Criteria for poorly soluble test substances: TS concentrations < solubility limit	X	
Oxygen concentration ≥ 60% throughout the test	X	
Variation of pH ≤ 0.3 units (OECD 202, part II, 1984)		X
Variation of pH ≤ 1.5 units (OECD 211, 1998)	X	
Test substance concentration ≥ 80% of nominal		X
First control offspring born after max. 9 days	X	

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.3.5/01:** Huber W, Mitchell GC, Zieris FJ, Neugebaur-Büchler K, Cascorbi U (2000) valuation of Effects of a 100 g/L SC Formulation (CF 06677) of AC 900049 (alphacypermethrin) on macroinvertebrates, zooplankton and algae in enclosures in ponds. Technical University of Munich Weihenstephan, Freising, Germany, Report no. ECO-97-144, October 01, 2000 (unpublished), BASF RDI No.: AL-560-031.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	None
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes Draft OECD guideline on freshwater lentic field tests, SETAC, EWOFFT, HARAP
2.2	**GLP**	No Whereas all aspects of this study were accurately recorded by qualified scientific staff and the general principles of GLP, this study was not strictly GLP compliant because there was no Quality Assurance oversight.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Alphacypermethrin 100 g a.s./L OESC (formulation code CF 06677)
3.1.1	Lot/Batch number	FAS 400001
3.1.2	Specification	Alphacypermethrin 100 g a.s./L OESC (formulation code CF 06677)
3.1.3	Purity	101 g/L alphacypermethrin (a.s.)
3.1.4	Composition of product	OESC formulation with a.i. content as specified under 3.1.3 above.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

3.1.5	Further relevant properties	The formulation is not considered to have any impact on the quality of the results, since it represents a neutral oil suspension. The overall purpose of this study was to evaluate possible effects of alphacypermethrin insecticide when applied according to Good Agricultural Practice (GAP) for this formulation in cereals, resulting in predicted initial environmental concentrations (PIEC) in adjacent ditches as predicted by the Ganzelmeier Drift Model. The application of the active substance in biocidal products may be expected to result in similar releases to the aquatic environment: use as an insecticide for domestic hygiene purposes may lead to pulsed / intermittent release, e.g. as consequence of cleaning operations in the treated premises. Thus, the study design and the endpoint are fully adequate in the context of a biocidal risk assessment based on the intended uses.
3.1.6	Method of analysis	Concentrations of a.s. in stock solutions were verified analytically using the analytical method SAMS 469-2 (see section A4.2). Concentrations of a.s. in the test enclosures, however, could not be verified due to the low test concentrations.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	No The test concentrations were below the limit of water solubility of alphacypermethrin.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not appropriate
3.4	**Testing procedure**	
3.4.1	Dilution water	Natural pond water
3.4.2	Test organisms	Semi-natural assemblages of freshwater macroinvertebrates, zooplankton and algae in pond-enclosures located at the Technical University of Munich, Freising, Germany.
3.4.3	Test system	The test system is described in Table A7.4.3.5- 1.
3.4.4	Test conditions	Test conditions in the aquatic phase are provided in Table A7.4.3.5- 2. In addition, sediment was characterised chemically and physically (particle size etc.) and found to be representative for natural sediments. Pebble baskets served as habitat and sampling sites for macroinvertebrates.
3.4.5	Duration of the test	145 days Treatment: June 6, 1997
3.4.6	Test parameter	Density of planktonic algae (measured as chlorophyll a), as an indicator of primary production performance; Zooplankton: abundance, species composition; Macroinvertebrates: abundance, species composition (live sampling and release into the original replicate upon determination of species identity).

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

3.4.7	Examination/ Sampling	- Oxygen, pH, alkalinity, conductivity: day 3, 7, 11, and then at 7-d intervals until day 144 (October 28). - Chlorophyll a: Water samples of 4 l volume were directly measured in a photometer after sieving (1 mm mesh width) to remove filamentous algae; sampling schedule as for water parameters given above. - Zooplankton: Water samples of 4 l volume were sampled at dawn (most representative point of time with respect to species composition in the water column); the samples were poured through a 63 µm mesh, which was rinsed with 200 mL tap water; formaldehyde was added gradually for fixation; after 24 sedimentation and the fixed plankton samples subjected to microscopic examination; sampling schedule: day 3, 7, 11, and then at 7-d intervals until day 116 (September 30). - Macroinvertebrates: Pebble baskets were removed from the enclosures, the substrate rinsed with approx. 1 l of tap water, the animals identified to at least the level of order, counted and returned alive to their original habitat; sampling schedule: day 4 and then at 7-d intervals until day 145 (October 29). - Macrophytes: visual mapping on October 21 (day 137).
3.4.8	Monitoring of TS concentration	No Instead, concentrations of stock solutions were analytically verified.
3.4.9	Statistics	Combined ANOVA / regression analysis.

4 RESULTS

4.1	**Results test substance**	
4.1.1	Initial concentrations of test substance	The study was invalidated following the discovery of a dosing error, as a result of which (it was subsequently determined) only 1/10 of the required nominal concentrations of active ingredient were applied to the pond-enclosures. The actual applied nominal concentrations, therefore, were approximately 10x lower than required, i.e., 0.0005, 0.001, 0.002, 0.003, 0.006, 0.012 and 0.06 µg a.s./L. Since these nominal concentrations were not verified analytically and could not be confirmed, this dosing error was considered to be a significant deviation from the Study Plan and as a result the study was invalidated.
4.1.2	Actual concentrations of test substance	Not determined.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

4.1.3	Effect data	For planktonic algae (chlorophyll a) and physical-chemical parameters, there were no effects at any test concentration. For zooplankton, a clear absence of effects was demonstrated at PIEC concentrations (0.005, 0.010, 0.020, 0.030, µg a.s./l). The no observed effect concentration (NOEC) for effects on zooplankton was 0.060 µg a.s./l (i.e., 2x highest PIEC value). Macroinvertebrates were generally unaffected at PIEC concentrations. However, based on the abundance of the aquatic larval stages of one dipteran genus, *Chaoborus* spp., the ghost (or phantom) midge, a lowest observed effect concentration (LOEC) of 0.005 µg a.s./l was obtained. There were no effects on any other macroinvertebrate species at PIEC concentrations. Macroinvertebrate community diversity was unaffected by the test substance. For *Chaoborus* spp., a temporary failure to increase in abundance relative to control values was observed at PIEC concentrations. However, this effect was relatively transient and reversible, occurring only in the period 30 to 60 DAT. Post 60 DAT, numbers of *Chaoborus* returned to normal (control) values and the organism was observed to exhibit development cycles indistinguishable from controls, until the termination of sampling at 145 DAT.
4.2	**Results of controls**	Controls formed the baseline for the evaluation of effects.
4.3	**Test with reference substance**	No reference substance was used.
4.3.1	Concentrations	Not applicable
4.3.2	Results	Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	An aquatic mesocosm study was conducted in order to derive an ecologically acceptable concentration of alphacypermethrin in surface water. The study design followed the draft OECD guideline on freshwater lentic field tests, SETAC, EWOFFT and HARAP recommendations. The overall purpose of this study was to evaluate possible effects of FASTAC OESC insecticide, a 100 g/L OESC (oil-enhanced suspension concentrate) formulation (CF 06677) of AC 900049 (alphacypermethrin), applied at aqueous concentrations as predicted by the Ganzelmeier drift model and Good Agricultural Practice (GAP) for this formulation in cereals, on macroinvertebrates, zooplankton and planktonic algae in enclosures in ponds. Pond-enclosures containing diverse biological communities were treated. The possible impact of drift on zooplanktonic organisms provided the primary focus of the study. Biological samples were collected prior to treatment on June 6, 1997 and until 145 days after treatment (DAT 145) on October 29, 1997. Environmental conditions were monitored during the test.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

5.2	**Results and discussion**	The pond-enclosures used for this study supported a representative diversity of freshwater organisms, comparable to that of natural ponds in the vicinity of the Test Facility. Biological diversity was unaffected by the test substance at all test concentrations. No effects on zooplankton were demonstrated at PIEC concentrations (0.005, 0.010, 0.020, 0.030 µg a.s./l), thereby fulfilling the primary objective of this pond-enclosure study. There were no effects on ecosystem function at PIEC concentrations, as demonstrated by chlorophyll a measurements and physical-chemical parameters. Macroinvertebrates were generally unaffected at PIEC concentrations. A transient effect was observed on the abundance of the dipteran genus *Chaoborus* spp. relative to controls, but even at exaggerated treatment rates of 0.060, 0.12 and 0.60 µg a.s./l, this effect was demonstrated to be fully reversible, with an observed return to control values during the course of the study. Some perturbation was noted in pond-enclosures treated with 0.060, 0.12 and 0.60 µg a.s./l (i.e., concentrations 2x, 4x and 10x highest PIEC value), thereby confirming the validity of the test system. This non-GLP study was invalidated following the discovery of a dosing error, as a result of which (it was subsequently determined) only 1/10 of the required nominal concentrations of active ingredient were applied to the pond-enclosures. The actual applied nominal concentrations, therefore, were approximately 10x lower than required, i.e., 0.0005, 0.001, 0.002, 0.003, 0.006, 0.012 and 0.06 µg a.s./l. Since these nominal concentrations were not verified analytically and could not be confirmed, this dosing error was considered to be a significant deviation from the Study Plan and as a result the study was invalidated.
5.2.1	NOEC	
5.2.2	LOEC	
5.3	**Conclusion**	As already noted above, the study was invalidated due to a retrospectively detected dosing error. However, these invalidated results provided the basis for the design of a new mesocosm study (ref. A7.4.3.5/02 ff. below) and supplementary laboratory studies with FASTAC OESC, as summarised in the current section below.
5.3.1	Reliability	4
5.3.2	Deficiencies	Yes Study invalidated as discussed above.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and Methods	BE CA agrees with the applicant's version
Results and discussion	BE CA agrees with the applicant's version
Conclusion	BE CA agrees with the applicant's version
Reliability	4
Acceptability	Study invalidated
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.3.5- 1: Test system.

Criteria	Details
Test type	Static mesocosm test
Renewal of test solution	–
Volume of test vessels	640 l per enclosure (replicate) Enclosures situated in a larger pond of 5 m diameter (20 000 l volume)
Volume/animal	Not appropriate (semi-natural community)
Number of animals/vessel	Not appropriate (semi-natural community)
Number of vessels/concentration	3 (controls, 0.005, 0.010, 0.020 and 0.030 µg/l, respectively) 1 (0.060, 0.12 and 0.60 µg/l, respectively)
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.5- 2: Test conditions.

Criteria	Details
Test temperature	Water temperatures in the in-life period: 18–23 °C
Dissolved oxygen	Within acceptable limits (for details see test report)
pH	8.1–9.3
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Natural sunlight
Photoperiod	Seasonally varying natural photoperiod

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

Official use only

1 REFERENCE

1.1 Reference

A7.4.3.5/02:

Huber W, Zieris F-J, Meyer-Tuve H, Nunn A, Sandmann E, Mitchell GC, Horton W (2000) Evaluation of possible effects of a 100 g/l SC formulation (CF 06677) of AC 900049 (alphacypermethrin) on macroinvertebrates, zooplankton and algae in pond-enclosures and determination of the ecologically acceptable concentration (EAC). Technical University of Munich-Weihenstephan, Germany, Report no. ETX-99-101, March 09, 2000 (unpublished), BASF RDI No.: AL-560-023.

A7.4.3.5/03:

Huber W (2000) Summary of an expert panel opinion of the study "Evaluation of possible effects of a 100 g/L SC formulation (CF 06677) of AC 900049 (alphacypermethrin) on macroinvertebrates, zooplankton and algae in pond-enclosures and determination of the ecologically acceptable concentration (EAC)". Institute of Aquatic Ecotoxicology, Buch am Erlbach, Germany, Report no. 2000/I, July 31, 2000 (unpublished), BASF RDI No.: AL-560-033.

A7.4.3.5/04:

Huber,W (2000) Evaluation of an Ecologically Acceptable Concentration (EAC) for alphacypermethrin in aquatic environments. Institute of Aquatic Ecotoxicology, Buch am Erlbach, Germany, Report no. 2000/II, October 01, 2000 (unpublished), BASF RDI No.: AL-529-002.

A7.4.3.5/05:

Huber W (2003) Expert opinion on the results from modified laboratory toxicity tests and population modeling with aquatic macroinvertebrates and FASTAC® OESC insecticide (alphacypermethrin). Institute of Aquatic Ecotoxicology, Buch am Erlbach, Germany, Report no. 03/1, January 28, 2003 (unpublished), BASF RDI No.: 2003/1012037.

A7.4.3.5/06:

Ratte H-T, Strauss T (2002) The response and recovery of a mesocosm population of *Chaoborus crystallinus* (Diptera) at multiple applications of 0.015 µg a.i./L FASTAC OESC (active ingredient; alphacypermethrin, BAS 310 I) – a modeling approach. Dept. of Biology V, Aachen University of Technology, Aachen, Germany, Report no. 145555, November 25, 2002 (unpublished), BASF RDI No.: 200311012038. X

A7.4.3.5/07:

Mitchell GC (2003) Alphacypermethrin: An overview of effects of FASTAC on aquatic macroinvertebrates, with particular reference to the midge Chaoborus crystallinus (Chaoboridae: Diptera). Ecotoxicology Services, Yardley, PA, USA, Report no. ES060301, June 27, 2003 (unpublished), BASF DocID: 2003/1024853.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

		Remark: The above references are, for convenience, merged into one single study summary since they are all based on the same aquatic mesocosm study (ref. A7.4.3.5/02), with the further references providing additional evaluation steps and expert opinions with respect to the derivation of an ecologically acceptable concentration (EAC).
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	None
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes Draft OECD guideline on freshwater lentic field tests, SETAC, EWOFFT, HARAP, CLASSIC
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Alphacypermethrin 100 g a.s./L OESC (formulation code CF 06677)
3.1.1	Lot/Batch number	166772
3.1.2	Specification	Alphacypermethrin 100 g a.s./L OESC (formulation code CF 06677)
3.1.3	Purity	101.7 g/L alphacypermethrin (a.s.)
3.1.4	Composition of product	OESC formulation with a.i. content as specified under 3.1.3 above.
3.1.5	Further relevant properties	The formulation is not considered to have any impact on the quality of the results, since it represents a neutral oil suspension. The overall purpose of this study was to evaluate possible effects of alphacypermethrin insecticide when applied according to Good Agricultural Practice (GAP) for this formulation in cereals, resulting in predicted initial environmental concentrations (PIEC) in adjacent ditches as predicted by the Ganzelmeier drift model. The application of the active substance in biocidal products may be expected to result in similar releases to the aquatic environment: use as an insecticide for domestic hygiene purposes may lead to pulsed / intermittent release, e.g. as consequence of cleaning operations in the treated premises. Thus, the study design and the endpoint are fully adequate in the context of a biocidal risk assessment based on the intended uses.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

3.1.6	Method of analysis	*Water:* GC-ECD method SAMS 469-2 (reference A4.2/07); in excess of the standard analytical method, a LoQ of 0.01 µg/l was established for the current study. *Sediment:* GC-ECD method SAMS 354-2 (reference A4.2/01) was validated for sediment; a LoQ of 0.01 mg/kg was established.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	No The test concentrations were below the limit of water solubility of alphacypermethrin.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not appropriate
3.4	**Testing procedure**	
3.4.1	Dilution water	Natural pond water
3.4.2	Test organisms	Semi-natural assemblages of freshwater macroinvertebrates, zooplankton and algae in pond-enclosures located at Technical University of Munich, Freising, Germany.
3.4.3	Test system	The test system is described in Table A7.4.3.5- 3. Baskets with artificial benthic substrate served as habitat patches and sampling sites for macroinvertebrates.
3.4.4	Test conditions	Test conditions in the aquatic phase are provided in Table A7.4.3.5- 4. In addition, sediment was characterised chemically and physically (particle size etc.) and found to be representative for natural sediments.
3.4.5	Duration of the test	126 days Treatment (start of assessment period): June 9, 1999
3.4.6	Test parameter	Density of planktonic algae, measured as chlorophyll a and as algal cell density, as an indicator of primary production performance; Zooplankton: abundance, species composition; Macroinvertebrates: abundance, species composition (live sampling and release into the original replicate upon determination of species identity).

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

3.4.7	Examination/ Sampling	- *Oxygen, pH, alkalinity, conductivity*: pre-treatment days –29, –23, -15, –8, and –2; post-treatment days 6, 13, and then at 7-d intervals (± 1 d) until day 110 (September 27); - *Chlorophyll a*: Water samples of 0.5 l volume were directly measured in a photometer after sieving (1 mm mesh width) to remove filamentous algae; sampling schedule pre-treatment days –29, –23, –15, –8, and –2; post-treatment days 2, 6, 13, and then at 7-d intervals (± 1 d) until day 110 (September 27); - *Phytoplankton*: 200 mL sub-samples were taken from the samples drawn for chlorophyll *a* determination, fixed with Lugol's solution and algae determined microscopically; sampling schedule as for water quality and chlorophyll *a*, except day –2, which was omitted; - *Zooplankton*: Two water samples of 3 l volume were sampled per sampling day per enclosure (one from the open-water body, one in close vicinity to macrophytes; the samples were poured through a 63 µm mesh, which was rinsed with tap water; formaldehyde and subsequently diethylenglycol were added for fixation; the fixed plankton samples subjected to microscopic examination; sampling schedule: pre-treatment days –29 and –23, post-treatment days 2, 6, 13, and then at 7-d intervals (± 1 d) until day 110 (September 27); - *Macroinvertebrates*: (i) artificial substrate for benthic invertebrates was exposed in sampling baskets, removed at the sampling dates, rinsed with tap water, the live animals identified to at least the level of order, counted and returned alive to their original enclosure; pre-treatment days –14, –7, –1, post-treatment days day 3, 7, and then at 7-d intervals until day 126 (October 13) (ii) pelagic and plant-associated macroinvertebrates were sampled using a plankton net pulled through the water body; sampling schedule as for benthic organisms above; (iii) emerging insects were collected using emergence traps; sampling schedule: weekly, starting on day 7 and ending on day 126; - *Macrophytes*: visual mapping (% coverage) at the beginning and the end of the in-life period.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

3.4.8	Monitoring of TS concentration	A rigorous analytical programme was conducted to establish, with certainty that the intended exposure to the in-life pond-enclosures occurred. Before application, the alphacypermethrin concentration was established in all stock solutions used to treat the pond-enclosures. Furthermore, the initial alphacypermethrin concentration in the pond was verified analytically for the four highest treatments (0.015, 0.075, 0.375, 1.875 µg a.s./l). Analysis of pond water from the lower treatments was not possible due to analytical limitations; the limit of quantitation (LoQ) of the analytical method was 0.01 µg a.i./l. In addition to the in-life pond-enclosures, separate but biologically equivalent pond-enclosures were established in an independent pond in order to assess the fate of alphacypermethrin and thus actual exposure throughout the course of the study. The dissipation of alphacypermethrin residues in the pond water and potential uptake of alphacypermethrin residues in pond sediment were measured analytically. The test substance was applied at an initial nominal concentration of 1.59 µg/l.
3.4.9	Statistics	Regression analysis, principal response curve (PRC) analysis
3.4.10	Further remarks	The results of the experimental mesocosm study itself were subject to an extensive review by an independent expert panel. The experts' opinion is also reflected in this summary, resulting in a recommendation of an overall ecologically acceptable concentration (EAC). Effects of alphacypermethrin were additionally evaluated in an individual-based community simulation study (A7.4.3.5/06).

4 RESULTS

4.1 Results test substance

4.1.1 Initial concentrations of test substance

Nominal [µg/l]:	0.015	0.075	0.375	1.875
1 h post-treatment [µg/l]:	0.017	0.0695	0.387	2.080
% of nominal	113	92	109	111

The nominal levels of 0.00006, 0.0006, 0.003 µg/l could not be verified due to analytical limitations (LoQ). However, the stock solutions were analysed and the concentrations confirmed to be within acceptable limits (87 ± 2% of nominal). Since the application procedure was equivalent across all treatment levels, it may be safely assumed that initial target concentrations were also achieved at the three lowest nominal levels.

In conclusion, the pond enclosures are confirmed to have received the target concentrations.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

4.1.2	Actual concentrations of test substance	The further fate of the applied active substance was monitored in a separate pond specifically designated to the dissipation of the test material (also see 3.4.8 above). Results from the dissipation experiment are provided in Table A7.4.3.5-5. A graph of the dissipation kinetics in the aqueous phase is presented in Figure A7.4.3.5- 1. Accordingly, the initial test concentrations in the enclosures matched the nominal levels. Dissipation from the aqueous phase was rapid (DT_{50} = 2.4 d). Partitioning to the sediment played a minor role: the maximum concentration in sediment (0.015 mg/kg) was reached after 30 days, thereafter declining to levels below the LoQ.
4.1.3	Effect data, general	The pond-enclosures used for this study supported a representative diversity of freshwater organisms, comparable to that of natural ponds in the proximity of the test facility. Significant perturbation was noted in pond-enclosures treated with 0.075, 0.375 and 1.875 µg a.s./l (the three highest concentrations), thereby confirming the validity of the test system.
4.1.4	Effect data, phytoplankton	Based on chlorophyll a measurements, there were transient treatment-related effects on planktonic algae only at the highest treatment concentration (1.875 µg a.s./l). Based on algal abundance, while the abundance of one group of algae (Chrysophyceae) was transiently affected in pond-enclosures treated with 0.375 and 1.875 µg a.s./L (i.e., 12.5x and 62.5x highest PIEC value), no adverse treatment-related effects on overall algal community structure were observed in any of the treated enclosures. The recommended EAC (Ecologically Acceptable Concentration) for algae, based on effects on abundance, is 0.075 µg a.s./l.
4.1.5	Effect data, zooplankton	No significant changes occurred in the overall numbers of zooplanktonic organisms in pond-enclosures treated at the three lowest concentrations (0.00006, 0.0006, 0.003 µg a.s./l). At the higher concentrations (0.015, 0.075, 0.375, 1.875 µg a.s./l), minor increases in abundance were observed on 13 DAT. By contrast, Principal Response Curve (PRC) analysis indicated two different effects in pond-enclosures treated with 0.075, 0.375 or 1.875 µg a.s./l. First, there appeared to be an acute effect directly after the treatment. Subsequently, the abundance of zooplankton increased probably due to reduced predation pressure induced by direct effects on larvae of the midge *Chaoborus crystallinus* (Diptera: Chaoboridae). These indirect effects were observed to continue until the end of the in-life assessment period. However, since effects on zooplankton were evident only at these highest test concentrations (0.075, 0.375, 1.875 µg a.s./l), the recommended EAC based on direct and indirect effects on zooplankton is 0.015 µg a.s./l.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

4.1.6	Effect data, macroinvertebrates	Macroinvertebrates were generally unaffected at concentrations of 0.00006, 0.0006, 0.003 and 0.015 µg a.s./l. In pond-enclosures treated with 0.075, 0.375 or 1.875 µg a.s./l, minor transient effects on the overall abundance of macroinvertebrates were noted. However, full recovery to normal (control) values was observed during the course of the in-life assessment period. By contrast, minor transient effects on the abundance of larval stages of one dipteran species, *C. crystallinus* (the ghost or phantom midge) provided a population LOEC (lowest observed effect concentration) of 0.00006 µg a.s./l, based on a temporary failure of this species to increase relative to control values at all tested concentrations. However, this effect did not appear to be dose-dependent (see Table A7.4.3.5- 6). Post 35 DAT at the lower concentrations (0.00006, 0.0006, 0.003 and 0.015 µg a.s./l), and post 105 DAT at 0.075 µg a.s./l, the numbers of *C. crystallinus* returned to normal (control) values (i.e., the populations exhibited development cycles indistinguishable from controls). Only in pond-enclosures treated at the two exaggerated highest concentrations there was no complete recovery by 126 DAT. Therefore, based on the demonstration of full recovery of *C. crystallinus* at environmental concentrations up to 0.075 µg a.s./l, and since *C. crystallinus*, the most sensitive organism in this study, is neither a protected organism nor a "keystone" species in natural fish-dominated aquatic systems, nor a "keystone" species in running waters, the receiving compartments following biocidal application, the recommended EAC based on effects on macroinvertebrates is 0.015 µg a.s./l is also considered to be valid in the biocides context. Macroinvertebrate community diversity was transiently affected only in pond-enclosures treated with 0.015, 0.075, 0.375 or 1.875 µg a.s./l. However, full recovery to control values was observed during the in-life assessment period, even at the highest concentrations. Therefore, based on effects on macroinvertebrate diversity, an EAC of 0.015 µg a.s./l is recommended.
4.1.7	Effect data, ecosystem function	There were no effects on overall ecosystem function at any test concentration, as demonstrated by the outcome of a series of physical-chemical measurements. However, the chlorophyll *a* concentration increased transiently at the highest concentration, possibly due to decreased predation pressure following direct effects on zooplankton at the highest concentration (1.875 µg a.s./l). Biological diversity was affected by the test substance only at the two highest test concentrations, 0.375 and 1.875 µg/l. Therefore, the recommended EAC based on community diversity and ecosystem function is 0.075 µg a.s./l.
4.2	**Results of controls**	Controls formed the baseline for the evaluation of effects.
4.3	**Test with reference substance**	No reference substance was used.
4.3.1	Concentrations	Not applicable
4.3.2	Results	Not applicable

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

An aquatic mesocosm study was conducted in order to derive an ecologically acceptable concentration (EAC) of alphacypermethrin in surface water.

The overall purpose of this study was to evaluate possible effects of FASTAC* OESC insecticide, a 100 g/L OESC (oil-enhanced suspension concentrate) formulation (CF 06677) of AC 900049 (alphacypermethrin), applied at aqueous concentrations as predicted by the Ganzelmeier drift model and current and proposed Good Agricultural Practice (GAP) for this formulation in cereals, on macroinvertebrates, zooplankton and planktonic algae in enclosures in ponds. Pond-enclosures containing diverse biological communities were singly treated by spray application to the water surface. The possible impact of drift on zooplanktonic organisms provided the primary focus of the study.

Biological samples were collected prior to treatment on June 9, 1999 and until 126 days after treatment (DAT 126) on October 13, 1999. Environmental conditions were monitored during the test.

5.2 Results and discussion

An overall EAC (Ecologically Acceptable Concentration) for alphacypermethrin of 0.015 µg/l was derived from this study, based on the demonstration of full recovery of transiently affected insect populations and intact ecological functioning of the pond systems at all environmentally relevant initial concentrations (PIECs). This recommendation is supported by a number of review reports and independent expert opinions as well as an individual-based ecosystem simulation study.

Whereas this study was originally designed in an agricultural context, i.e. a single short-term exposure of aquatic ecosystems following treatment of adjacent fields, the results are considered to be also valid in the context of a risk assessment for biocidal purposes for the following reasons:

- The intended biocidal use is described as insect control in domestic premises for hygiene purposes; due to the required prolonged action (6 months) of the product applied to surface areas in the treated premises, releases from the treated sites are expected to be intermittent, e.g. as a consequence of cleaning operations;
- The receiving water bodies following biocidal use typically are running waters; thus, intermittent releases result in rapid transport of such "pulsed" discharges; a given section of the watercourse will therefore be exposed to any residues of the active substance for a very limited period of time;
- In conclusion, the discharge patterns are very similar between a typical biocidal exposure scenario and the original agricultural application pattern under which the study was developed; thus, the EAC as recommended in the study and various subsequent review papers is equivalently applicable to intermittent releases to flowing water bodies.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **– Aquatic mesocosm study –**

5.2.1	EAC	0.015 µg/l
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	None

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Reference	A7.4.3.5/06 BASF RDI No.: 2003/1012038
Materials and Methods	The Applicant's version is considered to be acceptable
Results and discussion	The Applicant's version is considered to be acceptable
Conclusion	The Applicant's version is considered to be acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.3.5- 3: Test system.

Criteria	Details
Test type	Static mesocosm test
Renewal of test solution	–
Volume of test vessels	593 l per enclosure (replicate), calculated to provide a water level of 1 m Enclosures situated in a larger pond of 5 m diameter (29 000 l volume)
Volume/animal	Not appropriate (semi-natural community)
Number of animals/vessel	Not appropriate (semi-natural community)
Number of vessels/concentration	6 controls 2 replicates per treatment level
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.5- 4: Test conditions.

Criteria	Details
Test temperature	Water temperatures in the in-life period: 17.3–23.6 °C
Dissolved oxygen [% saturation]	22.1–133.2 (for details see test report)
pH	7.61–8.69
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Natural sunlight
Photoperiod	Seasonally varying natural photoperiod

Table A7.4.3.5- 5: Summary of the dissipation of alphacypermethrin in aquatic mesocosms: degradation in water, partitioning to and degradation in the sediment.

Sampling date	Mean concentration	
	Water [µg/l]	Sediment [mg/kg]
1 h	1.58	–
4 h	1.29	–
8 h	1.17	–
1 d	0.83	<0.003
1.5 d	0.62	–
2 d	0.59	(0.004)
3 d	0.28	(0.007)
7 d	0.17	0.013
14 d	0.02	0.014
30 d	(0.002)	0.015
61 d	<0.003	(0.007)

Values in parentheses: Mathematical estimate of alphacypermethrin residue below the validated limit of detection of the method (LoQ: 0.01 µg/l for water and 0.01 mg/kg for sediment)

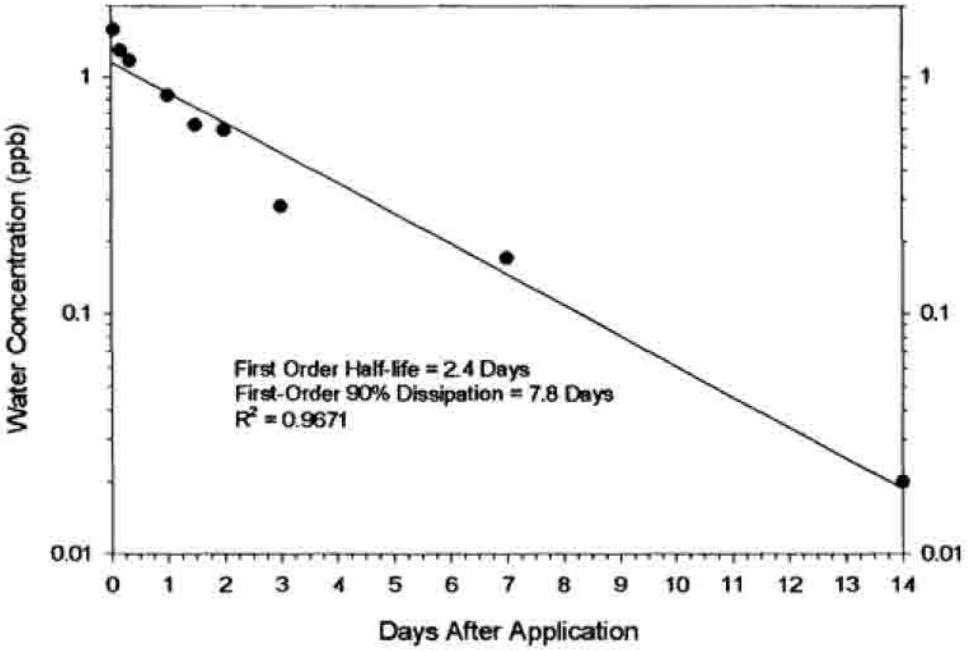


Figure A7.4.3.5- 1: Dissipation of alphacypermethrin in pond water (first order kinetics).

Table A7.4.3.5- 6: Effects of alphacypermethrin on abundance (total numbers) of the most sensitive organisms on selected days in the mesocosm study.

Taxon / Days after treatment	Concentration [µg/l]						
	0.00006	0.0006	0.003	0.015	0.075	0.375	1.875
	Mean difference from control [%]						
Copepoda/ nauplia							
2	20.2	–18.3	32.7	–6.7	–9.5	–62.8*	–76.8*
13	11.9	6.1	0.32	–9.4	201*	292*	–21.9
20	–0.99	4.9	2.2	11.4	27.4*	10.8	–4.3
34	8.3	107	–34.2	150	267*	154	226*
C. crystallinus							
3	–7.40	–9.40	10.10	9.60	–27.40	–41.90*	–42.40*
7	–36.80	–22.80	–18.30	–21.80	–48.80	–58.80	–58.80
14	–22.70	–29.20	–13.70	–18.70	–51.20	–58.20	–59.20
21	–12.90	–31.90	–31.90	–4.40	–42.40	–46.40	–46.40
C. crystallinus, instar III							
3	–10.60	–14.60	–6.10	–4.10	–17.10	–23.60*	–23.60*
7	–9.40	–5.40	–6.40	–5.40	–17.90	–20.40*	-20.40*
14	–15.50	–9.00	–9.50	–7.00	–19.50	–23.00*	–23.00*
21	–6.70	–11.20	–11.70	0.80	–14.70	–16.20	–16.20
C. crystallinus, instar IV							
3	1.50	9.00	4.50	6.00	–4.50	–9.50	–10.00
7	–6.30	5.20	1.20	–3.30	–6.30	–11.80	–11.80
14	–6.90	–3.90	2.10	–2.90	–9.40	–11.40	–11.40
21	–4.20	–9.70*	–8.70	–0.20	–12.70*	–14.20*	–14.20*

*) significantly different from control acc. to Dunnett's test

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **Aquatic mesocosm study**

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.3.5/08:** Huber W, Dawo U, Mitchell GC (2001) Evaluation of the effects of multiple applications of a 100 g/L SC formulation (FASTAC, OESC, CF 06677) of alphacypermethrin (AC 900049) on macroinvertebrates, zooplankton and algae in pond-enclosures. Technical University of Munich-Weihenstephan, Germany, Report no. ECO-00-268, August 05, 2001 (unpublished), BASF RDI No.: AL-560-056.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	None
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Not stated However, the study design is consistent to the principles outlined in the draft OECD guideline on freshwater lentic field tests, SETAC, EWOFFT, HARAP, CLASSIC
2.2	**GLP**	No However, GLP-like documentation was employed.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Alphacypermethrin 100 g a.s./L OESC (formulation code CF 06677)
3.1.1	Lot/Batch number	166772
3.1.2	Specification	Alphacypermethrin 100 g a.s./L OESC (formulation code CF 06677)
3.1.3	Purity	101.7 g/L alphacypermethrin (a.s.)
3.1.4	Composition of product	OESC formulation with a.i. content as specified under 3.1.3 above.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **Aquatic mesocosm study**

3.1.5	Further relevant properties	The formulation is not considered to have any impact on the quality of the results, since it represents a neutral oil suspension. The overall purpose of this study was to evaluate possible effects of alphacypermethrin insecticide when repeatedly applied according to Good Agricultural Practice (GAP) for this formulation, resulting in predicted initial environmental concentrations (PIEC) in adjacent ditches as predicted by the Ganzelmeier drift model. The application of the active substance in biocidal products may be expected to result in similar releases to the aquatic environment: use as an insecticide for domestic hygiene purposes may lead to pulsed / intermittent release, e.g. as consequence of cleaning operations in the treated premises. Thus, the study design and the endpoint are considered to be fully adequate in the context of a biocidal risk assessment based on the intended uses, particularly since repeated exposure is simulated in the current study.
3.1.6	Method of analysis	Residue analysis was performed in the parallel study no. ETX-99-101, utilising single applications of the test substance to equivalent outdoor ponds (ref. A7.4.3.5/02). Since the test conditions were thus equivalent, the actual test substance concentrations may be considered to be adequately verified also for the current study.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	No The test concentrations were below the limit of water solubility of alphacypermethrin.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not appropriate
3.4	**Testing procedure**	
3.4.1	Dilution water	Natural pond water
3.4.2	Test organisms	Semi-natural assemblages of freshwater macroinvertebrates, zooplankton and algae in pond-enclosures located at Technical University of Munich, Freising, Germany.
3.4.3	Test system	The test system is described in Table A7.4.3.5- 7. Baskets with artificial benthic substrate served as habitat patches and sampling sites for macroinvertebrates. The original macrophyte flora established since construction of the ponds was reduced to 20% surface cover on day –57.
3.4.4	Test conditions	Test conditions in the aquatic phase are provided in Table A7.4.3.5- 8. The sediment consisted of a mixture of natural sediment from adjacent ditches (1/3) and soil material obtained during construction of the ponds (2/3). The sediment was characterised chemically and physically (particle size etc.) and found to be structurally and biologically representative for natural sediments. Treatment: on days 0, 14, 28.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **Aquatic mesocosm study**

3.4.5	Duration of the test	119 days Treatment (start of assessment period): June 16, 1999
3.4.6	Test parameter	Density of planktonic algae, measured as chlorophyll a and as algal cell density, as an indicator of primary production performance; Zooplankton: abundance, species composition, diversity in terms of species number, Shannon index, Simpson's index and principal response curves (PRC); Macroinvertebrates: abundance, species composition, diversity in terms of species number, Shannon index, Simpson's index and principal response curves (PRC); (live sampling and release into the original replicate upon determination of species identity).
3.4.7	Examination/ Sampling	- *Oxygen, pH, alkalinity, conductivity*: pre-treatment days –35, –29, -21, –15, –8, and –1; post-treatment days 7, 13, 21, 27, 33 and then at 7-d intervals (± 1 d) until day 105 (September 29); - *Chlorophyll a*: Water samples of 0.5 l volume were directly measured in a photometer after sieving (1 mm mesh width) to remove filamentous algae; sampling schedule: pre-treatment days –35, –29, -21, –15, –8, and –1; post-treatment days 7, 13, 21, 27, 33 and then at 7-d intervals (± 1 d) until day 105 (September 29); - *Zooplankton*: Two water samples of 3 l volume were sampled per sampling day per enclosure (one from the open-water body, one in close vicinity to macrophytes; the samples were poured through a 63 µm mesh, which was rinsed with tap water; formaldehyde and subsequently diethylenglycol were added for fixation; the fixed plankton samples subjected to microscopic examination; sampling schedule: pre-treatment days–35, –29, –21, –15, –8, and –1, post-treatment days 1, 7, 13, 21, 27, 33, and then at 7-d intervals (± 1 d) until day 105 (September 29); - *Macroinvertebrates*: (i) artificial substrate for benthic invertebrates was exposed in sampling baskets, removed at the sampling dates, rinsed with tap water, the live animals identified to at least the level of order, counted and returned alive to their original enclosure; sampling schedule: pre-treatment days –29, –21, –14, –8, post-treatment days 1, 7, 13, 15, 21, 29, 34 and then at 7-d intervals until day 119 (October 13); (ii) pelagic and plant-associated macroinvertebrates were sampled using a plankton net pulled through the water body; sampling schedule as for benthic organisms above; (iii) due to the naturally low abundance of gammarids, 10 individuals of *Gammarus roeselii* were added to each enclosure on day –12; sampling schedule as under item (i) above; (iv) additionally, 10 *Gammarus roeselii* were introduced to each enclosure in 250 mL cages and their survival assessed weekly and on the day following each treatment, respectively.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **Aquatic mesocosm study**

3.4.8	Monitoring of TS concentration	The alphacypermethrin concentration in each treatment solution was confirmed analytically for the parallel single-application pond study (ref. A7.4.3.5/02). The overall average percent of nominal found in that study was 87 ± 2% for the treatment solutions. These data provide evidence that the alphacypermethrin concentration in the treatment solutions for the multiple applications study were as required to produce the targeted test substance concentrations in the pond enclosures.
3.4.9	Statistics	Regression analysis, diversity indices (Shannon, Simpson), principal response curve (PRC) analysis.
3.4.10	Further remarks	The results of this experimental mesocosm study itself were subject to an extensive review by an independent expert panel (references A7.4.3.5/05, 06, 07). The experts' opinion is also reflected in this and in the previous summary, resulting in a recommendation of an overall ecologically acceptable concentration (EAC). Effects of alphacypermethrin were additionally evaluated in an individual-based community simulation study (A7.4.3.5/06).

4 RESULTS

4.1	**Results test substance**	
4.1.1	Initial concentrations of test substance	Nominal test concentrations: 0.00006, 0.0006, 0.015, and 0.375 µg/l. The pond enclosures were treated three times at 14-day intervals (i.e. days 0, 14, and 28), aimed at achievement of the stated nominal concentrations for each treatment.
4.1.2	Actual concentrations of test substance	As already stated under 3.4.8 above, the alphacypermethrin concentration in each treatment solution was confirmed analytically for the parallel single-application pond study (ref. A7.4.3.5/02). The overall average percent of nominal found in that study was 87 ± 2% for the treatment solutions. These data provide evidence that the alphacypermethrin concentration in the treatment solutions for the multiple applications study were as required to produce the targeted test substance concentrations in the pond enclosures.
4.1.3	Effect data, general	The pond-enclosures used for this study supported a representative diversity of freshwater organisms, comparable to that of natural ponds in the proximity of the test facility. Significant perturbation was noted in the pond-enclosure treated with 0.375 µg a.s./l (i.e., 12.5 x the highest PIEC value), thereby confirming the validity of the test system.
4.1.4	Effect data, phytoplankton	Based on chlorophyll a measurements, there were transient treatment-related effects on planktonic algae only at the highest treatment concentration (0.375 µg a.s./l).

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **Aquatic mesocosm study**

4.1.5	Effect data, zooplankton	No significant changes occurred in the overall number of zooplankton organisms in pond-enclosures treated at the two lowest concentrations (0.00006, 0.0006 µg a.i./l). At a concentration of 0.015 µg a.i./l, however, minor increases in abundance were observed from 8 DAT to 35 DAT, possibly induced by reduced predation pressure. This indirect effect was markedly expressed at the highest, exaggerated concentration (0.375 µg a.i./l) where there was no recovery to control values by the end of the in-life assessment period. Principal Response Curve (PRC) analysis indicated indirect effects only in pond-enclosures treated with 0.015 or 0.375 µg a.i./l. The abundance of zooplankton increased at these concentrations, possibly due to reduced predation pressure induced by direct effects of treatment on larvae of the midge *Chaoborus crystallinus* (Diptera: Chaoboridae, the ghost, or phantom midge). These indirect effects on zooplankton were observed to continue until the end of the in-life assessment period. Since effects on zooplankton were evident only at these highest test concentrations (0.015 and 0.375 µg a.i./l), the recommended EAC based on direct and indirect effects on zooplankton is 0.015 µg a.i./l.
4.1.6	Effect data, macroinvertebrates	General: Effects on macroinvertebrates at concentrations of 0.00006 and 0.0006 µg a.i./l were both minor and transient. Transient effects on overall abundance were also noted at 0.015 and 0.375 µg a.i./l. Full recovery to normal (control) values was observed during the course of the in-life assessment period at a concentration of 0.015 µg a.i./l, but not at 0.375 µg a.i./l. *Chaoborus crystallinus*: Minor, transient effects on the abundance of larval stages of one dipteran species, *C. crystallinus*, indicated a population LOEC (lowest observed effect concentration) of 0.00006 µg a.i./l, based on a temporary failure of this dipteran species to increase relative to control values at all tested concentrations. However, post 50 DAT at the lowest concentrations (0.00006, 0.0006 µg a.i./l), and post 108 DAT at 0.015 µg a.i./l, the numbers of *C. crystallinus* returned to normal (control) values (i.e., the organism exhibited developmental cycles that were indistinguishable from controls). Only in pond-enclosures treated at the highest concentration (0.375 µg a.i./l) there was no complete recovery evident by the end of the in-life assessment period. Therefore, based on (a) full recovery of *C. crystallinus* at relevant environmental concentrations (and up to 12.5 times the highest PIEC value), and (b) since *C. crystallinus*, the most sensitive organism in this study, is neither a protected organism nor a 'keystone' in natural fish-dominated aquatic systems, the recommended EAC based on effects on this most sensitive organism is 0.015 µg a.i./l. Community structure: Macroinvertebrate community-structure was transiently affected in pond-enclosures treated with 0.00006, 0.0006 and 0.015 µg a.i./l, with full recovery to control values during the in-life assessment period. Only at the highest concentration (0.375 µg a.i./l) there was no re-alignment with control values observed at the end of the in-life assessment period (119 DAT). Therefore, based on effects on macroinvertebrate community-structure, an EAC of 0.015 µg a.i./l is recommended.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **Aquatic mesocosm study**

4.1.7	Effect data, ecosystem function	There were no effects on overall ecosystem function at any test concentration, as demonstrated by the outcome of a series of physical-chemical measurements. However, the chlorophyll *a* concentration increased transiently at the highest concentration, possibly due to decreased predation pressure following direct effects on zooplankton at the highest concentration (0.375 µg a.s./l). Only at the highest test concentration of 0.375 µg a.s./l (i.e., 12.5 times the highest PIEC value in an agricultural context) was biological diversity affected by three applications of FASTAC OESC. There were no such effects at any environmentally relevant concentration (PIECs). Therefore, the recommended EAC based on conservation of community diversity and function is between 0.015 and 0.375 µg a.i./l.
4.2	**Results of controls**	Controls formed the baseline for the evaluation of effects.
4.3	**Test with reference substance**	No reference substance was used.
4.3.1	Concentrations	Not applicable
4.3.2	Results	Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	An aquatic mesocosm study was conducted in order to derive an ecologically acceptable concentration (EAC) of alphacypermethrin in surface water. The overall objective of this study was to evaluate the possible effects of multiple applications (three treatments at 14-day intervals) of a 100 g/l SC (suspension concentrate) formulation (CF06677) of alphacypermethrin (AC 900049), applied at aqueous concentrations as predicted by the German Drift Model (GANZELMEIER et al., 1995) and current as well as proposed Good Agricultural Practice (GAP) parameters for this formulation, on macroinvertebrates and zooplankton in enclosures in ponds. The possible effects of multiple applications on macroinvertebrates and zooplanktonic organisms provided the primary focus of the study. Biological samples were collected prior to the first treatment on June 16, 1999 and until 119 days after treatment (DAT 119) on October 13, 1999. Environmental conditions were monitored during the test.

Section A7.4.3.5 **Effects on any other specific, non-target organisms:**
Annex Point IIIA 13.3.4 **Aquatic mesocosm study**

5.2	**Results and discussion**	An overall EAC (Ecologically Acceptable Concentration) for alphacypermethrin of 0.015 µg/l was derived from this study, based on the demonstration of full recovery of transiently affected insect populations and intact ecological functioning of the pond systems at all environmentally relevant initial concentrations (PIECs). This recommendation is also backed up by a number of review reports and independent expert opinions as well as an individual-based ecosystem simulation study, which were already considered in the previous study summary (references A7.4.3.5/05, 06, 07). Whereas this study was originally designed in an agricultural context, i.e. repeated pulsed release of the actives substance to aquatic ecosystems following treatment of adjacent fields, the results are considered to be also valid in the context of a risk assessment for biocidal purposes for the following reasons: - The intended biocidal use is described as insect control in domestic premises for hygiene purposes; due to the required prolonged action (6 months) of the product applied to surface areas in the treated premises, releases from the treated sites are expected to be intermittent, e.g. as a consequence of cleaning operations; - The receiving water bodies following biocidal use typically are running waters; thus, intermittent releases result in rapid transport of such "pulsed" discharges; a given section of the watercourse will therefore be exposed to any residues of the active substance for a very limited period of time; - In conclusion, the discharge patterns are very similar between a typical biocidal exposure scenario and the original agricultural application pattern under which the study was developed; thus, the EAC as recommended in the study and various subsequent review papers is equivalently applicable to intermittent releases to flowing water bodies.
5.2.1	EAC	0.015 µg/l
5.3	**Conclusion**	
5.3.1	Reliability	2
5.3.2	Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and Methods	The Applicant's version is considered to be acceptable
Results and discussion	The Applicant's version is considered to be acceptable
Conclusion	The Applicant's version is considered to be acceptable
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.3.5- 7: Test system.

Criteria	Details
Test type	Static mesocosm test
Renewal of test solution	–
Volume of test vessels	593 l per enclosure (calculated to provide a water level of 1 m) Enclosures situated in a larger pond of 5 m diameter (29 000 l volume)
Volume/animal	Not appropriate (semi-natural community)
Number of animals/vessel	Not appropriate (semi-natural community)
Number of vessels/concentration	2 controls No replication of treatments
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.5- 8: Test conditions.

Criteria	Details
Test temperature	Water temperatures in the in-life period: 15–23 °C
Dissolved oxygen [% saturation]	50–140 (see figures provided in the test report)
pH	7.6–8.6 (see figures provided in the test report)
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Natural sunlight
Photoperiod	Seasonally varying natural photoperiod

Section A7.4.3.5 Effects on any other specific, non-target organisms:

Annex Point IIIA 13.3.4 **– supportive data with respect to the EAC –**

The following reference is considered to contain additional information concerning the derivation of the EAC. The chronic toxicity of the active substance to various aquatic macroinvertebrates is addressed, in order to supplement the results obtained from the outdoor mesocosm studies presented above (A7.4.3.5/02, 08). Thus, the result of the current study are presented in an abbreviated format (adopted from the PPP-dossier) as supportive data:

Reference: **A7.4.3.5/09**

Huber W, Grünwald H (2002) Toxicity of Alphacypermethrin (BAS 310 I, AC 900049) in a 100 g/L OESC formulation (FASTAC OESC insecticide, CF 06677) to aquatic macroinvertebrates (*Cloëon, Gammarus*) in laboratory microcosms. Technical University of Munich-Weihenstephan, Germany, Report no. LSÖ 02/3, December 15, 2002, (unpublished), BASF RDI No.: 2002/1013889.

Guidelines: Non-guideline study

GLP: Non-GLP study

Material and methods:

Test substance: Alphacypermethrin 100 g a.s./L OESC (formulation code CF 06677), batch no. 214228/214130.

Test species: *Cloëon dipterum* larvae (Insecta, Ephemeroptera)
Gammarus roeseli (Crustacea, Amphipoda)

Test system: Laboratory microcosms in 5 l glass beakers, containing natural pond sediment and one shoot of the macrophyte *Myriophyllum*.

Test design: *Cloëon*: Single application of test material, test duration 7 days, sampling of test animals on days 1, 3, and 7.

Gammarus: Three applications of test material (day 0, 7, and 14); test duration 28 days, sampling of test animals only on day 28.

Test concentrations: 0.003, 0.0075, 0.015, 0.035, 0.075, 0.375 and 1.875 µg/l (nominal).

Test conditions: Temperature approx. 15 ± 2 °C; photoperiod 16:8 h (L:D), light intensity 10 000 lux, pH 8.7 (± 0.35) in the *Cloëon* test, and 8.6 (± 0.22) in the *Gammarus* test; oxygen saturation 150% (± 26.2%) in the *Cloëon* test, and 115.2% (± 8.2%) in the *Gammarus* test; conductivity 194 ± 33 µS/cm in the *Cloëon* test, and 181.8 ± 30.7 µS/cm in the *Gammarus* test.

Statistics: EC and LC-estimates determined from logistic dose-response curves.

Findings:

Nominal test concentrations of 0.003, 0.0075, 0.015, 0.035, 0.075, 0.375 and 1.875 µg/l were confirmed analytically, within 1 hour of application, to be approximately 79% of nominal values.

Results of chemical analysis demonstrated that alphacypermethrin in the water of the laboratory microcosms degraded completely before a subsequent application of the test chemical after 7 or 14 days. There was no accumulation of alphacypermethrin in the water, even after a maximum of three treatments.

In sediments, measurements for possible accumulation were made following each treatment, however, only in microcosms treated with an exaggeratedly high concentration of 1.875 µg a.i./l was any (minor) accumulation noted. There was no accumulation of alphacypermethrin in sediments at any other concentrations, including environmentally realistic concentrations.

Cloëon dipterum: Sensitivity to alphacypermethrin apparently increased with decreasing age of larvae and increasing exposure time. In tests with over-wintered (hibernated, LII-LIV) larvae, NOEC values ranged between 0.13 and 0.36 µg a.i./l. EC_{50} values ranged between 0.39 and 4.59 µg a.i./l. Young larvae from summer (July/August) populations were relatively more sensitive, with a mean NOEC of 0.005 µg a.i./l. EC_{50} and EC_{80} values were also lower than for the over-wintered larvae. Toxicity values for larvae from July and August populations were; 0.028 and 0.085 µg a.i./l (EC_{50}), and 0.056 and 0.54 µg a.i./l (EC_{80}), respectively. These data indicate differential sensitivities between the various tested larval stages of *C. dipterum*.

Gammarus roeseli: In laboratory microcosms, following two applications of FASTAC OESC separated by 14 days, the NOEC and EC_{50} were 0.048 µg a.i/l and 0.071 µg a.i./l, respectively. With two or three applications separated by a 7-day spray interval, the 28-day NOEC was ≤ 0.007 µg a.i./l. The lowest 28-day EC_{50} after two applications (with a 7-day spray interval) was 0.11 µg a.i./l and after three applications (with a 7-day spray interval) 0.048 µg a.i./l. Significantly, in microcosms treated repeatedly with the proposed EAC concentration of 0.015 µg a.i./l at 7-day spray intervals, pronounced production and survival of juveniles was observed at the end of the 28-day test period.

Conclusions:

Based on these data, an EAC (Ecologically Acceptable Concentration) of 0.015 µg alphacypermethrin/l is proposed for multiple applications of FASTAC OESC.

Section A7.4.3.5 Effects on any other specific, non-target organisms:

Annex Point IIIA 13.3.4 **– supportive data with respect to the EAC –**

The following reference is considered to contain additional information concerning the derivation of the EAC. The acute toxicity of the active substance to various aquatic macroinvertebrates is addressed, in order to supplement the results obtained from the outdoor mesocosm studies presented above (A7.4.3.5/02, 08). Thus, the result of the current study are presented in an abbreviated format (adopted from the PPP-dossier) as supportive data:

Reference: **A7.4.3.5/10**

Funk M, Huber W, Mitchell GC (2000) Acute toxicity of alphacypermethrin (AC900049) in a 100 g/L OESC formulation (CF06677) to aquatic macroinvertebrates. Technical University of Munich-Weihenstephan, Germany, Report no. 98-I, October 15, 2000 (unpublished), BASF RDI No.: AL-521-001.

Guidelines: Laboratory toxicity tests with non-standard organisms, no guidelines available

GLP: While all aspects of this study were accurately recorded by qualified scientific staff and the general principles of GLP, this study was not strictly GLP compliant because there was no Quality Assurance oversight.

Material and methods:

Test substance: Alphacypermethrin 100 g a.s./L OESC (formulation code CF 06677); Batch Number FAS 400001; 101 g/L alphacypermethrin (a.s.) and "Blank "OESC" formulation, i.e., formulation components without active ingredient (alphacypermethrin).

Species: The aquatic macroinvertebrates tested were:

the phantom midge (Diptera: *Chaoborus crystallinus*),
a mayfly (Ephemeroptera: *Cloëon dipterum*),
a caddis fly (Trichoptera: *Phryganea grandis*),
an amphipod crustacean (Amphipoda: *Gammarus roeseli*),
an isopod crustacean (Isopoda: *Asellus aquaticus*),
an aquatic butterfly (Lepidoptera: *Nymphula nymphaeata*),
backswimmers (Heteroptera: *Notonecta glauca* and *Plea leachi*)
dragonflies (Odonata: Zygoptera sp.).

Chaoborus, Cloëon, Phryganea, Nymphula, Notonecta, Plea and Zygoptera were collected from pond-enclosures operated by the contract facility (Technical University of Munich-Weihenstephan). *Gammarus* was collected from a river in the vicinity of the test ponds. *Asellus aquaticus* was collected from a pond near to the test facility. In the case of *Chaoborus*, several life-stages (eggs, larvae, pupae) were isolated for toxicity testing.

Test Conditions: For acclimation: 14 l glass aquaria in environmental chamber; temp. 18 ± 2 °C, 16-h light: 8 h dark photoperiod; light intensity 200 mE × m^{-2} × S^{-1}. Test organisms were acclimated > 3 days in environmental chamber before testing. Prior to testing, food for the test organisms included small planktonic organisms (e.g., small cladocerans) for *Chaoborus*, Trichoptera, Heteroptera, Zygoptera; detritus for *Gammarus* and *Asellus*; algae for Ephemeroptera, and vascular plant material for Lepidoptera.

For testing, 1000-mL beakers containing 1-L of filtered pond water were used with at least three replicates at each test concentration. Toxicity tests that incorporated sediment were conducted with 50 g of typical natural lake sediment. Glass beakers containing 1L of filtered (63 µm mesh) pond water were allowed to settle for 24 hours prior to introduction of tests organisms and application of the test substance.

Laboratory microcosms contained 50 g of lake sediment, 1 l of filtered (63 µm mesh) pond water and one shoot of *Myriophyllum spicatum*. Microcosms were equilibrated one week before use in toxicity testing.

Laboratory tests with *Chaoborus* eggs were conducted in glass bowls containing 100 mL of tests substance and 20 eggs from an egg package. For "hatch-success" tests with *Chaoborus*, egg-packages were collected from the surfaces of untreated ponds and used for testing the same day.

Concentrations: Nominal test concentrations ranged from 0.00001 to 100 µg a.s./l.

Treatment/Application: Applications of 100–1000 µL were made with an Eppendorff pipette (range 100–1000 µL), beginning with the lowest test concentration.

Observation: Acute toxicity values, i.e., 50% effect concentrations (EC_{50}), no observed effect concentrations (NOEC) and lowest observed effect concentrations (LOEC) for aquatic macroinvertebrates exposed in the laboratory to a 100 g/L FASTAC *OESC formulation containing alphacypermethrin (AC 900049) as active ingredient (a.i) were determined in a series of single-species toxicity tests.

Statistics: EC and LC estimates were determined from sigmoid dose response curves and linear regression. The evaluations were performed with the PC program MICROCAL ORIGIN Version 5, 1997.

Analytical Chemistry: Test concentrations were not analytically confirmed. Test results were based on nominal concentrations.

Findings:

"Blank " Formulation:

The "blank" OESC formulation, i.e., formulation ingredients without a.s., caused no detectable toxicological effect on any of the tested species.

Active Formulation:

Gammarus roeseli and larval stages of *Chaoborus crystallinus* were the most sensitive organisms, each with a NOEC value ≤ 0.01 µg a.s./l. The lowest EC_{50} was 0.042 µg a.s./l.

Chaoborus eggs were significantly less sensitive than other life stages to alphacypermethrin. Even at concentrations up to 100 µg a.s./l, approximately 50% of the eggs hatched.

Zygoptera spec. relatively less sensitive to alphacypermethrin than other tested macroinvertebrates, with a LOEC value of 2 µg a.s./l and EC/LC_{50} values of 4.7/9.8 µg a.s./l, respectively.

$EC_{50/100}$ values for all other testes macroinvertebrates were in the range 0.25 to 0.52 µg a.s./l and NOEC values were in the range 0.0011 to 0.3 µg a.s./l.

Conclusions:

These laboratory results corroborated data or observations from field (pond-enclosure) studies with FASTAC OESC (summarized above). There was a range of sensitivities to alphacypermethrin among selected, ecologically relevant aquatic invertebrates. The midge *Chaoborus crystallinus* was confirmed as the most sensitive species. *Chaoborus* eggs were significantly less sensitive than other life stages. Zygoptera were relatively less sensitive than other tested species, with EC_{50} values significantly higher than environmentally relevant concentrations of alphacypermethrin. Formulation ingredients did not contribute to the toxicity of FASTAC OESC.

Section A7.4.3.5 Effects on any other specific, non-target organisms:

Annex Point IIIA 13.3.4 **– supportive data with respect to the EAC –**

The following reference is considered to contain additional information concerning the derivation of the EAC. The acute toxicity of the active substance to various aquatic macroinvertebrates is addressed, in order to supplement the results obtained from the outdoor mesocosm studies presented above (A7.4.3.5/02, 08). Furthermore, the current study was evaluated independently in reference A7.4.3.5/05. Thus, the result of the current study are presented in an abbreviated format (adopted from the PPP-dossier) as supportive data:

Reference: **A7.4.3.5/11**

Hahn T (2002) Effects of Alphacypermethrin (BAS 310 03 I) applied as FASTAC OESC on aquatic macroinvertebrates (Ephemeroptera and Trichoptera) in extended "standard" laboratory tests. Zoologisches Institut der TU Braunschweig, Report no. 14-53-81, November 26, 2002 (unpublished), BASF Doc-ID.: 2003/1012036.

Guidelines: Laboratory toxicity tests with non-standard organisms, no guidelines available

GLP: No; however, all aspects of this study were accurately recorded by qualified scientific staff.

Material and methods:

Test substance: FASTAC 100 g a.s./L OESC (formulation code CF 06677); batch no. 214228; a.i. content 100 g/l alphacypermethrin and.

Species: The aquatic macroinvertebrates tested were removed from watercourses close to the test facility;

Caddis flies:
Anabolia nervosa (Trichoptera: Limnephilidae)
Limnephilus lunatus (Trichoptera: Limnephilidae)

Monovoltine mayfly species:
Ephemerella ignita (Ephemeroptera: Ephemerellidae)
Siphlonurus lacustris (Ephemeroptera: Siphlonuridae)

Plurivoltine mayfly species:
Baëtis vernus (Ephemeroptera: Baëtidae)
Caënis luctuosa (Ephemeroptera: Caënidae)

Test Conditions: Caddis flies: 500 mL non-chlorinated tap water (aerated prior to use) in 1000 mL laboratory beakers, no sediment, temperature 16 ± 1°C, 16:8 h light: dark cycle; random assignment to test vessels; test duration 96 h with inspections for mortality every 24 h.

Mayflies: 100 mL non-chlorinated tap water (aerated prior to use) in 300 mL crystallising dishes, 4 × 7 cm stainless-steel gaze (0.5 mm mesh) as artificial sediment, temperature 15 ± 1°C, 16:8 h light: dark cycle; random assignment to test vessels; test duration 48 h with additional inspection for mortality at 24 h.

Concentrations:

Anabolia nervosa:	0.1, 0.2, 0.4, 0.8, 1.6	µg a.s./l (nominal)
Limnephilus lunatus:	0.2, 0.4, 0.8, 1.6, 3.2	µg a.s./l (nominal)
Baëtis vernus:	0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8	µg a.s./l (nominal)
Caënis luctuosa:	1.6, 3.2, 6.4, 12.8, 25.6	µg a.s./l (nominal)
Ephemerella ignita:	0.8, 1.6, 3.2, 6.4, 12.8	µg a.s./l (nominal)
Siphlonurus lacustris:	5.5, 10.0, 18.0, 32.4, 58.3, 105.0	µg a.s./l (nominal)

Test parameters: Mortality, behavioural responses.

Analytical method: SAMS 469, as validated in section A4.2.

Statistics: Analysis of variance and Dunnett's test (Kruskal-Wallis test in case of non-normality) for differences in survival between treatments and control; EC_{50} (behavioural responses) and LC_{50} by probit analysis.

Findings:

Species	LC50 [µg/l]		(95% CI)
Anabolia nervosa:	0.53		(0.44–0.64)
Limnephilus lunatus:	1.03	BE CA1.02	(0.61–1.45)
Baëtis vernus:	1.50		(0.07–2.64)
Caënis luctuosa:	9.84		(7.1–13.08)
Ephemerella ignita:	1.83		((–1.74)–3.76)
Siphlonurus lacustris:	48.01		(38.5–60.55)

Besides mortality, all species tested exhibited behavioural responses upon alphacypermethrin exposure. In most cases these consisted of symptoms of paralysis, i.e., in affected larvae reactions to mechanic stimuli were highly retarded. In caddis flies, a further symptom was case leaving. These reactions were dose-dependent only after 24 hours of exposure. At later examinations patterns blurred, probably due to elevated mortality. Nevertheless, when 24-h behavioural response data were probit-analysed and median effect concentrations (EC_{50}) were compared to the respective LC_{50} values, a high degree of correspondence was found. In the trichoptera, EC_{50}s were 0.72 µg a.i./l (95% CI = 0.49–1.10.) in *A. nervosa*, and 1.50 µg a.i./l (95% CI = 1.13–2.58) in *L. lunatus*. Values for the monovoltine ephemeroptera were 1.54 µg a.i./l (95% CI = 1.33–1.89) in *E. ignita*, and 32.05 µg a.i./l (95% CI = 25.34–44.11) in *S. lacustris*. The plurivoltine species *B. vernus* and *C. luctuosa* exhibited EC_{50} values of 1.07 µg a.i./l (95% CI = 0.50–2.06), and 10.15 µg a.i./l (7.73–13.26), respectively.

Conclusions:

In general, the results of this study suggest that species differences in alphacypermethrin susceptibility are based more on physiological adaptations to environmental conditions than on life history traits (voltiny level). Those species exhibiting a lower susceptibility to alphacypermethrin exposure (i.e., *C. luctuosa* and *S. lacustris*) are preferably living on or in organic sediments, where water flow is low or absent. The other two ephemeropteran and trichopteran species under examination appear to be more dependent on low to medium flowing water, often settling on leaves of submerged macrophytes.

In the described laboratory tests with four species of mayflies and two species of caddis fly, it was demonstrated that there is comparable sensitivity between insects exhibiting single or multi-generational (uni-, multivoltine, respectively) life histories. 48-h/96-h LC_{50} values were in the range 100–3200 fold (for mayflies) and 35– 68 fold (for caddis flies) above the proposed EAC of 0.015 µg a.i/l for multiple applications of FASTAC OESC insecticide. The tests also demonstrated that 24-h laboratory data predict potential 48-h/96-h effects in the field.

In summary, it was clearly corroborated that 48-h and 96-h LC_{50} values occurred at concentrations far in excess of the proposed EAC value of 0.015 µg a.i./l (100–3200 fold for mayflies, 35–68 fold for caddis flies), indicating a sufficient margin of safety for these sensitive organisms at environmentally relevant concentrations of alphacypermethrin.

Section A7.4.3.5.1 **Effects on sediment dwelling organisms**
Annex Point IIIA 13.3.4

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.3.5.1/01:** Heintze A (1997) Alphacypermethrin (AC 900049): effects on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system. GAB, Niefern-Öschelbronn, Germany, Report no. 96178/02-ASCr, September 30, 1997 (unpublished), BASF RDI No.: AL-523-002.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	No
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 Proposal for a BBA-Guideline: Effects of plant protection on the development of sediment-dwelling larvae of *Chironomus* in a water-sediment system (1995)
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	50323
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	97%
3.1.4	Composition of product	Not applicable
3.1.5	Further relevant properties	Water solubility at 20°C: pH 4 4.59 µg/L pH 7 5.80 µg/L pH 9 7.87 µg/L Distilled water 2.06 µg/L Vapour pressure: 3.4×10^{-7} Pa at 25 °C.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

3.1.6	Method of analysis	Analytical verification of stock solution concentrations, by HPLC-DAD (210 nm). Due to the low test concentrations, which are generally difficult to measure for pyrethroids, it was decided to analyse stock solutions only. Thus, test substance concentrations in the test systems were based on nominal values. Sediment concentrations were not measured.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Yes; see Table A7.4.3.5.1- 1.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	
3.4	**Testing procedure**	(Non-entry field)
3.4.1	Dilution water	Elendt M4 medium; details are given in Table A7.4.3.5.1-2.
3.4.2	Test organisms	*Chironomus riparius*, as specified in Table A7.4.3.5.1-3.
3.4.3	Test system	The test system is described in Table A7.4.3.5.1-4.
3.4.4	Test conditions	The dilution water was spiked with test substance. The test sediment was an artificial substrate (AS), prepared according to OECD test guideline No. 207 and consisting of the following fractions (dry weight basis): 10 % sphagnum peat (pH 5.5 , with no visible plant remains, air dried and finely ground); 20 % kaolin clay (kaolinite content of 33 %); 70 % industrial sand (77.4 % of the particles between 63 and 2000 microns); pH of the final mixture was adjusted to 6.0 ± 0.5 by addition of $CaCO_3$ (chemically pure quality). Other relevant test conditions are presented in Table A7.4.3.5.1-5.
3.4.5	Duration of the test	28 d
3.4.6	Test parameter	Larval development time, emergence rate, survival
3.4.7	Examination/ sampling	Daily inspection for emerged midges.
3.4.8	Monitoring of TS concentration	No (also see 3.1.6 above for explanation)
3.4.9	Statistics	Arcsine transformation was applied to the emergence rate prior to statistical analysis. NOEC, LOEC: Dunnett's or Williams' multiple *t*-test; EC_{50}: probit analysis.

Section A7.4.3.5.1 **Effects on sediment dwelling organisms**
Annex Point IIIA 13.3.4

4 RESULTS

4.1	**Range finding test**	Not performed.
4.1.1	Concentration	
4.1.2	Number/percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	Two consecutive definitive tests with overlapping concentration ranges were performed since the first test did not result in sufficiently high mortality to allow calculation of an EC_{50}.
4.2.1	Initial concentrations of test substance	1st test: 0.006, 0.012, 0.024, 0.048, 0.096, 0.192, 0.348 µg/l (nominal) 2nd test: 0.048, 0.096, 0.192, 0.348, 0.768, 1.536 and 3.072 µg/l (nominal)
4.2.2	Actual concentrations of test substance	Not applicable (see 3.1.6 above). The effects assessment was based on nominal concentrations. The results of concentration verification confirmed that all stock solutions, used for application of alphacypermethrin to the test vessels in each definitive test, contained >80% of nominal concentrations.
4.2.3	Effect data	Total numbers of emerged midges in the first and the second definitive test are presented in Table A7.4.3.5.1- 6 and Table A7.4.3.5.1- 7. In addition, effects on the emergence rate and the development rate are presented in Table A7.4.3.5.1- 8 and Table A7.4.3.5.1- 9. The first definitive test provided an NOEC of 0.096 µg/l. However, mortality did not exceed 38% up to the highest concentration. Therefore, an EC_{50} value could not be determined. Consequently, a second definitive test was conducted. The second definitive test provided an EC_{50} value. However, due to the biological variance in the test system a NOEC could not be determined. Since both definitive tests overlapped in their concentration ranges with an identical geometric factor of 2.0 between all concentration steps, the data from both tests were pooled to provide the following effect concentrations: EC_{50} = 0.227 µg/l (95% CI = 0.120–1.066) NOEC = 0.024 µg/l LOEC = 0.048 µg/l
4.2.4	Concentration / response curve	See study report.
4.2.5	Other effects	In the 1st definitive test, significant differences in the numbers of emerged midges could be detected only at the two highest concentrations (0.192 and 0.384 µg/l). No differences in sex of emerged midges among treatments were observed. For development rate, again only at the two highest concentrations significant differences from control/solvent control were detected.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

4.3	**Results of controls**	See above.
4.4	**Test with reference substance**	Not performed.
4.4.1	Concentrations	
4.4.2	Results	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The toxicity of the test substance alphacypermethrin to sediment-dwelling organisms was tested using larvae of the midge *Chironomus riparius*, according to the proposal for a BBA Guideline ("Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water sediment system"). According to this draft guideline, *Chironomus* larvae were exposed to the test substance over 28 days via spiked water. Effects on emergence rate, development rate and survival were evaluated.
5.2	**Results and discussion**	Due to the methodological difficulties with analytical verification generally known for pyrethroids, monitoring of the test substance in the test medium was not possible. Instead, test substance concentrations in stock solutions were verified analytically, yielding mean recoveries of 93%. In view of this and the accurately documented application procedure, it was concluded that nominal concentrations were satisfactorily met. At some rare occasions, dissolved oxygen and pH were outside the range specified by the BBA guideline (see Table A7.4.3.5.1-5). However, this may be explained by unavoidable algal growth, which may result in excessive oxygen production and a shift of pH. Upon request, BBA had confirmed that these deviations would not necessarily compromise the validity of the study.
5.2.1	NOEC	0.024 µg/l (water phase)
5.2.2	LOEC	0.048 µg/l (water phase)
5.2.3	EC_{50}	0.227 µg/l (95% CI = 0.120–1.066) (water phase)
5.3	**Conclusion**	Apart from the variation in water quality parameters discussed under 5.2 above, no other deviations from the test guideline were reported. Thus, study is considered as valid.
5.3.1	Reliability	2

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

5.3.2	Deficiencies	Yes In the context of a chemicals/biocides risk assessment, usually a test design utilising exposure via spiked sediment is required. Since the current study was originally performed for risk assessment of plant protection products, spiked water was employed, which may be regarded as a deficiency in a biocidal framework. Nevertheless, since higher-tier studies (mesocosm) also focusing on sediment organisms are available, the *Chironomus* study is considered useful for comparison. Thus, a reliability indicator of 2 was allocated.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and Methods	The Applicant's version is considered to be acceptable
Results and discussion	The Applicant's version is considered to be acceptable
Conclusion	The Applicant's version is considered to be acceptable
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.3.5.1- 1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	Yes Acetone
Concentration of vehicle	0.01% (v/v)
Vehicle control performed	Yes 0.01% (v/v) solvent control in addition to blank control
Other procedures	None

Table A7.4.3.5.1-2: Dilution water.

Criteria	Details
Source	Elendt M4 medium, composed on the basis of deionised water
Hardness	14.5 °dH
pH	7.5 ± 0.3
Ca^{2+} / Mg^{2+} ratio	4/1
Na^{+} / K^{+} ratio	10/1
Holding water different from dilution water	No

Table A7.4.3.5.1-3: Test organisms.

Criteria	Details
Strain / Clone	Not applicable
Source	Egg masses from an in-house culture
Age	2–3 days (first instar)
Breeding method	Temperature controlled breeding area, 20–25 °C, illuminated by cool white lamps (Osram L30W/21-840), photoperiod 16:8 h (L:D)
Kind of food	Mixture of algae supplemented with an invertebrate food suspension
Amount of food	Not reported
Feeding frequency	Not reported
Pre-treatment	No (not required)
Feeding of animals during test	Yes Invertebrate food suspension at least three times per week

Table A7.4.3.5.1-4: Test system.

Criteria	Details
Test type	Static
Renewal of test solution	Not applicable
Volume of test vessels	2 l
Volume/animal	0.08 l
Number of animals/vessel	25
Number of vessels/concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.5.1-5: Test conditions.

Criteria	Details
Test temperature	20 ± 2 °C (temperature controlled room)
Dissolved oxygen	4.3–11.7 mg/l
pH	7.24–9.88
Adjustment of pH	No
Aeration of dilution water	Yes Gentle aeration (approx. 1 bubble/sec) through a glass pipette at about 2–3 cm above the substrate surface aeration was interrupted during addition of the test organisms, to allow them to settle undisturbed to the sediment; 24 h after addition of the larvae, immediately after application of the test substance, aeration was re-commenced
Quality/Intensity of irradiation	800–1200 Lux
Photoperiod	16:8 h (L:D)

Table A7.4.3.5.1- 6: Effect data: Numbers of emerged midges in the first definitive test.

c [µg/l]	Vessel 1	Vessel 2	Vessel 3	Vessel 4	Sum	Max.
Control	23	26	26	27	102	100
Solvent	27	25	25	23	100	100
0.006	24	25	21	24	94	100
0.012	28	25	22	25	100	100
0.024	23	25	26	23	97	100
0.048	24	23	27	24	98	100
0.096	22	25	26	26	99	100
0.192	14	19	25	4	62	100
0.384	14	13	17	20	64	100

Table A7.4.3.5.1- 7: Effect data: Numbers of emerged midges in the second definitive test.

c [µg/l]	Vessel 1	Vessel 2	Vessel 3	Vessel 4	Sum	Max.
Control	25	27	22	27	101	100
Solvent	23	24	22	22	91	100
0.048	14	11	23	8	56	100
0.096	10	20	27	23	80	100
0.192	19	11	0	7	37	100
0.384	0	0	0	0	0	100
0.768	0	0	0	0	0	100
1.536	0	0	0	0	0	100
3.072	0	0	0	0	0	100

Table A7.4.3.5.1- 8: Effect of alphacypermethrin on emergence and development rates (1st definitive test).

c [µg /l]	C	SC	0.006	0.012	0.024	0.048	0.096	0.192	0.384
Emergence rate	1.499	1.499	1.367	1.482	1.427	1.398	1.482	0.972	0.932
Development rate	0.065	0.068	0.069	0.067	0.065	0.063	0.062	0.056	0.054

C = Control, SC = Solvent Control

Table A7.4.3.5.1- 9: Effect of alphacypermethrin on emergence and development rates (2nd definitive test).

c [µg /l]	C	SC	0.048	0.096	0.192	0.384	0.768	1.536	3.072
Emergence rate	1.482	1.271	0.864	1.161	0.585	0.0	0.0	0.0	0.0
Development rate	0.065	0.067	0.059	0.060	0.058	–	–	–	–

C = Control, SC = Solvent Control