Section A7.4.1.1/2 Acute toxicity to fish

Annex Point IIA7.1

		Official use only
	~~5~~1 REFERENCE	
~~5~~1.1 1.1 **Reference**	[redacted] (1998), Acute Toxicity Study of Permethrin Technical in Common Carp, Cyprinus carpio, Department of Ecotoxicology, [redacted] unpublished report no.: 1598 Dates of experimental work: July 25, 1998 – July 29, 1998	
~~5~~1.2 1.2 **Data protection**	Yes	
~~5~~1.2.1 1.2.1 Data owner	Tagros Chemicals India Ltd.	
~~5~~1.2.2 1.2.2 Companies with letters of access	Not applicable	
~~5~~1.2.3 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/IA.	
	~~5~~2 GUIDELINES AND QUALITY ASSURANCE	
~~5~~2.1 2.1 **Guideline study**	Yes OECD test guideline 203: "Fish, Acute Toxicity Test".	
~~5~~2.2 2.2 GLP	Yes	
~~5~~2.3 2.3 **Deviations**	No	
	~~5~~3 MATERIALS AND METHODS	
~~5~~3.1 3.1 **Test material**	As given in section 2 (Permethrin 40:60)	
~~5~~3.1.1 3.1.1 Lot/Batch number	PH 01	
~~5~~3.1.2 3.1.2 Specification	As given in section 2 (Permethrin 40:60)	
~~5~~3.1.3 3.1.3 Purity	94.10%	

Formatted: Outline numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0 cm + Tab after: 1.25 cm + Indent at: 1.25 cm
Formatted: Bullets and Numbering
Comment [T2]: confidential
Formatted: Highlight
Formatted: Bullets and Numbering
Formatted
Formatted: Highlight
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering

Section A7.4.1.1/2 Acute toxicity to fish

Annex Point IIA7.1

~~53.1.4~~3.1.4 Composition of Product	Not applicable
~~53.1.5~~3.1.5 Further relevant properties	None
~~53.1.6~~3.1.6 Method of analysis	For the extraction of Permethrin, 100ml of the sample was taken with the addition of 1g of NaCl and extracted in hexane and chloroform. The combined organic layer was allowed to pass through a column containing sodium sulphate to remove any water. The eluate was concentrated to dryness by using rotary vacuum evaporator. The residue was further dissolved in 1ml of hexane. 20µl of this solution was injected into HPLC [Model LC-10AT] with column Shinwa ODS (25cm x 4.6 mm ID), and a UV-Vis detector set at wave length of 273 nm using hexane and tetra hydrofuran (1000:1.2) as a mobile phase. Concentrations of Permethrin were evaluated from the standard *vs*. sample response.
~~53.2~~3.2 **Preparation of TS solution for poorly soluble or volatile test substances**	Details are given in Table A7.4.1.1/2-1.
~~53.3~~3.3 **Reference substance**	No
~~53.4~~3.4 **Testing procedure**	
~~53.4.1~~3.4.1 Dilution water	Details are given in Table A7.4.1.1/2-2.
~~53.4.2~~3.4.2 Test organisms	Details are given in Table A7.4.1.1/2-3.
~~53.4.3~~3.4.3 Test system	Details are given in Table A7.4.1.1/2-4.
~~53.4.4~~3.4.4 Test conditions	Details are given in Table A7.4.1.1/2-5.
~~53.4.5~~3.4.5 Duration of the test	96 hours
~~53.4.6~~3.4.6 Test parameter	Mortality, behavioural responses and toxicity symptoms.
~~53.4.7~~3.4.7 Sampling	At 3, 6 and 24 hours and then at each subsequent 24-hour interval until test termination.
~~53.4.8~~3.4.8 Monitoring of TS concentration	Yes, at the start of the study (0 hr) and again after 24 hrs.
~~53.4.9~~3.4.9 Statistics	From the mortality data an estimate of the acute median lethal concentration (LC_{50}) of Permethrin at 96 hr exposure period, was calculated by using probit analysis method (Finney, 1971)

Formatted: Bullets and Numbering

Section A7.4.1.1/2 Acute toxicity to fish

Annex Point IIA7.1

		~~5~~4 RESULTS	Formatted: Bullets and Numbering
~~5~~4.1~~4~~.1	**Limit test**	Not performed	Formatted: Bullets and Numbering
~~5~~4.1.1~~4~~.1.1	Concentration	Not applicable	Formatted: Bullets and Numbering
~~5~~4.1.2~~4~~.1.2	Number/ percentage of animals showing adverse effects	Not applicable	Formatted: Bullets and Numbering
~~5~~4.1.3~~4~~.1.3	Nature of adverse effects	Not applicable	Formatted: Bullets and Numbering
~~5~~4.2~~4~~.2	**Results test substance**		Formatted: Bullets and Numbering
~~5~~4.2.1~~4~~.2.1	Initial concentrations of test substance	Details are given in Table A7.4.1.1/2-8.	Formatted: Bullets and Numbering
~~5~~4.2.2~~4~~.2.2	Actual concentrations of test substance	Details are given in Table A7.4.1.1/2-8.	Formatted: Bullets and Numbering
~~5~~4.2.3~~4~~.2.3	Effect data (Mortality)	Please refer to Table A7.4.1.1/2-6.	Formatted: Bullets and Numbering
~~5~~4.2.4~~4~~.2.4	Concentration / response curve	Not documented	Formatted: Bullets and Numbering
~~5~~4.2.5~~4~~.2.5	Other effects	Loss of equilibrium, restlessness	Formatted: Bullets and Numbering
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	Please refer to Table A7.4.1.1/2-6.	
4.3.2	Nature of adverse effects	No toxicity symptoms were observed in the negative control, or the vehicle control (0.06 ml acetone/ l water).	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	

Section A7.4.1.1/2 Acute toxicity to fish

Annex Point IIA7.1

	~~5~~5 APPLICANT'S SUMMARY AND CONCLUSION	Formatted: Bullets and Numbering
~~5.1~~5.1 **Materials and methods**	The test system used was a semi-static and *Poecilia reticulata* was chosen as the test organism. The test was conducted according to OECD test guideline 203: "Fish, Acute Toxicity Test" and is described under point 3.	Formatted: Bullets and Numbering
~~5.2~~5.2 **Results and discussion**	Please refer to Tables A7.4.1.1/2-6 and A7.4.1.1/2-7. No mortalities were observed in the 0.05 mg/l exposure treatment. Two mortalities were observed at the 0.08 mg/l treatment following 72 hrs of exposure, with no further mortalities for the duration of the treatment. The 0.13 and 0.20 mg/l treatments resulted in 40% and 70% mortality respectively by the end of the 96 hr exposure-period. 100% mortality was observed in fish exposed to 0.33 mg/l Permethrin/water. Fish exposed to 0.33 mg/l of Permethrin showed toxicity symptoms such as loss of equilibrium and restlessness. Loss of equilibrium only was observed in fish exposed to test concentrations 0.08, 0.13 and 0.20 mg Permethrin / l water. The acute median lethal toxicity (LC_{50}) of Permethrin in freshwater fish Common Carp, *Cyprinus carpio* at 96 h exposure period is 0.145 mg/l water with 95% fiducial limits of 0.104 and 0.203 mg/l water.	Formatted: Bullets and Numbering
~~5.2.1~~5.2.1 LC_0	Not documented	Formatted: Bullets and Numbering
~~5.2.2~~5.2.2 LC_{50}	0.145 mg/l	Formatted: Bullets and Numbering
~~5.2.3~~5.2.3 LC_{100}	Not documented	Formatted: Bullets and Numbering
~~5.3~~5.3 **Conclusion**	In accordance with Council Directive 67/548/EEC, Permethrin is very toxic to freshwater fish Common Carp, *Cyprinus carpio* and should be assigned the symbol N and the R phrase R50.	Formatted: Bullets and Numbering
~~5.3.1~~5.3.1 Reliability	1	Formatted: Bullets and Numbering
~~5.3.2~~5.3.2 Deficiencies	No	Formatted: Bullets and Numbering

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30 March 2009

Section A7.4.1.1/2 Acute toxicity to fish

Annex Point IIA7.1

Materials and Methods	Applicant recorded fish as '*Poecilia reticulata*'in applicants summary. Fish were *Cyprinus carpio*? No limit test recorded or threshold concentration calculated from other aquatics. Lack of detail on the 'Preparation of TS solution for poorly soluble or volatile test substances'.
Results and discussion	Accept applicants version
Conclusion	In accordance with directive 91/414, Permethrin should be classified very toxic to aquatic organisms.
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.1/2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not documented
Vehicle	Acetone
Concentration of vehicle	Not documented
Vehicle control performed	Yes; 0.06 ml acetone/l water
Other procedures	No

Table A7.4.1.1/2-2: Dilution water

Criteria	Details
~~5.3.3~~Source	Laboratory tap water
~~5.3.4~~Alkalinity	Not documented
~~5.3.5~~Hardness	182 mg/L (total hardness as $CaCO_3$)
~~5.3.6~~pH	7.3 – 7.5
~~5.3.7~~Oxygen content	82.4 – 83.0

Formatted: Bullets and Numbering

~~55.3.8~~Conductance	Not documented
~~55.3.9~~Holding water different from dilution	No

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Table A7.4.1.1/2-3: Test Organisms

Criteria	Details
Species/strain	Common carp (*Cyprinus carpio)*
Source	Assistant Director of Fisheries, Government of Gujarat, India
Wild caught	Not documented
Age/size	2.8 to 3.4 cm in length
Kind of food	"Red Sea" brand freeze dried tubifex worms
Amount of food	Not documented
Feeding frequency	Daily during the acclimatisation period, up to 24 h before exposure to the test substance
Pretreatment	Acclimation period – 12 days prior to testing
Feeding of animals during test	No

Table A7.4.1.1/2-4: Test System

Criteria	Details
Test type	Semi-static
Renewal of test solution	Daily
Volume of test vessels	20 litre aquaria (filled with 15 litres water)
Volume/animal	15 litres / 10 fish (0.62g of fish per litre of water)
Number of animals/vessel	10 fish / vessel
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	Not documented

Table A7.4.1.1/2-5: Test Conditions

Criteria	Details
Test temperature	22.2 – 22.7°C
Dissolved oxygen	82.4 – 83.0 %
pH	7.3 – 7.5
Adjustment of pH	Not documented
Aeration of dilution water	Not documented
Intensity of irradiation	Not documented
Photoperiod	16h light / 8h dark

Table A7.4.1.1/2-6: Mortality Data

Test-Substance Concentration (nominal/measured)[1] [µg/l]	~~5.3.9.1.1.1.1.1~~5.3.2.1.1.1.1.1 Mortality							
	Number				Percentage (cumulative)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control (0)	0	0	0	0	0	0	0	0
Vehicle control (0.6 acetone ml/l water)	0	0	0	0	0	0	0	0
0.05	0	0	0	0	0	0	0	0
0.08	0	0	2	0	0	0	20	20
0.13	0	1	2	1	0	10	30	40
0.20	0	3	3	1	0	30	60	70

Formatted

Formatted: Bullets and Numbering

0.33	0	5	3	2	0	50	80	100

N = 10

Table A7.4.1.1/2-7: Relationship Between per cent Mortality and Concentration of Permethrin

Exposure time (h)	LC50 value (mg/l water)	95% fiducial limits		Regression equation $Y = a + b\chi$
		Upper limit (mg/l water)	Upper limit (mg/l water)	
96	0.145	0.104	0.203	$Y = 7.885 + 3.445\chi$

Y = Probit value of mortality, χ = log concentration of Permethrin in µg/l,

a = intercept, b = slope, h = hour.

Table A7.4.1.1/2-8: Concentrations of Permethrin in Water during the Exposure Period.

Nominal concentration (mg/l)	~~55.3.9.1.25~~.3.2.1.2 Detected a.s. concentration	
	0h	24h
G1-Control	BDL	BDL
G2-Vehicle control	BDL	BDL
G3-0.05	0.0486	0.0449
G4-0.08	0.0779	0.0724
G5-0.13	0.1284	0.1177
G6-0.20	0.1933	0.1814
G7-0.33	0.326	0.3114

Formatted

Formatted: Bullets and Numbering

Detectable level: ≥0.001 ppm

BDL- below detectable limit,

G – group, h = hour

Table A7.4.1.1/2-10: Validity Criteria for Acute Fish Test According to OECD Guideline 203

	Fulfilled
Mortality of control animals <10%	Yes
Concentration of dissolved oxygen in all test vessels > 60% saturation	Yes
Concentration of test substance ≥80% of initial concentration during test	Yes

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

Official use only

~~5~~6**1** **REFERENCE**

~~5~~6.~~1~~1.1 **Reference** Sharma, V.G.S. (1998), 24 h EC_{50} acute immobilisation study of Permethrin Technical in *Daphnia magna*, Department of Ecotoxicology, JAI Research Foundation, Valvada - 396108, Dist. Valsad, Gujarat, India, unpublished report No.: 1597.

Dates of experimental work: July 25, 1998 – August 21, 1998.

~~5~~6.~~2~~1.2 **Data protection** Yes

~~5~~6.2.~~1~~1.2.1 Data owner Tagros Chemicals India Ltd.

~~5~~6.2.~~2~~1.2.2 Companies with letter of access Not applicable.

~~5~~6.2.~~3~~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/IA.

~~5~~7**2** **GUIDELINES AND QUALITY ASSURANCE**

~~5~~7.~~1~~2.1 **Guideline study** Yes

OECD test guideline 202, "*Daphnia* sp., Acute Immobilisation and Reproduction Test" (adopted April 1984).

This guideline has recently been updated (13th April 2004).

~~5~~7.~~2~~2.2 **GLP** Yes

~~5~~7.~~3~~2.3 **Deviations** No deviations from the 1984 guideline. The study does however deviate

Formatted: Outline numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0 cm + Tab after: 1.25 cm + Indent at: 1.25 cm

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

from the current 2004 guideline in the following respect:

~~1.~~• Test duration was 24 hours rather than the recommended 48 hrs.

However, this deviation is not considered to compromise the scientific validity of the study.

~~5~~8~~3~~ MATERIALS AND METHODS

~~58.1~~3.1 **Test material**	As given in section 2 (Permethrin 40:60)
~~58.1.1~~3.1.1 Lot/Batch number	PH 01
~~58.1.2~~3.1.2 Specification	As given in section 2 (Permethrin 40:60)
~~58.1.3~~3.1.3 Purity	92.50%
~~58.1.4~~3.1.4 Composition of Product	Not applicable
~~58.1.5~~3.1.5 Further relevant properties	None
~~58.1.6~~3.1.6 Method of analysis	HPLC and a UV
~~58.2~~3.2 **Preparation of TS solution for poorly soluble or volatile test substances**	Details are given in Table A7.4.1.2-1.
~~58.3~~3.3 **Reference substance**	No
~~58.3.1~~3.3.1 Method of analysis for reference substance	Not applicable
~~58.4~~3.4 **Testing procedure**	
~~58.4.1~~3.4.1 Dilution water	Details are given in Table A7.4.1.2-2.
~~58.4.2~~3.4.2 Test organisms	*Daphnia magna*, details are given in Table A7.4.1.2-3.
~~58.4.3~~3.4.3 Test system	Details are given in Table A7.4.1.2-4.

Formatted: Indent: Hanging: 0.86 cm, Bulleted + Level: 1 + Aligned at: 0.63 cm + Tab after: 1.27 cm + Indent at: 1.27 cm

Formatted: Bullets and Numbering

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

~~58.4.4~~3.4.4	Test conditions	Details are given in Table A7.4.1.2-5.
~~58.4.5~~3.4.5	Duration of the test	24 hours
~~58.4.6~~3.4.6	Test parameter	Immobility
~~58.4.7~~3.4.7	Sampling	Observations were made at 24 hours after test initiation.
~~58.4.8~~3.4.8	Monitoring of TS concentration	Yes Interval: 0 and 24 hours
~~58.4.9~~3.4.9	Statistics	The acute immobilization concentration (EC_{50}) of Permethrin at 24 hr exposure was made together with 95% fiducial limits using probit analysis method (Finney, 1971).
		~~5~~9.4 **RESULTS**
4.1	**Limit Test**	Not performed
~~59.1.1~~4.1.1	Concentration	Not applicable
~~59.1.2~~4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
~~59.1.3~~4.1.3	Nature of adverse effects	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Please refer to Table A7.4.1.2-6
4.2.2	Actual concentrations of test substance	Please refer to Table A7.4.1.2-6
4.2.3	Effect data (Immobilisation)	Please refer to Table A7.4.1.2-7. The 24hr EC_{50} value including fiducial limits is presented in Table A7.4.1.2-8
4.2.4	Concentration / response curve	Not documented
4.2.5	Other effects	Not documented

Formatted: Bullets and Numbering

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

4.3	**Results of controls**	Please refer to Table A7.4.1.2-7.
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not applicable
4.4.2	Results	Not applicable

~~6~~**5 APPLICANT'S SUMMARY AND CONCLUSION**

~~6~~**0.1~~5~~.1**	**Materials and methods**	The test system was static and *Daphnia magna* was chosen as the test organism. The test method used was conducted according to OECD test guideline 202: *Daphnia* sp., Acute Immobilisation Test (April 1984) and is described under point 3.
~~6~~**0.2~~5~~.2**	**Results and discussion**	Please refer to Table A7.4.1.2-7 and Table A7.4.1.2-8 The acute immobilisation, EC_{50} value of Permethrin in *Daphnia magna* at 24 h exposure period was 0.020 mg/L.
~~6~~**0.2.1~~5~~.2.1**	EC_0	Not documented
~~6~~**0.2.2~~5~~.2.2**	EC_{50}	0.020 mg/l
~~6~~**0.2.3~~5~~.2.3**	EC_{100}	Not documented
~~6~~**0.3~~5~~.3**	**Conclusion**	In accordance with Council Directive 67/548/EEC, Permethrin is very toxic to *Daphnia magna* and should be assigned the symbol N and the R phrase R50.
~~6~~**0.3.1~~5~~.3.1**	Reliability	1
~~6~~**0.3.2~~5~~.3.2**	Deficiencies	There are no deficiencies based on the 1984 guideline. The study does however deviate from the current 2004 guideline in the following respect:
		~~2.~~• Test duration was 24 hours rather than the recommended 48 hrs.

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Indent: Hanging: 0.86 cm, Bulleted + Level: 1 + Aligned at: 0.63 cm + Tab after: 1.27 cm + Indent at: 1.27 cm

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

However, this deviation is not considered to compromise the scientific 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	1 April 2009
Materials and Methods	Test duration 24 hr, should be 48 hr according to most recent guidelines. Little information on source of water, no alkalinity, Ca/Mg, Na/K concentrations. No limit test/range finding test performed. Confusion over strain and age of test organism. Strain '*Daphnia magna* (gravid)' if 'gravid' wouldn't be less than 24 hrs old.
Results and discussion	Adopt applicant's version.
Conclusion	Adopt applicant's version.
Reliability	~~2~~3
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Acetone
Concentration of vehicle	0.01% v/v
Vehicle control performed	Yes (0.1 ml acetone/ l water)
Other procedures	Not documented

Table A7.4.1.2-2: Dilution water

Criteria	Details
Source	Not documented
Alkalinity	Not documented
Hardness	196 mg/l as $CaCO_3$
pH	Ranged between 7.6 – 7.8
Ca / Mg ratio	Not documented
Na / K ratio	Not documented
Oxygen content	Ranged between 82.7 – 83.2%
Conductance	Not documented
Holding water different from dilution water	Not documented

Table A7.4.1.2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna* (Gravid)
Source	M/s. Asif Khan, Shop No. 2, Crawford Market, Mumbai.
Age	≤ 24 h old
Kind of food	Unicellular Algae
Amount of food	Not documented
Feeding frequency	Not documented
Pretreatment	Acclimatisation period – 24 hrs prior to testing
Feeding of animals during test	No

Table A7.4.1.2-4: Test system

Criteria	Details
Test Type	Static
Renewal of test solution	No
Volume of test vessels	Glass beaker 500ml capacity
Volume/animal	40 ml/daphnid
Number of animals/vessel	5 daphnids / replicate (4 replicates)
Number of vessels/ concentration	4 / concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2-5: Test conditions

Criteria	Details
Test temperature	Ranged between 21 – 21.3 °C
Dissolved oxygen	Ranged between 82.7- 83.2 %
pH	Ranged between 7.6 – 7.8
Adjustment of pH	No
Aeration of dilution water	Not documented
Quality/Intensity of irradiation	Not applicable
Photoperiod	Complete darkness

Table A7.4.1.2-6: Actual concentrations of test substance

Nominal concentrations (mg/l)	Mean measured concentration (mg/l)	
	0 hours	24 hours
Control	-	-
Vehicle control (0.1 ml/l water)	-	-
0.005	0.0048	0.0045
0.01	0.0095	0.0090
0.02	0.0192	0.0163
0.04	0.0379	0.0334

0.08	0.0771	0.0694

Table A7.4.1.2-7: Immobilisation data and test system conditions

Test-Substance Concentration (nominal/effective)[1] [mg/l]	Immobile *Daphnia*		Oxygen [%]	pH	Tempera-ture [°C]
	Number 24 h	Percentage 24 h	24 h	24 h	24 h
Control	0	0	82.9	7.7	21.1
Vehicle control (0.1 ml/l water)	0	0	82.7	7.7	21.2
0.005	0	0	82.7	7.8	21.2
0.01	5	25	82.8	7.7	21.2
0.02	9	45	82.7	7.7	21.2
0.04	16	80	82.7	7.7	21.3
0.08	20	100	82.7	7.7	21.3

Table A7.4.1.2-8: Acute Immobilisation, EC_{50} Value of Permethrin in *Daphnia magna*

~~60.3.2.1~~5.3.2.1 at 24 h Exposure Period

Formatted: Bullets and Numbering

Exposure time (h)	EC_{50} value (mg/l water)	95% Fiducial limits (mg/l water)		Regression equation (Y = a + bχ)
		Lower limit	Upper limit	
24	0.020	0.015	0.027	Y= 9.255 + 2.501χ

h = hour, y = probit value of immobility, χ = log concentration of Permethrin,

a = intercept and b = slope

Table A7.4.1.2-9: Validity criteria for acute *Daphnia* immobilisation test according to OECD Guideline 202

Criteria	Fulfilled
Immobilisation of control animals <10%	~~60.3.2.1.1.1.1.1~~5.3.2.1.1.1.1.1 Yes
Control animals not staying at the surface	Not documented

Formatted

Formatted: Bullets and Numbering

Concentration of dissolved oxygen in all test vessels >3 mg/l (≥ 60% of air saturation value)	Yes
Concentration of test substance ≥80% of initial concentration during test	Yes
Criteria for poorly soluble test substances	Yes

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

Official use only

	~~6~~1 REFERENCE
~~6.1.1~~1.1 **Reference**	Mead, C. (2003), Permethrin: Algae Inhibition Test. SafePharm Laboratories, Shardlow Business Park, London Road, Shardlow, Derbyshire, DE72 2GD, UK, unpublished report No.: 1667/001 Dates of experimental work: September 11, 2003 – October 10, 2003.
~~6.2.~~1.2 **Data protection**	Yes
~~6.2.1~~1.2.1 Data owner	Copyr s.p.a
~~6.2.2~~1.2.2 Companies with letter of access	Not applicable
~~6.2.3~~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/IA.
	~~6~~2 GUIDELINES AND QUALITY ASSURANCE
~~6.1~~2.1 **Guideline study**	**Yes** **OECD test guideline 201: "Alga, Growth Inhibition Test".**
~~6.2~~2.2 **GLP**	Yes
~~6.3~~2.3 **Deviations**	Yes, this study deviates from OECD Guideline 203 in the following respect: The concentration of test substance was ≤ 80% of initial concentration during test, which does not fulfil the validity criteria for the algal growth inhibition test according to OECD Guideline 201. However, this deviation is not considered to compromise the scientific validity of the study.
	~~6~~3 MATERIALS AND METHODS
~~6.1~~3.1 **Test material**	As given in section 2 (Permethrin 25:75)
~~6.1.1~~3.1.1 Lot/Batch number	P-127
~~6.1.2~~3.1.2 Specificati	As given in section 2 (Permethrin 25:75)

Formatted: Outline numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0 cm + Tab after: 1.25 cm + Indent at: 1.25 cm

Formatted: Bullets and Numbering

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

	on	
~~63.1.3~~3.1.3	Purity	Not documented
~~63.1.4~~3.1.4	Composition of Product	Not applicable
~~63.1.5~~3.1.5	Further relevant properties	None
~~63.1.6~~3.1.6	Method of analysis	The test material concentration in the test samples was determined by high performance liquid chromatography (HPLC) using an external standard. The test material gave a chromatographic profile consisting of two peaks. The results have been calculated using the total peak area of both peaks. The standards and samples were analysed by HPLC using the following conditions: HPLC System: Agilent Technologies 1050 or 1100, incorporating autosampler and workstation. Column: LUNA, 5µ, C18 (250 x 4.6 mm id) Column temp. : Ambient
~~63.2~~3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	No
~~63.3~~3.3	**Reference substance**	No
~~63.3.1~~3.3.1	Method of analysis for reference substance	Not applicable
~~63.4~~3.4	**Testing procedure**	
~~63.4.1~~3.4.1	Culture medium	The culture medium was prepared using reverse osmosis purified deionised water (ElganOptima 15+) and the pH adjusted to 7.5 ± 0.1 with 0.1N NaOH or HCL. The prepared media was sterilised by 0.2 µm membrane filtration. Please refer to Table A7.4 .1.3-1 for further details.
~~63.4.2~~3.4.2	Test organisms	*Scenedesmus subspicatus,* details are given in Table A7.4.1.3-2.
~~63.4.3~~3.4.3	Test system	Details are given in Table A7.4.1.3-3.
~~63.4.4~~3.4.4	Test conditions	Details are given in Table A7.4.1.3-4.

Formatted: Bullets and Numbering

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

~~6~~3.4.~~5~~3.4.5 Duration of the test	72 hours
~~6~~3.4.~~6~~3.4.6 Test parameter	The 72 hour EC_{50} and the NOEC with respect to growth inhibition.
~~6~~3.4.~~7~~3.4.7 Sampling	Cell densities were determined at 0, 24, 48 and 72 hours.
~~6~~3.4.~~8~~3.4.8 Monitoring of TS concentration	Yes; at 0 and 72 hours
~~6~~3.4.~~9~~3.4.9 Statistics	A Students t-test incorporating Bartlett's test for homogeneity of variance (Sokal and Rohlf 1981) was carried out on the area under the growth curve data at 72 hours for the control and the test concentration to determine any statistically significant differences between the test and control groups. All statistical analyses were performed using the SAS computer software package (SAS 1999-2001).

~~6~~4.4 **RESULTS**

~~6~~4.~~1~~4.1 **Limit Test** Performed

~~6~~4.1.~~1~~4.1.1 Concentration

The 0.095 mg/L concentration as decided upon based on results from the range finding study, was not reproduced in the definitive test. The measured concentrations were as follows:

Time 0 hr:	0.333 mg/l (measured concentration)
Time 72 hr:	0.0223 mg/l (measured concentration)
Mean:	0.022 mg/l (measured concentration)

~~6~~4.1.~~2~~4.1.2 Number/ percentage of animals showing adverse effects: No adverse effects were observed at the concentration tested.

~~6~~4.1.~~3~~4.1.3 Other observed effects:

Observations on cultures:

All test and control cultures were inspected microscopically at 72 hours.

There were no abnormalities detected in any of the control or test cultures.

Physico-chemical measurements:

The pH values of the control cultures were observed to increase from pH 7.5 at 0 hours to pH 8.4 – 8.6 at 72 hours. The pH deviation in the control cultures was less than 1.5 pH units after 72 hours and therefore was within the limits given in the test guidelines. Temperature was maintained at 24 ± 1°C throughout the test.

Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

Observations on test material solubility:

At the start of the test all control and test cultures were observed to be clear colourless solutions. After the 72 - hour test period all control and test cultures were observed to be green dispersions.

~~6.2.2~~4.2.2 Results test substance	
~~6.2.1~~4.2.1 Initial concentrations of test substance	Please refer to Tables A7.4.1.3-5 and A7.4.1.3-6 and Figure A7.4.1.3-1. The difference in measured concentrations in the range-finding and definitive tests was considered to possibly be due to a small amount of dispersed test material being present in the saturated solution prepared for the range-finding test that had not been completely removed during centrifugation of the initial test material dispersion.
~~6.2.2~~4.2.2 Actual concentrations of test substance	Please refer to Tables A7.4.1.3-5 and A7.4.1.3-6.
~~6.2.3~~4.2.3 Growth curves	Please refer to Figure A7.4.1.3-2.
~~6.2.4~~4.2.4 Concentration / response curve	Not applicable
~~6.2.5~~4.2.5 Cell concentration data	Details are provided in Tables A7.4.1.3-7 and A7.4.1.3-8.
~~6.2.6~~4.2.6 Effect data (cell multiplication inhibition)	E_bC_{50} (72 h): >0.022 mg/l E_rC_{50} (0 - 72 h): >0.022 mg/l NOEC: >0.022 mg/l
~~6.2.7~~4.2.7 Other observed effects	None documented.
~~6.3~~4.3 Results of controls	
~~6.4~~4.4 Test with reference substance	Not performed
~~6.4.1~~4.4.1 Concentrations	Not applicable
~~6.4.2~~4.4.2 Results	Not applicable

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

~~6~~5 APPLICANT'S SUMMARY AND CONCLUSION

~~6~~5.1 **Materials and methods**

Scenedesmus subspicatus was used as the test organism. The test was conducted according to OECD test guideline 201: "Alga, Growth Inhibition Test" and is described under point 3.

~~6~~5.2 **Results and discussion**

The concentration chosen for the limit test was 0.022 mg/l. This value was lower than the highest concentrations tested in the range finding test. As no inhibition was detected at any of the concentrations tested in the range finding study, a value greater than those tested should have been chosen for the limit test.

Neither the growth or the biomass of *Scenedesmus subspicatus* were effected by the presence of test material over the 72-hr exposure period.

~~6~~5.2.1 NOE_rC > 0.022 mg/l

~~6~~5.2.2 E_rC_{50} > 0.022 mg/l

~~6~~5.2.3 E_bC_{50} > 0.022 mg/l

~~6~~5.3 **Conclusion**

The effect of the Permethrin on the growth of *Scenedesmus subspicatus* has been investigated and based on the mean measured test concentration gave EC_{50} values of greater than 0.022 mg/l. Correspondingly, the No Observed Effect Concentration (NOEC) was 0.022 mg/l.

Therefore, in accordance with Council Directive 67/548/EEC, Permethrin is classified as acutely toxic to Algae.

~~6~~5.3.1 Reliability 2

~~6~~5.3.2 Deficiencies Yes,

Analysis of the test solution used in the definitive test showed the measured concentration to be 0.033 mg/ml at time 0 and 0.0113 mg/ml at 72 hours (Mean = 0.022 mg/ml). It is therefore thought that the initial test material solution concentration of 0.095 mg/l was incorrect. (Due to problems with solubility it is likely that an unrepresentative sample was taken for analysis, yielding a much higher value than was correct).

Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2 April 2009
Materials and Methods	The concentration chosen for the limit test was 0.022 mg/l. This value was lower than the highest concentrations tested in the range finding test. As no inhibition was detected at any of the concentrations tested in the range finding study, a value greater than those tested should have been chosen for the limit test.
Results and discussion	Analysis of the test solution used in the definitive test showed the measured concentration to be 0.033 mg/ml at time 0 and 0.0113 mg/ml at 72 hours (Mean = 0.022 mg/ml). It is therefore thought that the initial test material solution concentration of 0.095 mg/l was incorrect. (Due to problems with solubility it is likely that an unrepresentative sample was taken for analysis, yielding a much higher value than was correct).
Conclusion	E_rC_{50}, E_bC_{50} and NOE_rC of > 0.022 mg/l is unreliable.
Reliability	3
Acceptability	Acceptable despite a poor reliability factor as toxicity is probably greater than water solubility.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Formatted

Formatted

~~6.5.3.2.1~~5.3.2.1 Table A7.4.1.3-1: Composition of algal nutrient medium

Formatted

Formatted: Bullets and Numbering

Compound	Concentration (mg/l)
$NaNO_3$	25.5
$MgCl_2.6H_2O$	12.164
$CaCl_2.2H_2O$	4.41
$MgSO_4.7H_2O$	14.7
K_2HPO_4	1.044
$NaHCO_3$	15.0
H_3BO_3	0.1855
$MnCl_2.4H_2O$	0.415
$ZnCl_2$	0.00327
$FeCl_3.6H_2O$	0.159
$CoCl_2.6H_2O$	0.00143
$Na_2MoO_4.2H_2O$	0.00726
$CuCl_2$. $2H_2O$	0.000012
Na_2EDTA. 2H2O	0.30
$Na_2SeO_3.5H_2O$	0.000010

Table A7.4.1.3-2: Test organisms

Criteria	Details
Species	Scenedesmus subspicatus
Strain	CCAP 276 / 20
Source	Culture Collection of Algae and Protozoa (CCAP), Institute of Freshwater Ecology, The Ferry House, Far Sawrey, Ambleside, Cumbria.
Laboratory culture	Yes
Method of cultivation	The flasks/vessels were plugged with polyurethane foam bungs and incubated (Gallenkamp INR – 401 – 010W incubator) at 24 ± 1°C under continuous illumination (intensity approx. 7000 lux) and constantly shaken at approx. 100 rpm for 72 hours.
Pretreatment	Cultures were maintained in the laboratory at a temperature of 21 ± 1°C under continuous illumination (intensity approx. 7000 lux) and constant aeration.
Initial cell concentration	Initial cell density was 10^4 cells/ml of Scenedesmus subspicatus.

Formatted

Formatted

Table A7.4.1.3-3: Test system

Criteria	Details
Volume of culture flasks	100mls of solution were prepared in 250 ml culture flasks.
Culturing apparatus	Gallenkamp INR – 401-010W incubator
Light quality	Continuous illumination (intensity was approximately 7000 lux)
Procedure for suspending algae	Continuous shaking at approx. 100 rpm for 72 hours.
Number of vessels/ concentration	Time 0 hr: 0.333 mg/l (measured conc.) Time 72 hr: 0.0223 mg/l (measured conc.) Mean: 0.022 mg/l (measured conc.) Six vessels were used at the above concentration.
Test performed in closed vessels due to significant volatility of TS	Yes, the flasks/vessels were plugged with polyurethane foam bungs prior to incubation

Formatted

Table A7.4.1.3-4: Test conditions

Criteria	Details
Test temperature	24 ± 1°C
pH	The pH for the definitive test 0 hrs – 7.5 72 hrs – (8.3 - 8.6)
Aeration of dilution water	Not documented
Light intensity	Continuous illumination (intensity approximately 7000 lux)
Photoperiod	Continuous illumination throughout the 72 hour period of the test.

Formatted

Formatted: Bullets and Numbering

~~6.5.3.2.1.1.1.1~~**Table A7.4.1.3-5: Verification of Test Concentrations**

Fortification (mg/l)	Recoveries		
	(mg/l)	(%)	Mean %
0.0970	0.0817	84	83
0.0970	0.0801	83	
0.0970	0.0796	82	NA

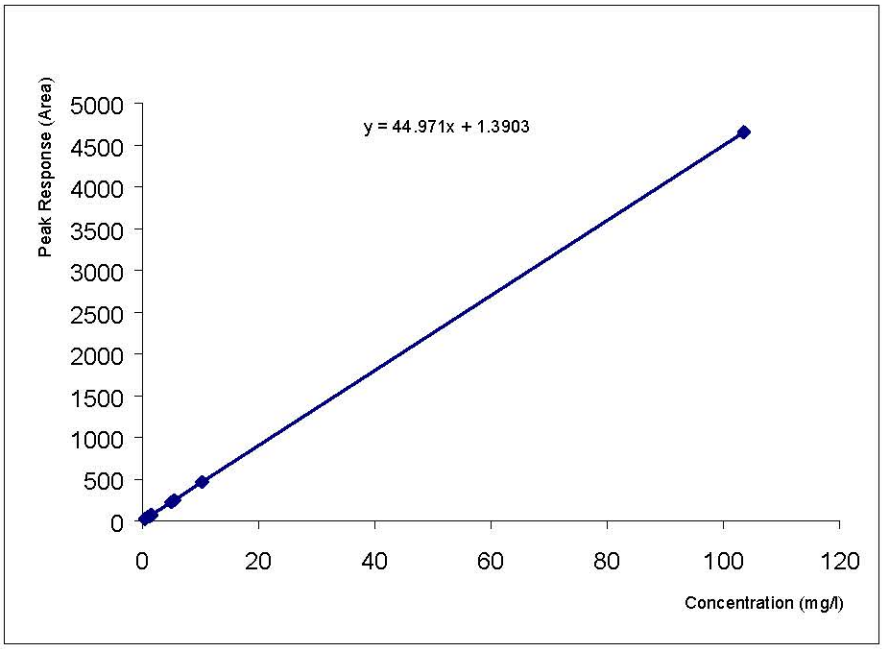

Figure A7.4.1.3-1: Verification of Test Concentrations Linearity of Detector Response

Table A7.4.1.3-6: Verification of Test Concentrations in the Definitive Test

Test Series	Measured concentrations (mg/l)		Mean Measurerd Test Concentration (mg/l)
	0 Hours	72 Hours	
Test material (R1-R3)	0.0330	0.0113	0.022
Test material (R4-R6)	0.0336	0.0110	

Table A7.4.1.3-7: Cell Densities and Percentage Inhibition of Growth from the Range-Finding Test

Nominal concentrations (mg/l)*		Cell Densities** (Cells per ml)		
		0 Hours	72 Hours	% Inhibition (Area under curve at 72 h)
Control	R1	1.19E + 04	6.92E + 05	-
	R2	1.12E + 04	6.93E + 05	
	Mean	1.16E + 04	6.93E + 05	
0.00095	R1	1.17E + 04	7.05E + 05	[1]
	R2	1.17E + 04	6.95E + 05	
	Mean	1.17E + 04	7.00E + 05	
0.0095	R1	1.13E + 04	7.08E + 05	[2]
	R2	1.10E + 04	7.07E + 05	
	Mean	1.11E + 04	7.08E + 05	
0.095	R1	1.09E + 04	6.97E + 05	0
	R2	1.43E + 04	6.93E + 05	
	Mean	1.26E + 04	6.95E + 05	

* Concentrations based on analysis of the saturated solution use to prepare the test concentrations.

** Cell densities represent the mean number of cells per ml calculated from the mean of the cell counts from 3 counts for each replicate flasks.

R_1-R_2 = Replicates 1 and 2

[Increase in growth compared to controls]

~~6.5.3.2.1.1.1.1.1.1~~5.3.2.1.1.1.1.1 **Table A7.4.1.3-8: Cell Densities and pH Values in the Definitive Test**

Formatted

Formatted: Bullets and Numbering

Mean measured Test Concentration (mg/l)		pH	Cell Densities* (cells per ml)				pH
		0 h	0 h	24 h	48 h	72 h	72 h
Control	R1	7.5	1.20E+04	2.63E+04	8.67E+04	1.01E+06	8.4
	R2	7.5	1.21E+04	2.90E+04	7.63E+04	8.64E+05	8.6
	R3	7.5	1.25E+04	2.75E+04	7.96E+04	8.84E+05	8.5
	Mean		1.22E+04	2.76E+04	8.09E+04	9.20E+05	
0.0095	R1	7.5	1.17E+04	2.63E+04	8.89E+04	8.11E+05	8.4
	R2	7.5	1.22E+04	2.83E+04	9.41E+04	1.01E+06	8.4
	R3	7.5	1.13E+04	2.95E+04	8.14E+04	8.51E+05	8.3
	R4	7.5	1.06E+04	2.78E+04	8.26E+04	9.38E+05	8.6

R5	7.5	1.01E+04	2.39E+04	9.31E+04	9.34E+05	8.5
R6	7.5	1.05E+04	2.91E+04	9.31E+04	9.87E+05	8.5
Mean		1.11E+04	2.75E+04	8.89E+04	9.22E+05	

* Cell densities represent the mean number of cells per ml calculated from the mean of the cell counts from 3 counts for each of the replicate flasks.

R1-R6 = Replicates 1 to 6

Formatted

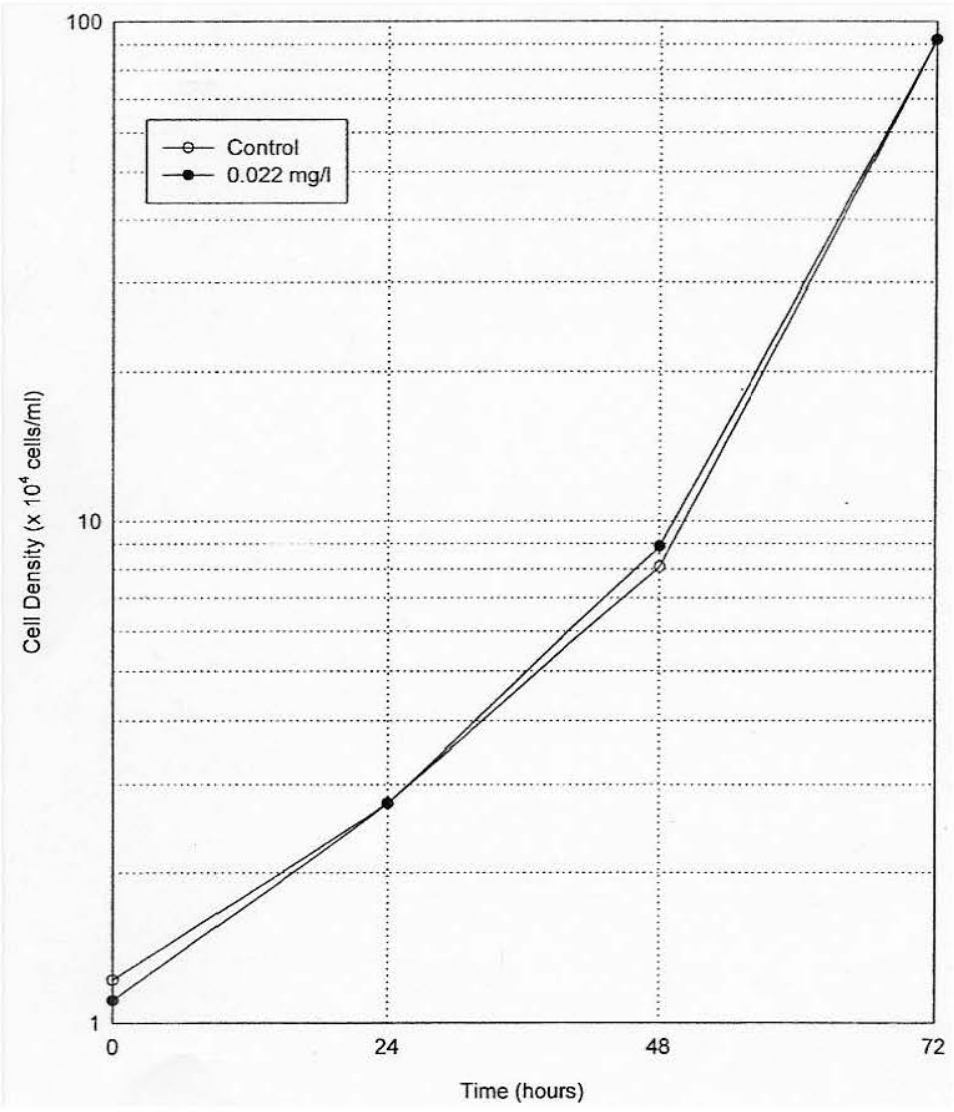

Figure A7.4.1.3-2: Mean Cell Densities v Time of the Definitive Test

Formatted: Bullets and Numbering

~~6.5.3.2.1.1.1.1.2~~5.3.2.1.1.1.1.2 Table A7.4.1.3-9: Inhibition of Growth Rate and Biomass

Formatted

Mean measured test concentration (mg/l)	Area under the curve at 72h	% Inhibition	Growth Rate (0-72h)	% Inhibition
Control	1.29E+07	-	0.060	-
0.022	1.32E+07	[2]	0.061	[2]

3. Tables for Applicant's Summary and Conclusion

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

	Fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	Yes
Concentration of test substance ≥80% of initial concentration during test	No
Criteria for poorly soluble test substances	Yes

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

	~~6~~6.1 REFERENCE
~~6~~6.1.1 Reference	Clarke, N. (2003), Permethrin: Assessment of the Inhibitory Effect on the Respiration of Activated Sewage Sludge. Safepharm Laboratories Limited, P.O. Box No. 45, Derby, DE1 2BT, U.K, unpublished report No.: 1667/002. Dates of experimental work: July 29, 2002 – September 23, 2002.
~~6~~6.2 1.2 Data protection	Yes
~~6~~6.2.1 1.2.1 Data owner	Copyr s.p.a
~~6~~6.2.2 1.2.2 Companies with letter of access	Not applicable
~~6~~6.2.3 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/IA.
	~~6~~7.2 GUIDELINES AND QUALITY ASSURANCE

Official use only

Formatted: Outline numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0 cm + Tab after: 1.25 cm + Indent at: 1.25 cm

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

~~67.1~~2.1 **Guideline study**	Yes OECD test guideline 209: "Activated sludge – respiration inhibition test", and US EPA Draft Ecological Effects test guidelines OPPTS 850.6800.
~~67.2~~2.2 **GLP**	Yes
~~67.3~~2.3 **Deviations**	Yes, this study deviates from OECD Guideline 209 in the following respects: 1. The test report does not contain information on the chemical identification data of Permethrin. 2. Information on the purity of Permethrin has not been documented. However, these deviations are not considered to compromise the scientific validity of the study.
	~~68~~3 **MATERIALS AND METHODS**
~~68.1~~3.1 **Test material**	As given in section 2 (Permethrin 25:75)
~~68.1.1~~3.1.1 Lot/Batch number	P-127
~~68.1.2~~3.1.2 Specification	As given in section 2 (Permethrin 25:75)
~~68.1.3~~3.1.3 Purity	Not documented
~~68.1.4~~3.1.4 Composition of Product	~~Not applicable~~Cis/trans ratio 25:75
~~68.1.5~~3.1.5 Further relevant properties	None
~~68.1.6~~3.1.6 Method of analysis	Not applicable
~~68.2~~3.2 **Preparation of TS solution for poorly soluble or volatile test substances**	No
~~68.3~~3.3 **Reference substance**	Yes; 3,5-dichlorophenol (DCP - Sigma-Aldrich Batch No. 10330 H102611ES).
~~68.3.1~~3.3.1 Method of analysis for	Two stock solutions of 50 and 160 mg/l were prepared by dissolving the reference material directly in water through the aid of ultrasonic

Formatted: Bullets and Numbering

Formatted: Numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0.63 cm + Tab after: 1.27 cm + Indent at: 1.27 cm

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

reference substance	disruption. Aliquots of the 160 mg/l stock solution were removed and dispersed with activated sewage sludge, synthetic sewage and water to give the final concentrations of 3.2 and 32 mg/l. A 100 ml aliquot of the 50 mg/l stock solution was used to prepare the 10mg/l concentration. The volumetric flasks containing the reference material were inverted several times to ensure homogeneity. A reference material, 3,5-dichlorophenol, was included in the initial range-finding test at concentrations of 3.2 and 32 mg/l in order to confirm the suitability of the inoculum.	
~~68.4~~3.4 Testing procedure		Formatted: Bullets and Numbering
~~68.4.1~~3.4.1 Culture medium	Synthetic sewage feed as described in OECD guideline 209.	Formatted: Bullets and Numbering
~~68.4.2~~3.4.2 Inoculum / test organism	Details are given in Table A7.4.1.4-1.	Formatted: Bullets and Numbering
~~68.4.3~~3.4.3 Test system	Details are given in Table A7.4.1.4-2.	Formatted: Bullets and Numbering
~~68.4.4~~3.4.4 Test conditions	Details are given in Table A7.4.1.4-3.	Formatted: Bullets and Numbering
~~68.4.5~~3.4.5 Duration of the test	3 hours	Formatted: Bullets and Numbering
~~68.4.6~~3.4.6 Test parameter	Respiration inhibition.	Formatted: Bullets and Numbering
~~68.4.7~~3.4.7 Analytical parameter	Oxygen consumption rate.	Formatted: Bullets and Numbering
~~68.4.8~~3.4.8 Sampling	0 hours, 30 minutes and 3 hours	Formatted: Bullets and Numbering
~~68.4.9~~3.4.9 Monitoring of TS concentration	No	Formatted: Bullets and Numbering
~~68.4.10~~3.4.10 Controls	Two controls were used containing 16 ml of synthetic sewage, diluted to 300 ml with water and 200 ml of inoculum.	Formatted: Bullets and Numbering
~~68.4.11~~3.4.11 Statistics	To calculate the inhibitory effect of the test and reference materials in the initial range finding and definitive tests the respiration rate was expressed as a percentage of the two control respiration rates. The inhibitory effect of the test material in the second range finding test was expressed as a percentage of the solvent control respiration rate. The percentage inhibition values were plotted against concentration, a line fitted using a computer program and the EC_{50} values determined from the equation for the fitted line.	Formatted: Bullets and Numbering

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

	~~6~~9~~4~~ RESULTS	
~~69.1~~4.1 **Preliminary test**	Performed	
~~69.1.1~~4.1.1 Concentration	Oxygen consumption rates (mg O2/l/min) from the initial and the second range-finding test are illustrated in Table A7.4.1.4-2.	
~~69.1.2~~4.1.2 Effect data	No significant effect on respiration was observed at any of the test concentrations employed in either of the range-finding tests. Based on the data generated, a single test concentration (in triplicate) of 1000 mg/l was selected for the definitive test.	
~~69.2~~4.2 **Results test substance**		
~~69.2.1~~4.2.1 Initial concentrations of test substance	1000 mg/l	
~~69.2.2~~4.2.2 Actual concentrations of test substance	Not documented	
~~69.2.3~~4.2.3 Growth curves	Not documented.	
~~69.2.4~~4.2.4 Cell concentration data	Not documented.	
~~69.2.5~~4.2.5 Concentration/ response curve	Not documented	
~~69.2.6~~4.2.6 Effect data	The effect of the Permethrin on the respiration of activated sewage sludge micro-organisms gave a 3-Hour EC50 of greater than 1000 mg/l. The No Observed Effect Concentration (NOEC) after 3 hours exposure was 1000 mg/l.	
~~69.2.7~~4.2.7 Other observed effects	None	
~~69.3~~4.3 **Results of controls**	The validation criteria for the control respiration rates were satisfied. Refer to Table A7.4.1.4-4, A7.4.1.4-5 and Table A7.4.1.4-6.	
~~69.4~~4.4 **Test with reference substance**	Performed	
~~69.4.1~~4.4.1 Concentrations	The reference item, DCP was applied at 3.2, 10 and 32 mg/l.	
~~69.4.2~~4.4.2 Results	The 3 hour EC_{50} for DCP was 12 mg/l. This result is within the expected range of 5 to 30 mg/l.	

Formatted: Bullets and Numbering

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

~~7~~5 APPLICANT'S SUMMARY AND CONCLUSION

~~7~~5.1 **Materials and methods**

The study was conducted to determine the inhibitory effect of Permethrin on the respiration of activated sewage sludge. The test was conducted according to OECD guideline 209 and US EPA Draft Ecological Effects Test Guidelines OPPTS 850.6800 and is described under point 3.

Deviations from the prescribed guidelines are as follows:

~~1)~~1. The test report does not contain information on the chemical identification data of Permethrin.

~~2)~~2. Information on the purity of Permethrin has not been documented.

The deviations outlined do not compromise the scientific validity of this study.

~~7~~5.2 **Results and discussion**

The validity criteria for the control respiration rates and reference material EC_{50} values were satisfied. The 3-hour NOEC was determined to be 1000 mg/l.

~~7~~5.2.1 EC_{20} Not documented

~~7~~5.2.2 EC_{50} A 3-Hour EC_{50} of greater than 1000 mg/l.

~~7~~5.2.3 EC_{80} Not documented

~~7~~5.3 **Conclusion**

The effect of the Permethrin on the respiration of activated sewage sludge microorganisms gave a 3-Hour EC_{50} of greater than 1000 mg/l. The No Observed Effect Concentration (NOEC) after 3 hours exposure was 1000 mg/l.

~~7~~5.3.1 Reliability 1

~~7~~5.3.2 Deficiencies One deviation was noted:

The test report does not contain information on the chemical identification data and purity of Permethrin.

This deviation does not compromise the scientific validity of this study.

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0.63 cm + Tab after: 1.27 cm + Indent at: 1.27 cm

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Formatted: Bullets and Numbering

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2 April 2009
Materials and Methods	Deviations from the prescribed guidelines are as follows: The test report does not contain information on the chemical identification data of Permethrin. Information on the purity of Permethrin has not been documented. *According to TM II 06 and TM II 08, for substances with low water solubility and if no effects on microorganisms are observed at the highest tested concentration, then water solubility is set as the NOEC*~~Applicants version acceptable~~.
Results and discussion	~~Adopt applicant's version~~EC50 >0.42 mg/L NOEC = 0.00495 mg/L.
Conclusion	Adopt applicant's version.
Reliability	1
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.4-1: Inoculum / Test organism

Criteria	Details
Nature	Activated sewage sludge
Species	Not relevant
Strain	Not relevant
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	Severn Trent Water Plc sewage treatment plant at Loughborough, Leicestershire, UK.
Laboratory culture	Yes
Method of cultivation	Japanese Industrial Standard JISK 0102-1981
Preparation of inoculum for exposure	The activated sewage sludge sample was continuously aerated in the laboratory at a temperature of 21°C and was used on the day of collection. The pH of the sample was 7.5. Determination of the suspended solids level of the activated sewage sludge was carried out by filtering a sample of the activated sewage sludge by suction through a piece of filter paper. This was then dried and cooled prior to weighing.
Pretreatment	Not documented
Initial cell concentration	Suspended solid concentration was equal to 4.0g/l prior to use.

Table A7.4.1.4-2: Test system

Criteria	Details
Culturing apparatus	Volumetric and conical flasks
Number of culture flasks/concentration	The test was carried out in triplicate
Aeration device	Compressed air via narrow bore glass tubes at a rate of approximately 0.5 – 1 litre per minute.
Measuring equipment	pH values were determined using a WTW pH 320 pH meter. The rate of respiration was determined using a Yellow Springs dissolved oxygen meter fitted with a BOD probe.
Test performed in closed vessels due to significant volatility of TS	Not documented

Table A7.4.1.4-3: Test conditions

Criteria	Details
Test temperature	The test was conducted in a temperature-controlled room at 21°C.
pH	pH values of the test preparation at the end of the exposure period (3 hours): Test vessel- pH- a) Control R_1 7.8 b) Control R_2 7.9 c) Permethrin R_1 7.8 d) Permethrin R_2 7.8 e) Permethrin R_3 7.8 f) 3, 5-dichlorophenol 3.2 7.9 g) 3, 5-dichlorophenol 10 8.0 h) 3, 5-dichlorophenol 32 8.1 The pH of the sample, during the preparation of the inoculum, was measured at 7.5 using a WTW pH 320 pH meter.
Aeration of dilution water	Not documented
Suspended solids concentration	Suspended solid concentration was equal to 4.0 g/l prior to use.

~~70.3.2.1.1~~Table A7.4.1.4-4: Oxygen consumption rates and percentage inhibition values in the definitive test after 30 minutes contact time

Formatted: Bullets and Numbering

Nominal concentration (mg/L)	Initial O_2 reading (mgO_2/L)	Measurement period (minutes)	Final O_2 reading (mgO_2/L)	O_2 consumption rates ($mgO_2/L/min$)	% inhibition
Control					
R_1[b]	5.3	7	1.0	0.61	-
R_2[b]	6.7	10	1.3	0.54	-
Natural Pyrethrum					
32	4.5	6	1.0	0.58	[1][a]
100	4.5	6	1.2	0.55	4
320	4.4	6	1.2	0.53	8
1000	4.6	7	1.1	0.50	13
3200	3.7	5	1.4	0.46	20
3,5-DCP					

3.2	6.1	9	1.5	0.51	11
10	6.7	10	2.4	0.43	25
32	8.2	10	6.9	0.13	77

[a][ ] = increase in respiration rate as compared to controls

[b] R_1 and R_2 = Replicates 1 to 2

~~70.3.2.1.2~~

Formatted: Bullets and Numbering

Table A7.4.1.4-5: Oxygen Consumption Rates in the Range-Finding Test

Nominal Concentration (mg/l)		O_2 Consumption Rates (mg O_2 /l/min) in the Initial Range-Finding Test	% Inhibition	O_2 Consumption Rates (mg O_2 /l/min) in the Second Range-Finding Test	% Inhibition
Control	R_1 [b]	0.63	-	0.44	-
	R_2 [b]	0.59	-	0.51	-
Permethrin	10	0.62	[2]	0.46	3
	100	0.63	[3]	0.46	3
	1000	0.65	[7]	0.48	[1]
3, 5-dichlorophenol	3.2	0.48	21	0.38	20
	32	0.11	82	0.10	79

[Increase in respiration rate as compared to controls]

[b] R_1 and R_2 = Replicates 1 to 2

Table A7.4.1.4-6: Oxygen Consumption Rates in the Definitive Test

Nominal Concentration (mg/l)		O_2 Consumption Rates (mg O_2 /l/min) in the Definitive Test after 30 Minutes Contact Time	% Inhibition	O_2 Consumption Rates (mg O_2 /l/min) in the Definitive Test after 3 Hours Contact Time	% Inhibition
Control	R_1 [b]	0.49	-	0.42	-
	R_2 [b]	0.42	-	0.39	-
Permethrin	1000 R_1	0.48	[5]	0.40	1
	1000 R_1	0.47	[3]	0.40	1
	1000 R_1	0.46	[1]	0.38	6
3, 5-dichlorophenol	3.2	0.42	8	0.36	11
	10	0.28	38	0.23	43
	32	0.10	78	0.07	83

[Increase in respiration rate as compared to controls]

[b] R_1 and R_2 = Replicates 1 to 2

Section 7.4.2 Annex Point IIA 7.5	Effects on Aquatic Organisms Bioconcentration	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	It is proposed that this point is not relevant to Permethrin as the product is intended for direct application to the wood surface and is not applied directly to the soil or to watercourses. Exposure of aquatic organisms to Permethrin is considered to be unlikely as the product is intended for low volume, localised application and is recommended not to be used on or near water. Permethrin has an estimated log K_{ow} of 5.9 (indicating the potential to	

Section 7.4.2 Annex Point IIA 7.5	Effects on Aquatic Organisms Bioconcentration
	bioaccumulate) and the BCF for fish estimated by the environmental fate modelling system USES 4.0 is 2.07 x 10^4 l/kg. However, an investigation into the potential for secondary poisoning in mammals following consumption of fish exposed to Permethrin indicated a maximum potential concentration of 5.11 mg/kg Permethrin in fish tissues and an ultimate risk quotient of 2.68 for mammalian species. Although, this result is slightly above the trigger value of 1, considering the exaggerated PEC_{water} values used in the estimation of bioaccumulation, it can be assumed that there is little risk of bioaccumulation in the aquatic compartment as a result of exposure to Permethrin. It is also worth noting that according to the Technical Guidance Document (TGD) on Risk Assessment (ECB Part II, 2003), substances with a log K_{ow} between 5 and 8 typically result in a BCF of >5000. When comparing this default value to the estimated BCF provided by USES (2.07 x 10^4 l/kg), it is clear that there is a wide margin for variation. Measured bioconcentration factors for Permethrin are reported in Sheepshead minnows (*Cyprinodon variegates*) exposed to Permethrin at concentrations between 1.25 and 10 µg/litre for 28 days from hatching (WHO Permethrin EHC 94, 1990; Hansen *et al*, 1983). The BCF in this case varied between 290 and 620. Maximum bioconcentration occurred after exposure at 2.5 µg/litre, and a maximum residue of 5.7 mg/kg (in whole fish) occurred after exposure at 10 µg/litre. Clearly then, by calculating the risk to aquatic species based on an estimated BCF value of 2.07 x 10^4 l/kg, a worst-case result is obtained. In the absence of a measured BCF (which would most likely be much lower than the default calculation) and using a worst-case calculation, it can be concluded that little or no risk can be expected as a result of bioconcentration in the aquatic compartment.
Undertaking of intended data submission [ ]	Not applicable
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2 April 2009
Evaluation of applicant's justification	Justification acceptable.
Conclusion	Justification acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*

Section 7.4.2 Annex Point IIA 7.5	Effects on Aquatic Organisms Bioconcentration
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.4.3.1 Annex Point IIIA, XIII.2.1	Effects on Aquatic Organisms – Further Studies Prolonged Toxicity to an Appropriate Species of Fish		
	JUSTIFICATION FOR NON-SUBMISSION OF DATA		Official use only
Other existing data [ ] Limited exposure [ ]	Technically not feasible [ ] Other justification [X]	Scientifically unjustified [ ]	

Section 7.4.3.1 Annex Point IIIA, XIII.2.1	Effects on Aquatic Organisms – Further Studies Prolonged Toxicity to an Appropriate Species of Fish
Detailed justification:	According to the "Data requirements for biocidal product types, Version 4.3.2 (October 2000)", this test is usually not required, as it does not add information as needed in the risk assessment and the current test guidelines are not sufficient. Therefore, no studies are presented to address this point.
Undertaking of intended data submission [ ]	Not applicable
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2 April 2009
Evaluation of applicant's justification	Justification acceptable.
Conclusion	Justification acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.4.3.2 Effects on reproduction and growth rate with an appropriate species of fish

Annex Point IIIA XIII 2.2

		Official use only
	~~7~~1 REFERENCE	
~~7.1.1~~1.1 Reference	[redacted] (2006a), Zebrafish (*Danio rerio*), Early Life Stage Toxicity Test (OECD 210) with Permethrin technical, [redacted] unpublished report No.: GAB-012/4-18.	
	Dates of experimental work: March 3, 2006 – April 7, 2006.	
~~7.2.1~~1.2 Data protection	Yes	
~~7.2.1~~1.2.1 Data owner	Tagros Chemicals India Ltd.	
~~7.2.2~~1.2.2 Companies with letter of access	Not applicable.	
~~7.2.3~~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/IA.	
	~~7~~2 GUIDELINES AND QUALITY ASSURANCE	
~~7.2.1~~2.1 Guideline study	Yes OECD test guideline 210, "Fish, Early-life Stage Toxicity Test"	
~~7.2.2~~2.2 GLP	Yes	
~~7.2.3~~2.3 Deviations	Yes, this study deviates from OECD Guideline 210 in the following respect: ~~1.~~1) The validity criterion relating to test item concentration was not met. The concentration of test item in the test solutions varied by > 20%. The variability in test item concentration was attributed to the specific properties of the test item. However, low endpoint variability was demonstrated indicating high statistical power of the test. Therefore it is proposed that the scientific validity of the test was not compromised.	

Formatted: Outline numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0 cm + Tab after: 1.25 cm + Indent at: 1.25 cm
Formatted: Bullets and Numbering
Comment [T3]: Confidential
Formatted: Highlight
Formatted: Bullets and Numbering
Formatted: Highlight
Formatted
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Bullets and Numbering
Formatted: Numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0.09 cm + Tab after: 0.72 cm + Indent at: 0.72 cm

Section A7.4.3.2

Annex Point IIIA XIII 2.2

Effects on reproduction and growth rate with an appropriate species of fish

	~~7~~3 MATERIALS AND METHODS
~~7~~3.1 **Test material**	As given in section 2 (Permethrin 25:75)
~~7~~3.1.1 Lot/Batch number	P - 38
~~7~~3.1.2 Specification	As given in section 2 (Permethrin 25:75)
~~7~~3.1.3 Purity	93.61%
~~7~~3.1.4 Composition of Product	Not applicable
~~7~~3.1.5 Further relevant properties	None
~~7~~3.1.6 Method of analysis	GC-MS/MS
~~7~~3.2 **Preparation of TS solution for poorly soluble or volatile test substances**	Details are given in Table A7.4.3.2-1.
~~7~~3.3 **Reference substance**	No
~~7~~3.3.1 Method of analysis for reference substance	Not applicable
~~7~~3.4 **Testing procedure**	
~~7~~3.4.1 Dilution water	Details are given in Table A7.4.3.2-2.
~~7~~3.4.2 Test organisms	*Danio rerio*, (Zebrafish) details are given in Table A7.4.3.2-3.
~~7~~3.4.3 Handling of embryos and larvae (OECD 210)	At test initiation, 50 freshly fertilised and randomised eggs were placed on stainless steel nets forming the bottom of fry cages fixed at the water surface of each test vessel. Each aquarium was equipped with two cages. 200 eggs were used for each test concentration. Larvae were fed daily *ad libitum* from day 6 onwards with breeding food (Tetra AZ 000). From day 9 on, brine shrimp nauplii (*Artemia salina*) were added *ad libitum* and from day 16 on, ground TetraMin flake food was added *ad libitum* to the daily food.
~~7~~3.4.4 Test system	Details are given in Table A7.4.3.2-4.