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

Annex Point IIIA XIII 2.2

~~7.3.4.5~~3.4.5	Test conditions	Details are given in Table A7.4.3.2-5.
~~7.3.4.6~~3.4.6	Duration of the test	35 days
~~7.3.4.7~~3.4.7	Test parameter	Effects on hatching, larval survival and larval growth
~~7.3.4.8~~3.4.8	Examination / Sampling	Observations were made daily on hatching and survival as well as any other abnormalities in appearance and behaviour. Dead embryos, larvae and juvenile fish were removed as soon as observed. Larvae/juvenile fish were photographed after 6, 14, 21, 28 and 35 days and the survival rates and lengths of the animals were determined using digital image processing.
~~7.3.4.9~~3.4.9	Monitoring of TS concentration	Yes; Samples were taken from the inflow of all vessels before adding the eggs and twice weekly thereafter. On day 35, the fry cages were sampled once to monitor the test item concentration.
~~7.3.4.10~~3.4.10	Statistics	For each treatment level and for the controls (n = 4), the arithmetic means of the endpoints were calculated. The data were analysed for statistical differences as compared with untreated controls by ANOVA, followed by Williams's test or appropriate non-parametric approaches to calculate the NOEC and LOEC for growth (length and group weight after 35 d) and survival over 35 days. Probit-analysis was applied on survival data to calculate the LC_{10} and LC_{50}. The calculations were performed by using the ToxRat® computer program.
		~~7~~4.4 **RESULTS**
4.1	**Range finding test**	Not performed
~~7.4.1.1~~4.1.1	Concentrations	Not applicable
~~7.4.1.2~~4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
~~7.4.1.3~~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 A7.4.3.2-6
4.2.2	Actual	Please refer to Table A7.4.3.2-6

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Section A7.4.3.2 Annex Point IIIA XIII 2.2

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

	concentrations of test substance	
4.2.3	Effect data	Please refer to Table A7.4.3.2-7.
4.2.4	Concentration / response curve	Please refer to Figure A7.4.3.2-1
4.2.5	Other effects	Not documented
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	Please refer to Table A7.4.3.2-7.
4.3.2	Nature of adverse effects	Please refer to Table A7.4.3.2-7.
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not applicable
4.4.2	Results	Not applicable

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

~~7.1~~5.1 Materials and methods

The test system was flow-through and *Danio rerio* was chosen as the test organism. The test was conducted according to OECD test guideline 210: "Fish, Early-life Stage Toxicity Test", and is described under point 3.

~~7.2~~5.2 Results and discussion

The measured test item concentrations were very variable. This is due to the properties of the test item, which made the daily generation of reproducible stock solutions complicated. Difficulties also arose in maintaining the test concentration due to adsorption and biodegradation. The concentrations at the lowest treatment level were below the LOQ of 0.3 µg/L on most sampling dates. The second test concentration was measured as 55% of nominal concentrations on average. For the three highest test concentrations, mean measured concentrations were 80% to 84% of nominal, ranging from 40% to 150% of nominal in single samples. The variability around the mean decreased with increasing test item concentration. Taking into account the properties of Permethrin, a coefficient of variation of 35% and 30% as determined for the two highest concentrations, can be regarded as satisfactory. On this basis, test effects were based on the mean measured concentrations, rather than nominal concentrations.

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Section A7.4.3.2
Annex Point IIIA XIII 2.2

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

No clinical signs of intoxication were observed at any test concentration. Hatch was not influenced by the test item and exceeded mean values of 93% at all test concentrations. No trend was observed in survival or growth up to the mean measured concentration of 0.41 µg Permethrin/L, at which level a slight (but not significant) reduction in survival was observed (total survival rate just under 70% in one replicate chamber). A significant reduction of approximately 30% was observed at the highest treatment level. Mortality caused by the test item occurred in the second and third week of exposure and is thought to be due to heightened sensitivity of the Zebrafish larvae as a response to feeding stress.

No significant difference was recorded in individual length measurements. Dry weights of fish tended to decrease in a concentration-dependent manner. However, this effect was not statistically significant.

Data are present in Tables A7.4.3.2-6 and A7.4.3.2-7.

~~75.2.1~~5.2.1	NOEC	0.41 µg/L
~~75.2.2~~5.2.2	LC_{10}	0.59 µg/L
~~75.3~~5.3	**Conclusion**	Based on mean measured concentrations, the most sensitive NOEC for Permethrin was determined to be 0.41 µg/L for a reduction of survival. The NOEC is clearly below the LC_{10}, demonstrating low endpoint variability and high statistical power in the test. For the purpose of the aquatic risk assessment, the LC_{10} of 0.59 µg/L should be used, as it is the more statistically reliable effect threshold concentration.
~~75.3.1~~5.3.1	Reliability	1
~~75.3.2~~5.3.2	Deficiencies	Yes, this study deviates from the prescribed guideline in the following respect: 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.

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	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	3 April 2009

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

Annex Point IIIA XIII 2.2

Materials and Methods	Adopt applicant's version.
Results and discussion	~~Adopt applicant's version. LC_{10} of 0.59 µg/L to be used in risk assessment.~~ RMS disagree with Applicant's sugestion of using the LC_{10} of 0.59 µg/L for risk assessment purposes. Instead the NOEC value of 0.41 µg/L will be used.
Conclusion	~~Adopt applicant's version.~~ RMS disagree with Applicant's sugestion of using the LC_{10} of 0.59 µg/L for risk assessment purposes. Instead the NOEC value of 0.41 µg/L will be used.
Reliability	2 *(validity criterion relating to test item concentration was not met.)*
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 A7.4.3.2-1: 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	The test item was initially dissolved in acetone, however during preparation of the stock solutions this was evaporated under a gentle stream of nitrogen. Consequently there is no need for a vehicle control to be included in the study.
Other procedures	Not documented

Table A7.4.3.2-2: Dilution water

Criteria	Details
Source	Purified drinking water
Alkalinity	1.4 – 1.5 mmol/L
Hardness	0.9 mmol/L
pH	7.8 – 8.0
Ca / Mg ratio	3.5:1.0
Na / K ratio	Not documented
Oxygen content	95 – 97%
Conductance	184 – 185 µS/cm
Holding water different from dilution water	No

Table A7.4.3.2-3: Test organisms

Criteria	Details
Species/strain	*Danio rerio* (Hamilton-Buchanan 1822)
Source	Laboratory bred, from West Aquarium GmbH, 37431 Bad Lauterberg, Germany.
Wild caught	No
Age/size	Freshly fertilised sampled eggs <30 mins after fertilisation
Kind of food	TetraMin® Hauptfutter and brine shrimp nauplii (*Artemia salina*)
Amount of food	*ad libitum*
Feeding frequency	daily
Post-hatch transfer time	Not necessary for Zebrafish according to OECD Guideline 210
Time to first feeding	3 days post-hatch
Feeding of animals during test	Yes Larvae were fed daily *ad libitum* from day 6 on breeding food (Tetra, AZ 000). From day 9 on, brine shrimp nauplii were added *ad libitum* and from day 16 on, ground TetraMin flake food was added *ad libitum* to the daily food.
Treatment for disease within 2 weeks preceeding test	No

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Table A7.4.3.2-4: Test system

Criteria	Details
Test Type	Flow-through
Renewal of test solution	2.1 l/h (daily turnover of 5 volumes)
Volume of test vessels	12 litres
Volume/animal	100 ml / fertilised egg
Number of animals/vessel	50 freshly fertilised eggs / cage 100 eggs / vessel
Number of vessels/ concentration	2 vessels / concentration
Test performed in closed vessels due to significant volatility of TS	Not documented

Table A7.4.3.2-5: Test conditions

~~7.5.3.2.1~~Criteria	Details
Test temperature	24.2 – 25.4 °C
Dissolved oxygen	84 – 99 %
pH	7.9 – 8.2
Adjustment of pH	Not documented
Aeration of dilution water	Yes
Quality/Intensity of irradiation	Not documented
Photoperiod	12 hour photoperiod daily

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Table A7.4.3.2-6: Actual concentrations of test substance

Nominal concentrations (µg/L)	Mean measured concentration (µg/L)			
	Initial (Day 0)	Aged (Day 35)	Geometric mean (all time points)	
			µg/L	% nom
Control (untreated dilution water)	<LOQ*	<LOQ	<LOQ	NA
0.06	<LOQ	<LOQ	< 0.03	< 48%
0.13	0.04	0.16	0.07	55%
0.25	0.13	0.26	0.21	84%
0.50	0.28	0.50	0.41	82%
1.0	0.47	0.83	0.80	80%

* <LOQ (0.03 µg a.s./l) calculated with 0.5*LOQ = 0.015 µg/l
NA = Not applicable

Table A7.4.3.2-7: Survival, growth and weight data

Mean measured Concentration (µg/L)	~~75.3.2.1.1.1.1.1~~5.3.2.1.1 Survivors ~~75.3.2.1.1.1.1.2~~5.3.2.1 n day 35	Length on day 35	Dry weight on day 35
	~~75.3.2.1.1.1.1.3~~% ± SD	Mean ± SD (mm)	Mean ± SD (mg)
Control	82.4 ± 4.2	10.2 ± 0.4	2.5 ± 0.4
<0.03	86.7 ± 4.3	10.7 ± 0.5	2.7 ± 0.3
0.07	86.3 ± 7.0	10.8 ± 0.4	2.5 ± 0.4
0.21	86.2 ± 4.7	10.5 ± 0.6	2.5 ± 0.5
0.41	80.8 ± 8.8	10.9 ± 0.2	2.3 ± 0.3
0.80	59.4 ± 13.8*	10.5 ± 0.8	2.1 ± 0.8

*significant negative deviation from control by means of Williams' test ($p < 0.05$)

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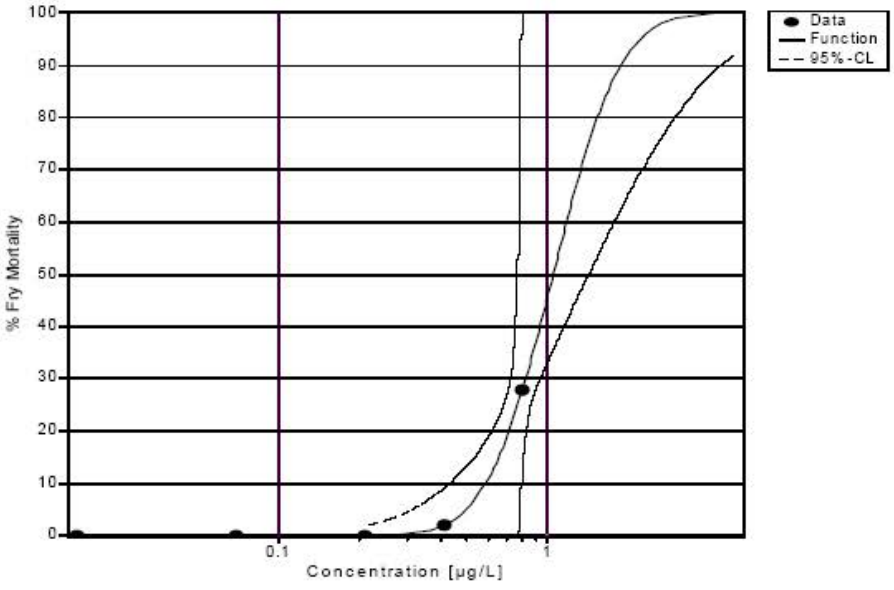

Figure A7.4.3.2-1: Concentration-effect curve showing the influence of the test item on survival of the introduced *Danio rerio* as observed after 35 days.

Table A7.4.3.2-8: Validity criteria for invertebrate reproduction test according to OECD Guideline 210

Criteria	Fulfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	~~7.5.3.2.1.1.1.1.4~~Yes
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	~~7.5.3.2.1.1.1.1.5~~Yes
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	~~7.5.3.2.1.1.1.1.6~~Yes
Test substance concentrations maintained within ± 20% of mean measured values	No
No effect on survival nor any other adverse effect found in solvent control	Yes

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Section 7.4.3.3.1 **Annex Point IIIA, XIII.2.3**	**Effects on aquatic organisms, further studies** **Bioaccumulation in an appropriate species of fish**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Existing information is available on the bioconcentration of Permethrin in fish and is discussed under point IIIA, 7.4.2. It is proposed that further studies are not required at this time. 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.	
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	3 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		

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Section 7.4.3.3.2 Annex Point IIIA, XIII.2.3	**Bioaccumulation in an Aquatic Organism** **Bioaccumulation in an Appropriate Invertebrate Species**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	According to the "Data requirements for biocidal product types, Version 4.3.2 (October 2000)", this test is only required for certain product types, especially if a direct release into marine/brackish water occurs. The product Permethrin 10 EC is not intended for release into marine/brackish water. Furthermore, exposure of aquatic organisms to Permethrin is considered to be unlikely as the product is intended for direct application to the wood surface and is not applied directly to the soil or to watercourses. However, in the unlikely event that the product enters watercourses by accidental discharge, an investigation into the potential for secondary poisoning following exposure 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. Based on the information above, it is therefore proposed that a study to address this point is not required.	
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	6 April 2009	
Evaluation of applicant's justification	Justification acceptable. As permethrin has not been classified for use as a wood preservative in Hazard Class 5 (saltwater) defined in the standard EN 335-1 (CEN, 1992), then a bioaccumulation study in an appropriate invertebrate species conducted in seawater and covering brackish water is not required for permethrin.	
Conclusion	Justification acceptable.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	

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Section 7.4.3.3.2 Annex Point IIIA, XIII.2.3	Bioaccumulation in an Aquatic Organism Bioaccumulation in an Appropriate Invertebrate Species
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 A7.4.3.4

Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an appropriate invertebrate species

Daphnia magna

Official use only

	~~7~~6 1 REFERENCE
~~7~~6.~~1~~1.1 **Reference**	Schäfers, C. (2006b), *Daphnia magna*, Reproduction test (OECD 211) Semi-static exposure, Permethrin technical, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), 57377 Schmallenberg, Germany, unpublished report No.: GAB-012/4-21.
	Dates of experimental work: April 5, 2006 – April 26, 2006.
~~7~~6.~~2~~1.2 **Data protection**	Yes
~~7~~6.2.~~1~~1.2.1 Data owner	Tagros Chemicals India Ltd.
~~7~~6.2.~~2~~1.2.2 Companies with letter of access	Not applicable.
~~7~~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.
	~~7~~7 2 GUIDELINES AND QUALITY ASSURANCE
~~7~~7.~~1~~2.1 **Guideline study**	Yes
	OECD test guideline 211, "*Daphnia magna*, Reproduction Test"
~~7~~7.~~2~~2.2 **GLP**	Yes
~~7~~7.~~3~~2.3 **Deviations**	No
	~~7~~8 3 MATERIALS AND METHODS
~~7~~8.~~1~~3.1 **Test material**	As given in section 2 (Permethrin 25:75)
~~7~~8.1.~~1~~3.1.1 Lot/Batch number	P - 38
~~7~~8.1.~~2~~3.1.2 Specification	As given in section 2 (Permethrin 25:75)
~~7~~8.1.~~3~~3.1.3 Purity	93.61%
~~7~~8.1.~~4~~3.1.4 Composition of Product	Not applicable

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Section A7.4.3.4

Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an appropriate invertebrate species

Daphnia magna

~~7.8.1.5~~3.1.5 Further relevant properties	None	Formatted: Bullets and Numbering
~~7.8.1.6~~3.1.6 Method of analysis	GC-NCl/MS	Formatted: Bullets and Numbering
~~7.8.2~~3.2 Preparation of TS solution for poorly soluble or volatile test substances	Details are given in Table A7.4.3.4-1.	Formatted: Bullets and Numbering
~~7.8.3~~3.3 Reference substance	No	Formatted: Bullets and Numbering
~~7.8.3.1~~3.3.1 Method of analysis for reference substance	Not applicable	Formatted: Bullets and Numbering
~~7.8.4~~3.4 Testing procedure		Formatted: Bullets and Numbering
~~7.8.4.1~~3.4.1 Dilution water	Details are given in Table A7.4.3.4-2.	Formatted: Bullets and Numbering
~~7.8.4.2~~3.4.2 Test organisms	*Daphnia magna*, details are given in Table A7.4.3.4-3.	Formatted: Bullets and Numbering
~~7.8.4.3~~3.4.3 Handling of offspring	Newborn daphnids were counted daily, inspected for abnormalities and removed. This procedure was performed daily between days 6 and 12 and three times weekly thereafter at the times of renewal of the test solutions.	Formatted: Bullets and Numbering
~~7.8.4.4~~3.4.4 Test system	Details are given in Table A7.4.3.4-4.	Formatted: Bullets and Numbering
~~7.8.4.5~~3.4.5 Test conditions	Details are given in Table A7.4.3.4-5.	Formatted: Bullets and Numbering
~~7.8.4.6~~3.4.6 Duration of the test	21 days	Formatted: Bullets and Numbering
~~7.8.4.7~~3.4.7 Test parameter	Effects on growth and reproductive performance	Formatted: Bullets and Numbering
~~7.8.4.8~~3.4.8 Examination / Sampling	Daphnids were observed daily for immobility and any other abnormalities in appearance and behaviour. Newborn daphnids were counted (daily from day 6 – 12 and three times weekly thereafter), inspected for abnormalities and removed. At study termination, the length of the adults was measured and compared with the control *Daphnia*.	Formatted: Bullets and Numbering
~~7.8.4.9~~3.4.9 Monitoring of TS concentration	Yes; Fresh preparations of three of the five test concentrations, (lowest, median, highest) and the control were sampled before each distribution	Formatted: Bullets and Numbering

Section A7.4.3.4
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an appropriate invertebrate species
Daphnia magna

		to the replicate vessels (3 times weekly). Aged test solutions including feed algae were analysed once weekly for residues of the test substance.
~~7~~8.4.10 3.4.10	Statistics	The evaluation of the concentration-effect-relationships and the calculations of effect concentrations were based on mean measured test item concentrations. For each endpoint, the NOEC, LOEC and (if possible), the EC_{50} and EC_{10} were determined. An NOEC was calculated by using an analysis of variance between groups (ANOVA) followed by Williams' test or an appropriate non-parametric test suggested by the ToxRat program. When the test results showed a concentration-response relationship, the data were analysed by regression to determine the EC_{50} including the 95% confidence interval as well as the EC_{10} using Probit-analysis, assuming log-normal distribution of the values by using the ToxRat computer program.
		~~7~~9 4 **RESULTS**
4.1	**Range finding test**	Performed
~~7~~9.1.1 4.1.1	Concentrations	Test concentrations for the main study were based on a non-GLP acute *Daphnia magna* range-finding test. Concentrations from this study were not documented in the report.
~~7~~9.1.2 4.1.2	Number/ percentage of animals showing adverse effects	Not documented
~~7~~9.1.3 4.1.3	Nature of adverse effects	Not documented
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Please refer to Table A7.4.3.4-6
4.2.2	Actual concentrations of test substance	Please refer to Table A7.4.3.4-6
4.2.3	Effect data	Please refer to Table A7.4.3.4-7
4.2.4	Concentration / response curve	Please refer to Figure A7.4.3.4-1
4.2.5	Other effects	Not documented
4.3	**Results of controls**	Please refer to Tables A7.4.3.4-6 and A7.4.3.4-7.
4.4	**Test with**	Not performed

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Section A7.4.3.4

Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an appropriate invertebrate species

Daphnia magna

	reference substance	
4.4.1	Concentrations	Not applicable
4.4.2	Results	Not applicable

~~8~~05 APPLICANT'S SUMMARY AND CONCLUSION

~~8~~0.1~~5~~.1 Materials and methods

The test system was semi-static and *Daphnia magna* was chosen as the test organism. The test method used was conducted according to OECD test guideline 211: "*Daphnia magna* Reproduction Test, and is described under point 3.

~~8~~0.2~~5~~.2 Results and discussion

Please refer to Tables A7.4.3.4-6 and A7.4.3.4-7.

The mean measured test item concentrations of the freshly prepared test solution were between 130% and 160% of the nominal concentrations. During the time interval until renewal of the test solution, test item concentrations decreased considerably to 10 – 20% of nominal at the higher concentrations. At the low concentration, most results obtained were below the LOQ of 10 ng/l, consequently the calculated mean is likely to overestimate the real concentrations. The geometric means of initial and aged concentrations at test solution renewal were 38 – 51% of nominal at the higher concentrations.

Neither adult mortality nor clinical signs were observed in any replicate at any concentration tested. Adult body length exhibited no significant differences between treatments.

Age at the first brood was between 8.4 and 9.2 days at concentrations ≤ 11 ng/l, the difference was not statistically significant. At 4.7 and 26 ng/l there was a slight, but significant increase to 9.2 and 9.6 days, but there was no clear dose-response relation and the highest test concentration showed no significant difference to the control. At the two highest test concentrations, offspring mortality was observed for 4 females. The dead offspring were not considered in the calculation of the cumulative number of offspring per female. The cumulative number of offspring per replicate ranged from 83.7 to 90.7 in the control and test groups exposed to ≤ 4.7 ng/l. There was a concentration-related decrease starting at 4.7 ng/l (7.7%), being significant at 11 ng/l (15.7%), and exhibiting an effect of 32% at the highest test concentration. Thus, the reproduction NOEC was 4.7 ng/l test item. However, the concentration-response relationship was flat and the EC_{50} (187.4 ng/l) had to be extrapolated from the EC_{10} (7.2 ng/l).

The NOEC for reproduction was clearly below the EC_{10}, indicating low

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

Annex Point IIIA XIII 2.4 — *Daphnia magna*

		data variability and sufficient statistical power. Even at the highest test concentration, no effect was observed with the exception of a 32% decrease of reproductive success which was deemed to be of limited ecological significance.	
~~80.2.1~~5.2.1	NOEC	4.7 ng/l	Formatted: Bullets and Numbering
~~80.2.2~~5.2.2	EC_{10}	7.2 ng/l	Formatted: Bullets and Numbering
~~80.2.3~~5.2.3	EC_{50}	187.4 ng/l	Formatted: Bullets and Numbering
~~80.3~~5.3	**Conclusion**	The mean 21 d NOEC for reproduction in *Daphnia magna* was determined to be 4.7 ng/l.	Formatted: Bullets and Numbering
~~80.3.1~~5.3.1	Reliability	1	Formatted: Bullets and Numbering
~~80.3.2~~5.3.2	Deficiencies	None	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	6 April 2009
Materials and Methods	Applicants version acceptable.
Results and discussion	Adopt applicant's version.
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*

Section A7.4.3.4 Annex Point IIIA XIII 2.4	**Effects on reproduction and growth rate with an appropriate invertebrate species** ***Daphnia magna***
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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

Criteria	Details
Dispersion	Not documented
Vehicle	Acetone
Concentration of vehicle	Not documented
Vehicle control performed	The test item was initially dissolved in acetone, however during preparation of the stock solutions this was evaporated under a gentle stream of nitrogen. Consequently there is no need for a vehicle control to be included in the study.
Other procedures	Not documented

Table A7.4.3.4-2: Dilution water

Criteria	Details
Source	Purified drinking water
Alkalinity	1.5 – 1.6 mmol/l
Hardness	0.9 – 1.0 mmol/l
pH	7.9 – 8.1
Ca / Mg ratio	2.2:1.0
Na / K ratio	Not documented
Oxygen content	Saturation point
Conductance	185 – 192 µS/cm
DOC	0 mg/l
Holding water different from dilution water	No

Table A7.4.3.4-3: Test organisms

Criteria	Details
Strain	*Daphnia magna* Straus
Source	Umweltbundesamt (German Federal Environment Agency), bred in the laboratory of the Fh-IME
Age	≤ 24 h old
Breeding method	Adults at least 3 weeks old were separated from the stock population by sieving. Batches of 30 to 50 animals were held at room temperature in *ca.* 1800 ml dilution water. Newborn *Daphnia* were separated by sieving and the first generation was discarded.
Kind of food	Algal suspension (*Scenedesmus subspicatus*) and LiquizellR (HOBBY).
Amount of food	30 ml suspension / 1L *Daphnia* medium
Feeding frequency	Daily
Pretreatment	Not documented
Feeding of animals during test	Yes; suspensions of *Desmodesmus subspicatus*

Table A7.4.3.4-4: Test system

Criteria	Details
Test Type	Semi-static
Renewal of test solution	Yes; 3 times weekly
Volume of test vessels	Glass beaker 60ml capacity
Volume/animal	50 ml / daphnid
Number of animals/vessel	1 daphnid / vessel (10 replicates)
Number of vessels/ concentration	10 / concentration
Test performed in closed vessels due to significant volatility of TS	Yes; beakers were covered with a glass lid

Table A7.4.3.4-5: Test conditions

~~80.3.2.1~~Criteria	Details
Test temperature	Ranged between 20.0 – 20.2 °C
Dissolved oxygen	Ranged between 96 – 97 %
pH	Ranged between 8.1 – 8.2
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Ranged between 688 – 746 lux
Photoperiod	16/8 hours light/dark cycle

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Table A7.4.3.4-6: Actual concentrations of test substance

Nominal concentrations (ng/l)	Mean measured concentration (ng/l)					
	Initial		Aged		Geometric mean	
	ng/l	%nom	ng/l	%nom	ng/l	%nom
Control (untreated dilution water)	<LOQ*	NA	<LOQ	NA	<LOQ	NA
3.0	4.8	160	1.7	56	2.8	94
22	31.0	141	4.1	19	11.2	51
159	202	127	17.8	11	60.0	38

* <LOQ calculated as 0.5*LOQ = 0.5 ng/l
NA = Not applicable

Table A7.4.3.4-7: Survival, growth and reproduction data

Mean measured Concentration [ng/L]	~~80.3.2.1.1.1.1~~ aren tal surv ival	~~80.3.2.1.1.1.1.2~~5.3.2.1. rowth (length on day 21)	Age at first brood	Cumulative offspring per female
	~~80.3.2.1.1.1.1~~ %)	Mean ± SD (mm)	Mean ± SD (days)	Mean ± SD (#)
Control	100	5.37 ± 0.44	8.4 ± 0.5	90.7 ± 8.2
1.9	100	5.45 ± 0.19	8.5 ± 0.5	91.8 ± 8.8
4.3	100	5.44 ± 0.37	9.2 ± 0.6*	83.7 ± 13.5
11	100	5.47 ± 0.16	8.9 ± 1.1	76.5 ± 9.2*
26	100	5.45 ± 0.24	9.6 ± 1.0*	70.8 ± 11.1*
60	100	5.23 ± 0.37	9.1 ± 1.0	61.7 ± 13.5*

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*significant deviation from control by Williams' test or Bonferroni-U-test

n = 10

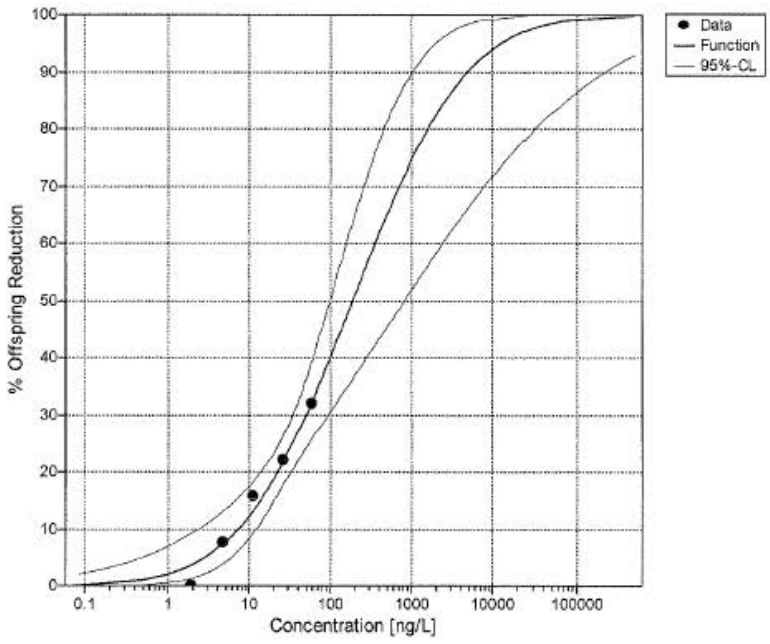

Figure A7.4.3.4-1: Percentage cumulative offspring at day 21 – concentration-effect curve using mean measured concentrations

Table A7.4.3.4-8: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

Criteria	Fulfilled
Mortality of parent animals <20% at test termination	~~8.9.3.2.1.1.1.1.4~~5.3.2.1.1.1.1.4 Yes
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	~~8.9.3.2.1.1.1.1.5~~5.3.2.1.1.1.1.5 Yes
Criteria for poorly soluble test substances	5.3.2.1.1.1.1.6 Yes

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Section 7.4.3.5.1 Annex Point IIIA, XIII.3.4	Effects on Any Other Specific, Non-Target Organisms (Flora and Fauna) Believed to be at Risk Effects on Sediment Dwelling Organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
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 therefore is unlikely to enter soil or watercourses during its normal use pattern.	
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	
Date	EVALUATION BY RAPPORTEUR MEMBER STATE 6 April 2009	

Section 7.4.3.5.1 Annex Point IIIA, XIII.3.4	**Effects on Any Other Specific, Non-Target Organisms (Flora and Fauna) Believed to be at Risk** **Effects on Sediment Dwelling Organisms**
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 7.4.3.5.2 Annex Point IIIA, XIII.3.4	Effects on Any Other Specific, Non-Target Organisms (Flora and Fauna) Believed to be at Risk Aquatic Plant Toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Permethrin is applied as a wood-preservative and is not intended for direct application to surface waters. In the unlikely event that Permethrin were introduced to the aquatic compartment *via* accidental exposure, it is not believed that Permethrin will pose any risk to plant species as its mode of action is insecticidal in nature. As the biocidal product Permethrin 10 EC is intended for direct application to the wood surface it is considered highly unlikely that Permethrin will be allowed to enter watercourses during its normal use pattern and on this basis it is proposed that a study is not required to address this point. Furthermore, studies were carried out on the toxicity of Permethrin to terrestrial plants in seedling emergence and vegetative vigour studies. In the seedling emergence study, Permethrin was found to potentially affect the emergence of species such as *Helianthus annuus* above concentrations of 0.0128 mg/kg dry soil and it was found that biomass reduction can occur for non-target plants like *Avena sativa* above 8 mg/kg dry soil. However, the results of the vegetative vigour study indicated that since the effects on biomass for all species was < 20%, Permethrin can be classified as a low risk to terrestrial plants. It can therefore be assumed that Permethrin would demonstrate a similar absence of adverse effects on aquatic plants.	
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	

Section 7.4.3.5.2 Annex Point IIIA, XIII.3.4	Effects on Any Other Specific, Non-Target Organisms (Flora and Fauna) Believed to be at Risk Aquatic Plant Toxicity
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	7 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 A7.5.1.1 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

		Official use only
	~~8.1~~ REFERENCE	
~~8.1.1~~1.1 **Reference**	Kölzer, U. (2006), Assessment of the side effects of Permethrin Technical on the activity of the soil microflora, GAB Biotechnologie GmbH & GAB Analytik GmbH, Eutinger Str. 24, D-75223 Niefern-Oschelbronn, Germany, unpublished report No.: 20051446/01-ABMF. Dates of experimental work: March 9, 2006 –April 25, 2006	
~~8.2.1~~1.2 **Data protection**	Yes	
~~1.2.1~~1.2.1 Data owner	Tagros Chemicals India Ltd.	
~~8.2.2~~1.2.2 Companies with letter of access	Not applicable.	
~~8.2.3~~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.	
	~~8.2~~2 GUIDELINES AND QUALITY ASSURANCE	
~~8.2.1~~2.1 **Guideline study**	Yes OECD test guideline 216, "Soil Microorganisms: Nitrogen Transformation Test" OECD test guideline 217, "Soil Microogranisms: Carbon Transformation Test"	
~~8.2.2~~2.2 **GLP**	Yes	
~~8.2.3~~2.3 **Deviations**	No	
	~~8.3~~3 MATERIALS AND METHODS	
~~8.3.1~~3.1 **Test material**	As given in section 2 (Permethrin Technical)	
~~8.3.1.1~~3.1.1 Lot/Batch number	P-37	
~~8.3.1.2~~3.1.2 Specification	As given in section 2 (Permethrin Technical)	

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Section A7.5.1.1 **Inhibition to microbial activity (terrestrial)**

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~~3.1.3~~3.1.3	Purity	93.61%
~~3.1.4~~3.1.4	Composition of Product	Not applicable
~~3.1.5~~3.1.5	Further relevant properties	None
~~3.1.6~~3.1.6	Method of analysis	Not documented
~~3.2~~3.2	**Reference substance**	Yes Dinoterb
~~3.2.1~~3.2.1	Method of analysis for reference substance	Not applicable
~~3.3~~3.3	**Testing procedure**	
~~3.3.1~~3.3.1	Soil sample / inoculum / test organism	Please refer to Table A7.5.1.1-1
~~3.3.2~~3.3.2	Test system	Not applicable
~~3.3.3~~3.3.3	Application of TS	Please refer to Table A7.5.1.1-2
~~3.3.4~~3.3.4	Test conditions	Please refer to Table A7.5.1.1-3
~~3.3.5~~3.3.5	Test parameter	Nitrogen turnover and short – term respiration
~~3.3.6~~3.3.6	Analytical parameter	Not applicable
~~3.3.7~~3.3.7	Duration of the test	28 days for short-term respiration; 42 days for nitrogen turnover
~~3.3.8~~3.3.8	Sampling	Short-term respiration: days 0, 7, 14, and 28 after application. Nitrogen turnover: days 0, 7, 14, 28, and 42 after application.
~~3.3.9~~3.3.9	Monitoring of TS concentration	No
~~3.3.10~~3.3.10	Controls	Two control groups were prepared. The first control consisted of soil treated with deionized water. The second control consisted of deionized water, acetone, and quartz sand.
~~3.3.11~~3.3.11	Statistics	The results of the nitrification and short-term respiration measurements were tested for normality using Shapiro-Wilk's Test and residual analysis. Homogeneity of variances was tested using Bartlett-Test. For the short-term respiration the data were analyzed using the Dunnett's t-

Section A7.5.1.1 **Inhibition to microbial activity (terrestrial)**

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Test. For the nitrogen turnover, the Jonckheere Terpstra Test was used to analyze the data for significance. SAS® release 9.1.3 was used for the statistical analyses.

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~~8.~~4.4 RESULTS

4.1	**Range finding test**	Not performed
~~8.1.1~~4.1.1	Concentration	Not applicable
~~8.1.2~~4.1.2	Effect data	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	1.83 mg/ kg (Permethrin technical/dry soil) 9.17 mg/kg (Permethrin technical/dry soil)
4.2.2	Actual concentrations of test substance	Not documented
4.2.3	Growth curves	Not applicable
4.2.4	Cell concentration data	Not applicable
4.2.5	Concentration/ response curve	Not applicable
4.2.6	Effect data	Please refer to Table A7.5.1.1-4 for nitrogen turnover results and Tables A7.5.1.1-7 and A7.5.1.1-8 for short-term respiration results.
4.2.7	Other observed effects	Please refer to Table A7.5.1.1-6 for nitrate formation rate.
4.3	**Results of controls**	Please refer to Tables A7.5.1.1-4, A7.5.1.1-5 and A7.5.1.1-7.
4.4.	**Test with reference substance**	Performed
4.4.1	Concentrations	13.3 mg/kg Dinoterb/dry soil
4.4.2	Results	The toxic standard, dinoterb, had distinct effects (deviation from the control > 25%) on the short term respiration and nitrogen turnover after 28 days, 47.8% and 56.4% deviation from the control, respectively.

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~~8.~~5.5 APPLICANT'S SUMMARY AND CONCLUSION

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~~8.~~5.1 **Materials and** The effects of Permethrin Technical on soil microflora were assessed in a

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	methods	test that measured nitrogen turnover and short-term respiration following an application of Permethrin Technical to soil. The test was conducted according to OECD test guideline 216, "Soil Microorganisms: Nitrogen Transformation Test" and OECD test guideline 217, "Soil Microogranisms: Carbon Transformation Test", and are described under point 3.
~~8.2~~5.2	**Results and discussion**	Please refer to Table A7.5.1.1-4 for nitrogen turnover results and Tables A7.5.1.1-7 and A7.5.1.1-8 for short-term respiration results. No statistical significant effect was observed for the short term respiration (produced CO_2) at both concentrations of Permetrhin Technical 28 days after application. All values were below the threshold value provided by the OECD guideline 217 (≤ 25% variation between the treatments and the control). The percent deviations between soil treated with Permethrin Technical and the acetone control were +4.27% for the 1.83 mg/kg concentration and +4.70% for the 9.17 mg/kg concentration. Twenty-eight days after the test was initiated, the deviation of the highest test item group (9.17 mg/kg) from the acetone control for the nitrogen transformation rate was above 25%. After 42 days, the effect was below the threshold value provided by the OECD guideline 216 (25%) and the study was terminated. For the nitrogen turnover, a statistical significant effect was observed for the 9.17 mg/kg concentration 42 days after application. The values for both concentrations were below the threshold value as outlined in the OECD guideline 216 (≤25%). The percent deviation between soil treated with the test substance and the acetone control was +0.957% for the 1.83 mg/kg concentration and +5.98% for the 9.17 mg/kg concentration. For the rate of nitrogen formation, the deviations between the soils treated with the test substance and the acetone control were +1.20% and +7.89%, respectively.
5.2.1	NOEC	Not applicable
5.2.2	EC_{10}	Not applicable
5.2.3	EC_{50}	Not applicable
~~8.3~~5.3	**Conclusion**	Based on the results of this study and in accordance with OECD guidelines 216 and 217, Permethrin Technical had no effects on the soil nitrogen turnover and the short term respiration in a field soil tested up to 6.875 kg of Permethrin Technical/ha (corresponding to 9.17 mg/kg concentration) 42 days after application. Further, the study is considered valid since the variation between replicate control samples was < +15%.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

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Section A7.5.1.1 Inhibition to microbial activity (terrestrial)

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	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	9 April 2009
Materials and Methods	Applicants version acceptable.
Results and discussion	Adopt applicant's version.
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 A7.5.1.1-1: Soil parameters

Criteria	Details
Nature	Loamy sand
Sampling site:	
Geographical reference on the sampling site	D-76877 Offenback, "Im Bildgarten", Nr. 586
Data on the history of the site	Plant protection products were not used on site for 1 year; no organic and mineral fertilizers had been applied to site for 3 – 6 months
Use pattern	Agricultural soil
Depth of sampling [cm]	0 – 20 cm
Sand / Silt / Clay content [% dry weight]	70.2%/ 24.4%/ 5.4%
pH	6.80
Organic carbon content [% dry weight]	1.19
Total Nitrogen (mg/kg)	919
Cation exchange capacity [mval/kg]	8.38
Initial microbial biomass	1.04
Reference of methods	OECD 216 and 217
Collection / storage of samples	Soil was collected and sieved to a particle size of 2mm. Sample was stored for a total of 19 days at an average storage temperature of 4°C (min temp: 3.6 °C and max temp 4.5°C)
Preparation of inoculum for exposure	Moisture content and amount of water needed to bring the soil moisture content to 45% WHC max were determined (maximum water holding). For the nitrogen turnover, soil was thoroughly mixed with ground Lucerne meal to a final concentration of 0.5% of the soil dry weight. For the short-term respiration, the amount of glucose needed to obtain maximal short term rates in the test soil were determined. A concentration of 400 mg glucose/100g soil wet weight was used.
Pretreatment	Soil was conditioned at 20° C ± 2 in the dark. Moisture content of the soil and amount of water needed to bring the soil moisture content to 45% WHC_{max} (maximum water holding capacity) were determined.

Table A7.5.1.1-2: Application of test substance

Criteria	Details
Application procedure	The calculated amount of test substance was dissolved in 20 g of acetone. For each test, 2 g of acetone solution was applied to quartz sand (amount: 1% the soil dry weight). The acetone was allowed to evaporate and the sand was thoroughly mixed and added to the soil.
Carrier	Acetone
Concentration of liquid carrier [% v/v]	Not documented
Liquid carrier control	Acetone and water
Other procedures	41.5 g of deionized water was added to each kg of test soil resulting in a final water content of 45% WHC_{max}.

Table A7.5.1.1-3: Test conditions

Criteria	Details
Organic substrate	The test soil was a loamy sand which was amended with ground lucerne meal (0.5% of soil dry weight) for the nitrogen turnover test and with glucose (400mg /100g soil wet) for the short term respiration test.
Incubation temperature	20° C ± 2
Soil moisture	9.15% initial content; brought up to 45% WHC_{max}; final moisture content not documented
Method of soil incubation	Three equally sized subsamples per treatment group were incubated in glass bottles.
Aeration	No

Table A7.5.1.1-4: Nitrogen turnover results

	0 d	7 d	14 d	28 d	42 d
Control (acetone)					
NH_4^+-N	1.29	<LOQ	<LOQ	<LOQ	<LOQ
NO_3^--N	10.40	9.28	15.7	23.6	41.8
N_{min}	11.7	9.28	15.7	23.6	41.8
Control (water)					
NH_4^+-N	1.25	<LOQ	<LOQ	<LOQ	<LOQ
NO_3^--N	9.9	8.58	14.8	21.4	40.3
N_{min}	11.2	8.58	14.8	21.4	40.3
Deviation from the acetone control (%)					
NH_4^+-N	-3.10	-	-	-	-
NO_3^--N	-4.81	-7.54	-5.73	-9.32	-3.59
N_{min}	-4.27	-7.54	-5.73	-9.32	-3.59
1.83 mg/kg Permethrin technical/dry soil weight					
NH_4^+-N	1.50	<LOQ	<LOQ	<LOQ	<LOQ
NO_3^--N	10.5	7.96	15.5	24.0	42.2
N_{min}	12.0	8.0	15.5	24.0	42.2
Deviation from the acetone control (%)					
NH_4^+-N	16.3	-	-	-	-
NO_3^--N	0.962	-14.2	-1.27	1.69	0.957
N_{min}	2.56	-13.8	-1.27	1.69	0.957
9.17 mg/kg Permethrin technical/dry soil weight					
NH_4^+-N	1.80	<LOQ	<LOQ	<LOQ	<LOQ
NO_3^--N	10.9	10.7	19.6	28.6	44.3
N_{min}	12.7	10.7	19.6	28.6	44.3
Deviation from the acetone control (%)					
NH_4^+-N	39.5	-	-	-	-
NO_3^--N	4.81	15.3	24.8	21.2	5.98
N_{min}	8.55	15.3	24.8	21.2	5.98

<LOQ = below the limit of quantification (0.890 mg/kg soil dry weight)

Table A7.5.1.1-5: Effects of controls on nitrate formation rate

Assessment Interval	Control (acetone)	Control (water)

	NO_3^--N [mg/kg dry weight /day][1]	NO_3^--N [mg/kg dry weight /day][1]	Deviation from acetone control [%][2]
0-7	-0.160	-0.189	+18.1
0-14	0.379	0.350	-7.65
0-28	0.471	0.411	-12.7
0-42	0.748	0.712	-4.81

[1] + = stimulation; - = inhibition

[2] + = effects more marked than control; - = effects less marked than control

Table A7.5.1.1-6: Effects of permethrin technical on nitrate formation rate

Assessment Interval	1.83 mg/kg Permetrhin technical/dry soil weight		9.17 mg/kg Permetrhin technical/dry soil weight	
	NO_3^--N [mg/kg dry weight /day][1]	Deviation from acetone control [%][2]	NO_3^--N [mg/kg dry weight /day][1]	Deviation from acetone control [%][2]
0-7	-0.363	+127	-0.0300	-81.3
0-14	0.357	-5.80	0.621	+63.9
0-28	0.482	+2.34	0.632	+34.2
0-42	0.757	+1.20	0.807	+7.89

[1] + = stimulation; - = inhibition

[2] + = effects more marked than control; - = effects less marked than control

Table A7.5.1.1-7: Results of short term respiration test (mg CO2/h/kg dry soil weight)

Time (days)	Control (acetone)	Control (water)	1.83 mg/kg Permetrhin technical/dry soil weight	9.17 mg/kg Permetrhin technical/dry soil weight
0	5.23	4.92	5.25	5.29
7	6.09	6.82	6.72	6.88
14	4.85*	5.28	4.96	4.76
28	4.68	5.31	4.88	4.90

*Due to a defective measuring unit this value was calculated on the basis of only two instead of three individual values.

Table A7.5.1.1-8: Short term respiration test, % deviation from the acetone control

Time (days)	Control (water)	1.83 mg/kg Permetrhin technical/dry soil weight	9.17 mg/kg Permetrhin technical/dry soil weight
0	-5.93	+0.382	+1.15
7	+12.0	+10.3	+13.0
14	+8.87	+2.27	-1.86
28	+13.5	+4.27	+4.70

- = inhibition

+ = stimulation

Section A7.5.1.2 **Earthworm, acute toxicity test**
Annex Point IIIA XIII 3.2

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	~~8~~6.1 REFERENCE
~~8~~6.1.1 Reference	Sunil Dutt, M. (2006), Toxicity of Permethrin Technical to Earthworm, *Lampito mauritii,* International Institute of Biotechnology and Toxicology (IIBAT), Padappai – 601301, Kancheepuram District, Tamil Nadu, India, unpublished report no.: 06039 Dates of experimental work: June 15, 2006 - June 29, 2006.
~~8~~6.2.1.2 Data protection	Yes
~~8~~6.2.1.1.2.1 Data owner	Tagros Chemicals India Ltd.
~~8~~6.2.2.1.2.2 Companies with letter of access	Not applicable
~~8~~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.

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Section A7.5.1.2 **Earthworm, acute toxicity test**
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	~~8~~7~~2~~ GUIDELINES AND QUALITY ASSURANCE
~~8~~7.~~1~~2.1 **Guideline study**	Yes
	OECD test guideline 207: "Earthworm, Acute Toxicity Tests".
~~8~~7.~~2~~2.2 **GLP**	Yes
~~8~~7.~~3~~2.3 **Deviations**	Yes, this study deviates from OECD Guideline 207 in the following respects: ~~3~~1. The recommended test species is *Eisenia foetida*, however *Lampito mauritii* were used. This species is the most prolific in Indian soil and has a higher susceptibility to pesticides. It was therefore considered to be the most appropriate choice of species. ~~4~~2. The test temperature was maintained at 24.2 – 25.6°C throughout the test, whereas the guideline recommends the temperature should be 20°C ± 2°C. 24.2 – 25.6°C is the most suitable temperature for the earthworm species *Lampito mauritii*. However, these deviations are not considered to compromise the scientific validity of the study.
	~~8~~8~~3~~ METHOD
~~8~~8.~~1~~3.1 **Test material**	As given in section 2 (Permethrin 25:75)
~~8~~8.~~1.1~~3.1.1 Lot/Batch number	P- 40
~~8~~8.~~1.2~~3.1.2 Specification	As given in section 2 (Permethrin 25:75)
~~8~~8.~~1.3~~3.1.3 Purity	93.01 %
~~8~~8.~~1.4~~3.1.4 Composition of Product	Not applicable
~~8~~8.~~1.5~~3.1.5 Further relevant properties	None
~~8~~8.~~1.6~~3.1.6 Method of analysis	Not applicable
~~8~~8.~~2~~3.2 **Reference substance**	Yes; Chloroacetamide
~~8~~8.~~2.1~~3.2.1 Method of analysis for reference substance	Not applicable

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Section A7.5.1.2 **Earthworm, acute toxicity test**
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~~8.3.3~~3.3	**Testing procedure**		Formatted: Bullets and Numbering
~~8.3.1~~3.3.1	Preparation of the test substance	Details are provided in Table A7.5.1.2-1	Formatted: Bullets and Numbering
~~8.3.2~~3.3.2	Application of the test substance	Different concentrations of Permethrin were prepared in acetone and uniformly applied to 1kg of artificial soil medium and mixed thoroughly.	Formatted: Bullets and Numbering
~~8.3.3~~3.3.3	Test organisms	*Lampito mauritii* (Details are given in Table A7.5.1.2-2)	Formatted: Bullets and Numbering
~~8.3.4~~3.3.4	Test system	Details are given in Table A7.5.1.2-3	Formatted: Bullets and Numbering
~~8.3.5~~3.3.5	Test conditions	Details are given in Table A7.5.1.2-4	Formatted: Bullets and Numbering
~~8.3.6~~3.3.6	Test duration	14 days	Formatted: Bullets and Numbering
~~8.3.7~~3.3.7	Test parameter	Mortality and weight change	Formatted: Bullets and Numbering
~~8.3.8~~3.3.8	Examination	Days 7 and 14 of the 14-day exposure period.	Formatted: Bullets and Numbering
~~8.3.9~~3.3.9	Monitoring of test substance concentration	No	Formatted: Bullets and Numbering
~~8.3.10~~3.3.10	Statistics	Not documented	Formatted: Bullets and Numbering
			Formatted: Bullets and Numbering
		~~8~~9.4 **RESULTS**	
4.1	**Filter paper test**	Not performed	
~~8.1.1~~4.1.1	Concentration	Not applicable	Formatted: Bullets and Numbering
~~8.1.2~~4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	Formatted: Bullets and Numbering
~~8.1.3~~4.1.3	Nature of adverse effects	Not applicable	Formatted: Bullets and Numbering
4.2	**Soil test**		
4.2.1	Initial concentrations of test substance	75, 150, 300, 600, 1200 mg Permethrin/kg dry weight artificial soil.	
4.2.2	Effect data	Details are provided in Tables A7.5.1.2-5 and A7.5.1.2-6.	

Section A7.5.1.2 **Earthworm, acute toxicity test**
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	(Mortality)	
4.2.3	Concentration / effect curve	Not documented
4.2.4	Other effects	Bleeding was observed in the dead earthworms. A slight reduction in weight of earthworms was noted in all treatments compared with the control.
4.3	**Results of controls**	
4.3.1	Mortality	Details are provided in Table A7.5.1.2-5.
4.3.2	Number/ percentage of earthworms showing adverse effects	Details are provided in Table A7.5.1.2-5 and A7.5.1.2-7
4.3.3	Nature of adverse effects	Details are provided in Table A7.5.1.2-5 and A7.5.1.2-7
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	Not documented
4.4.2	Results	LC_{50} value for Chloracetamide was 197.54 mg/kg dry weight of soil.

~~9~~5 APPLICANT'S SUMMARY AND CONCLUSION

~~90.1~~5.1 Materials and methods	An acute toxicity test was carried out in order to assess the effects of Permethrin on earthworms. The test was conducted according to OECD guideline 207 and is described under point 3.
~~90.2~~5.2 Results and discussion	No mortality was observed in the acetone solvent control group throughout the study. Following 7 days of exposure, mortality ranged from 0% to 10%. On the 14th day of exposure, mortality rates ranged from 0% in the 75 mg/kg concentration group up to 15% in the 1200 mg/kg concentration group. In the 150 – 1200 mg/kg concentration gourps, bleeding was observed in the dead earthworms. A slight reduction in weight of earthworms was also noted in all treatments compared with the control.
~~90.2.1~~5.2.1 LC_0	Not documented
~~90.2.2~~5.2.2 LC_{50}	LC_{50} for *Lampito mauritii* is >1200 mg/kg.

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Section A7.5.1.2 **Earthworm, acute toxicity test**
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~~90.2.3~~5.2.3	LC_{100}	Not documented
~~90.3~~5.3	**Conclusion**	The LC_{50} for *Lampito mauritii* was determined to be >1200 mg Permethrin/kg dry weight soil. The validity criteria for acute earthworm test according to OECD 207 were fulfilled.
~~90.3.1~~5.3.1	Other Conclusions	None
~~90.3.2~~5.3.2	Reliability	2
~~90.3.3~~5.3.3	Deficiencies	Yes, the following deficiencies were noted:

~~7.4~~1 The recommended test species is *Eisenia foetida*, however *Lampito mauritii* were used. This species is the most prolific in Indian soil and has a higher susceptibility to pesticides. It was therefore considered to be the most appropriate choice of species.

~~7.5~~2 The test temperature was maintained at 24.2 – 25.6°C throughout the test, whereas the guideline recommends the temperature should be 20°C ± 2°C. 24.2 – 25.6°C is the most suitable temperature for the earthworm species *Lampito mauritii*.

However, these deviations are 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	9 April 2009
Materials and Methods	Applicants version acceptable.
Results and discussion	Adopt applicant's version.
Conclusion	Adopt applicant's version.
Reliability	2
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*

Section A7.5.1.2 **Earthworm, acute toxicity test**
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Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.5.1.2-1: Preparation of TS solution

In case of the use of an organic solvent	
Dispersion	Not documented
Vehicle	Yes; Acetone
Concentration of vehicle	Not documented
Vehicle control performed	Yes; Acetone was mixed with the untreated control soil.
Other procedures	The glass trays were covered with perforated plastic film to prevent the test medium from drying out.

Table A7.5.1.2-2: Test organisms

Criteria	Details
Species/strain	*Lampito mauritii*
Source of the initial stock	Earthworm breeding stock, IIBAT
Culturing techniques	Earthworms were bred on 50:50 mixture of decomposed farm yard manure and soil
Age/weight	Worms were 6 weeks old and weighed 0.613 – 0.712 g at study commencement. Details of worm weight before treatment at 7 and 14 days after treatment are provided in Table A7.5.1.2-7.
Pre-treatment	A 7 day acclimation period was observed

Table A7.5.1.2-3: Test system

Criteria	Details
Artificial soil test substrate	1kg of artificial medium contains farmyard manure, sand and soil in the ratio 70:20:10 (weight:weight) respectively.
Test mixture	Test solutions of Permethrin were uniformly applied in one kg of artificial soil medium.
Size, volume and material of test container	Glass tray (Dimensions not documented)
Amount of artificial soil (kg)/ container	1 kg
Nominal levels of test concentrations	75, 150, 300, 600, 1200 mg/kg artificial soil
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Not documented
Test performed in closed vessels due to significant	The glass trays were covered with perforated plastic

volatility of TS	film to prevent the test medium from drying out although this is not due to volatility of the test substance

Table A7.5.1.2-4: Test conditions

Criteria	Details
Test temperature	24.2 – 25.6°C
Moisture content	28.6% at test initiation 28.04 – 28.13% at test termination
pH	pH of soil ranged from 7.70 – 7.72 at study commencement.
Adjustment of pH	No
Light intensity / photoperiod	A light intensity of 597 – 694 Lux was provided continuously throughout the experimental period.
Relevant degradation products	Not documented

Table A7.5.1.2-5: Mortality data (test substance)

Test Substance Concentration (nominal) [mg/kg artificial soil]	Mortality (based on 40 worms/concentration)			
	Number		Percentage	
	7 d	14 d	7 d	14 d
Untreated Control	0	0	0.00	0.00
75	0	0	0.00	0.00
150	2	2	2.50 (2.50)	2.50 (2.50)
300	2	4	2.50 (2.50)	5.00 (2.89)
600	4	4	7.50 (2.50)	10.00 (0.00)
1200	8	6	10.00 (0.00)	15.00 (2.89)
Temperature [°C]	24.2 – 25.6	24.2 – 25.6		
pH	7.70 – 7.72	ND		
Moisture content	ND	ND		

Figures in parentheses are standard error

ND = not documented

Table A7.5.1.2-6: Effect data

	14 d [mg/kg soil][1]	95 % c.l.
LC_{10}	ND	ND
LC_{50}	>1200	ND
LC_{90}	ND	ND

[1] nominal concentrations

Permethrin **(Tagros Chemicals India Ltd.)**	**Product-type 8**	~~August 2009~~March 2011

ND = not documented

Table A7.5.1.2-7: Group mean bodyweight changes

Test Substance Concentration (nominal) [mg/kg artificial soil]	Average Weight of Earthworm (g)*			Mean % bodyweight change
	Before Treatment	7th day	14th day	
75	0.666	0.693	0.681	-2.21
150	0.654	0.664	0.632	-3.37
300	0.677	0.687	0.638	-5.77
600	0.678	0.656	0.578	-14.75
1200	0.613	0.572	0.471	-23.17
Untreated Control	0.712	0.749	0.766	+7.58

* - Mean of 4 replications

Table A7.5.1.2-8: Validity criteria for acute earthworm test according to OECD 207

	fulfilled
Mortality of control animals < 10%	Yes

Section 7.5.1.3/1
Annex Point IIIA XIII 3.4

Terrestrial plant toxicity
Seedling emergence test

Official use only

~~91~~1 REFERENCE

~~91.1~~1.1 Reference

Balluff, M. (2006a), Seedling emergence dose-response test for non-target plants following multiple rate applications of Permethrin Technical 25/75, eurofins-GAB GmbH, Eutinger Str. 24, D-75223

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Section 7.5.1.3/1 Annex Point IIIA XIII 3.4	**Terrestrial plant toxicity** **Seedling emergence test**
	Niefern-Öschelbronn, Germany, unpublished report No.: 20064034/S1-FGSE.
	Dates of experimental work: June 30, 2006 – July 25, 2006.
~~91.2~~1.2 Data protection	Yes
~~91.2.1~~1.2.1 Data owner	Tagros Chemicals India Ltd.
~~91.2.2~~1.2.2 Companies with letter of access	Not applicable.
~~91.2.3~~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.
	~~92~~2 GUIDELINES AND QUALITY ASSURANCE
~~92.1~~2.1 Guideline study	Yes Draft Guideline OECD-208: Terrestrial (Non-Target) Plant Test 208A: Seedling emergence and seedling growth test (2000).
~~92.2~~2.2 GLP	Yes
~~92.3~~2.3 Deviations	No
	~~93~~3 MATERIALS AND METHODS
~~93.1~~3.1 Test material	As given in section 2 (Permethrin 25:75)
~~93.1.1~~3.1.1 Lot/Batch number	P - 165
~~93.1.2~~3.1.2 Specification	As given in section 2 (Permethrin 25:75)
~~93.1.3~~3.1.3 Purity	93.07
~~93.1.4~~3.1.4 Composition of Product	Not applicable
~~93.1.5~~3.1.5 Further relevant properties	None
~~93.1.6~~3.1.6 Method of	Not applicable

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