Section A7.4.2, A7.4.3.3.1

Annex Point IIA7.5, IIA8.2.3

Bioconcentration in aquatic organisms

Bioaccumulation in an appropriate species of fish

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]; Draft report on flow-through test in rainbow trout to determine the bioaccumulation potential of cypermethrin; [redacted] Dates of work: 2 March 1990 – 4 July 1990	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD guideline (1981) 305E	
2.2	**GLP**	Unclear from the final report (no copy of certificate however GLP is mentioned on page 8 of the study protocol)	X
2.3	**Deviations**	Yes. The volume of the aquaria allowed a fish weight:water volume ratio of c. 8 g/L which was around half of that specified in the guideline.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin technical	
3.1.1	Lot/Batch number	FSG 08611 J	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	94.2%	
3.1.4	Further relevant properties	Low water solubility (<5-10 ppb), log Pow of 4.47. Therefore the test substance was first dissolved in acetone before being introduced into the test system. See Table A7_4_3_3_1-1	X
3.1.5	Radiolabelling	Not applicable,	
3.1.6	Method of analysis	Samples of test chamber water and fish homogenate were analysed by Gas Chromatography (4mm I.D. glass column packed with 3% OV-101 on chromosorb W HP 100-200 mesh, nitrogen carrier gas flow rate 40ml/min) with Electron Capture Detection.	
3.2	**Reference substance**	No	
3.2.1	Method of analysis for reference substance	Not applicable	

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

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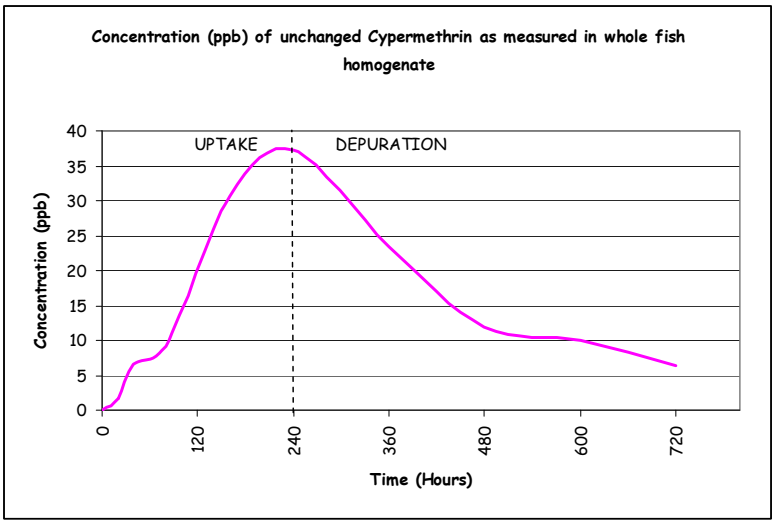
4.1.3	Concentrations of test material during test	See Table A7_4_3_3_1-7. Measurement of test chamber concentration showed that 0.1 ppb was attained with minute deviation. During the uptake phase the mean (±S.D.) concentration was found to be 0.09 (±0.007) ppb. The concentration of cypermethrin was below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

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

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

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Reliability	**2** **2.2 GLP: Even if the GLP certificate is not provided, the study report is very well documented and seems to be performed with similar level of quality as for a full GLP study. However, the RMS was not able to find a proof that the laboratorium who had performed the test were GLP in 1990.** **2.3 This deviation is not considered to be of concern for the validity of the test.**
Acceptability	**Acceptable:** **Due to the consideration above regarding the test duration, the result of the test is considered as valid but with some reservations.**
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_3_3_1-1: Preparation of TS

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

Table A7_4_3_3_1-2: Dilution water

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

Table A7_4_3_3_1-3: Test Organism

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

Table A7_4_3_3_1-4: Test system

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

Table A7_4_3_3_1-5: Test conditions

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

Table A7_4_3_3_1-6: Sampling schedule for fish bioaccumulation study

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

Table A7_4_3_3_1-7: Results for fish bioaccumulation study

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

ND=Not Determined