	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*19 April 2005*
Materials and Methods	The open literature paper does not provide sufficient information on the test materials, such as batch/lot number.
Results and discussion	Adopt applicant's version.
Conclusion	Adopt applicant's version.
Reliability	3
Acceptability	~~acceptable~~ / not acceptable Consider as additional information only.
Remarks	This published study on the soil column test of permethrin as affected by anhydrous ammonia should be treated as additional supporting data only. This study is not strictly required to address this data point as permethrin has been shown to be rapidly and strongly adsorped to a wide variety of different soil and sediment types, and from these to be not readily desorbed. Please refer to the applicants justification for Data Point A7.2.3.2 (Adsorption and mobility in soil, further studies). Indeed, in the adsorption/desorption study (Davis, 1991) K_{foc} values determined for the adsorption phase in five soil types ranged from 28200 to 194000, indicating considerable adsorption. Furthermore, in the water/sediment study conducted under aerobic conditions (Robinson and Ryan, 1996a) permethrin was observed to partition rapidly to the sediment phase by up to ~97.3% and ~97.7% for the acid- and alcohol-labelled permethrin, respectively, on Day 0. As a result permethrin remains in the water column for less than a day, owing to the rapid adsorption to sediment. Furthermore, supporting data from a field aquatic dissipation study (Hatfield, 1996) on two small pond systems at two trial locations in the USA supported the water/sediment study and indicated a similar rapid adsorption of permethrin to the sediment phase. It is considered unlikely that permethrin would reach groundwater under normal use conditions of this biocidal product and, therefore, poses a very limited contamination risk to groundwater.
	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 1:Classification and physico-chemical properties of soil

	Soil
Soil series	Thermic typic hapludalf
Classification	Silt-loam
Location	Mississippi
Horizon	30-90 cm
Sand [%]	23
Silt [%]	67
Clay [%]	10
Organic matter [%]	0.31
pH	6.8

Water movement was found to be very slow, because the clay content was found to be predominantly expanding lattice. Therefore builders sand was mixed with the soil to raise the sand content to 50%.

Figure 1: Distribution of *cis*-permethrin in soil columns as affected by anhydrous NH_3.

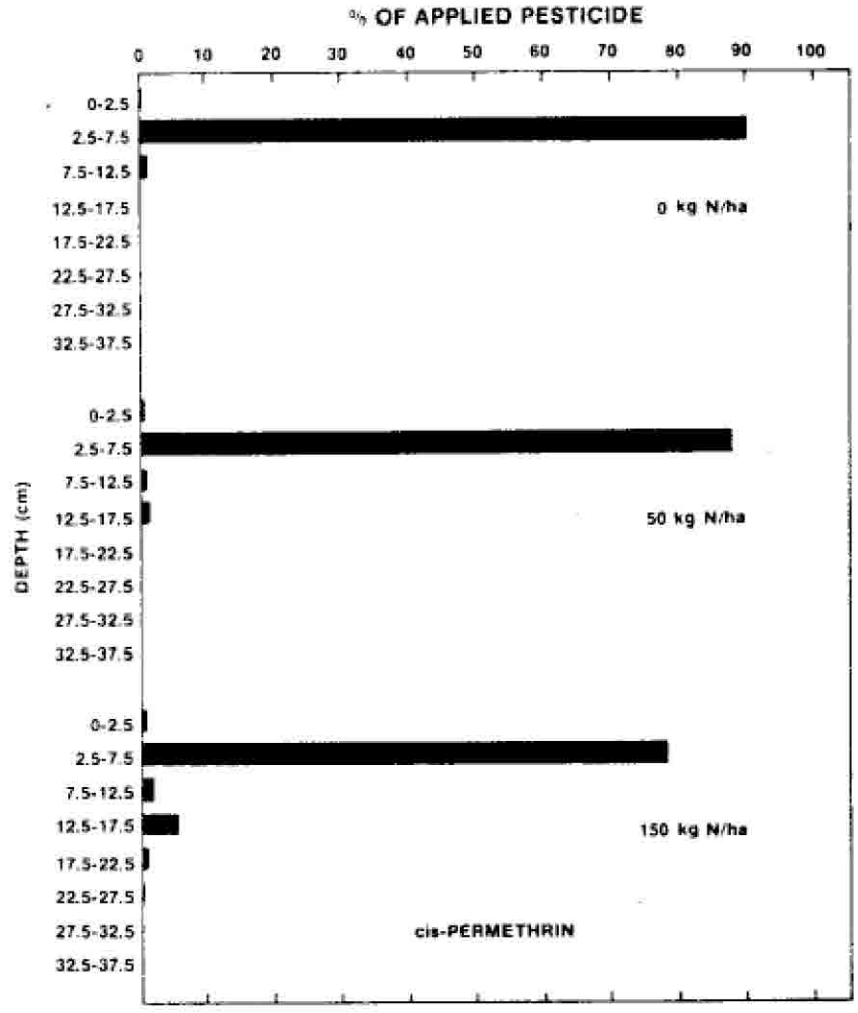

Fig. 2. Distribution of *cis*-permethrin in soil columns as affected by anhydrous NH_3.

Figure 2: Distribution of *trans*-permethrin in soil columns as affected by anhydrous NH_3.

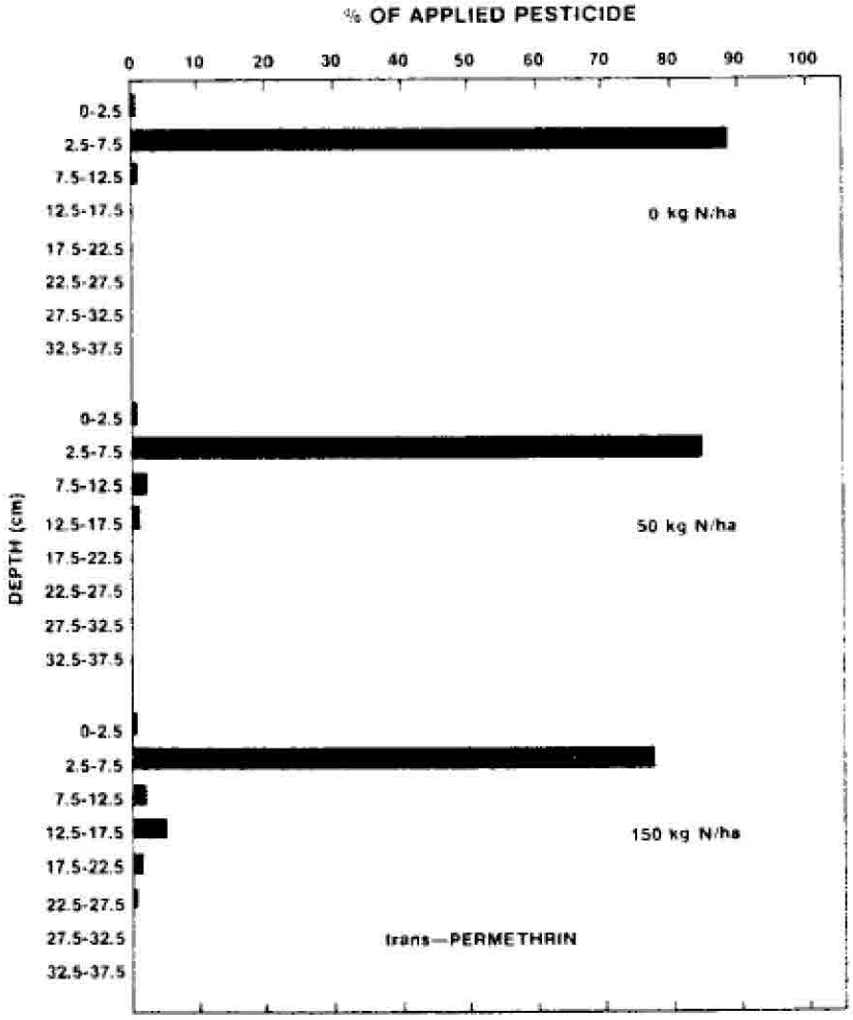

Fig. 3. Distribution of *trans*-permethrin in soil columns as affected by anhydrous NH_3.

Section A7.3.1	Phototransformation in air (estimation method)	
BPD Data set IIIA/ Annex Point VII.5	, including identification of breakdown products	
	Key Study	Official use only
	8 REFERENCE	
8.1 Reference	Hellpointer, E. (2007). Permethrin : Calculation of the chemical lifetime in the troposphere. BAYER CropScience AG, Report No.: MEF-07/395; September 17, 2007.unpublished	
8.2 Data protection	Yes	
8.2.1 Data owner	Bayer CropScience AG	
8.2.2 Companies with letter of access		
8.2.3 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	
	9 GUIDELINES AND QUALITY ASSURANCE	
9.1 Guideline study	No	
9.2 GLP	No, GLP is not relevant for a theoretical estimation method	
9.3 Deviations	Not relevant, as not a guideline study	
	10 MATERIALS AND METHODS	
10.1 Estimation procedure		
10.1.1 Estimation method	The Atmospheric Oxidation Program for Microsoft Windows (AOP version 1.4, US EPA) estimates the rate constant for the atmospheric gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. It also estimates the rate constant for the gas-phase reaction between ozone and olefinic/acetylenic compounds. The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radicals and ozone. AOPWINTM requires only a chemical structure and atmospheric concentrations of the potential reaction partners to make these predictions. The AOPWINTM estimation method adds up the partial reaction rates constant *ki* of the reaction of photochemically generated active species with subgroups of the test molecule (increments), resulting in the overall reaction rate. The following hydroxyl and/or ozone reactions are considered: • hydrogen abstraction • addition to double bonds • addition to triple bonds • reaction with N, S and –OH • addition to aromatic rings • addition to fused rings	X
10.2 Test performance	The accuracy of the method used by AOPWINTM was examined by comparison of estimated and experimentally determined hydroxyl	

Section A7.3.1	Phototransformation in air (estimation method)	
BPD Data set IIIA/ Annex Point VII.5	, including identification of breakdown products	

Key Study

radical rate constants. Over 90 percent of the estimated rate constants for 647 different chemicals were within a factor of two of the experiment value. Over 95 percent of the estimates were within a factor of three of the experimental.

11 RESULTS

11.1 Calculations

X

The overall hydroxyl radical reaction rate of permethrin as calculated by the model was 22.885 × 10^{-12} cm^3×$molecule^{-1}$×s^{-1}. The result was derived from increments such as hydrogen abstraction (1.9059 cm^3×$molecule^{-1}$× s^{-1}) and addition reaction to olefinic bond and aromatic rings (3.8323 and 17.1468 cm^3×$molecule^{-1}$×s^{-1}), respectively.

Based on the overall OH rate constant and using the "short term" daylight scenario (1.5x10^{+6} OH radicals/cm^3) half-life ($t_{1/2}$) and chemical lifetime (τ) were derived as follows:

- half-life ($t_{1/2}$) of permethrin in air = 0.47 days
- chemical lifetime (τ) of permethrin in air = 0.67 days

These estimates should be understood as worst-case assumptions which do not consider any contribution of attack by further reactive species other than hydroxyl radicals (i.e. by nitrate radicals). Whenever the active substance is applied during early afternoon (as opposed to early morning or late afternoon), it is to be expected that the chemical lifetime is shorter at that moment, since during the day the OH radical concentration in the troposphere may increase up to 5 x 10^{+6} radicals/cm^3.

11.2 Degradation product(s)

Not determined by this theoretical estimation method. However this is a standard and widely accepted method.

12 APPLICANT'S SUMMARY AND CONCLUSION

12.1 Materials and methods

Based on an estimation according to structure-activity relationship (SAR) methods developed by Roger Atkinson and co-workers, the half-life time in air of the fungicide active substance permethrin was assessed by the computer program AOPWINTM (v 1.91).

12.2 Results and discussion

X

A half-life time ($t_{1/2}$) of at the most 0.5 to 0.7 days was estimated, depending on the model input parameter of the mean concentration of hydroxyl radicals present in the troposphere ("short-term" scenario: typical OH radical concentration during daylight hours / "long-term" scenario: typical OH radical concentration averaged over day and night-times).

permethrin may be expected to be susceptible for reactions with hydroxyl radicals, which will contribute significantly to the overall degradation of the substance in the atmosphere. Various moieties of the molecule were identified as possible targets for radical reactions. Attack by hydroxyl radicals should result in the formation of multiple primary radicals. These may lead to secondary oxidation products, which can be eliminated from the air by wet and/or dry deposition.

Section A7.3.1	Phototransformation in air (estimation method)
BPD Data set IIIA/ Annex Point VII.5	, including identification of breakdown products

Key Study

A minor contribution to overall degradation was assessed by reaction with tropospheric ozone ($t_{1/2}$ = 49 days), only.

12.3 Conclusion	Concluded from the short half-life time of in air, it is to be expected that permethrin cannot be transported in gaseous phase over large distances and cannot accumulate in the atmosphere. Furthermore, only limited quantities of permethrin will enter the atmosphere, due to the low vapour pressure of the substance.
12.3.1 Reliability	1
12.3.2 Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2009
Materials and Methods	Applicant's version is acceptable with the addition of the following information. Section 10.1.1 The software used for the calculation was AOPWIN v1.91 (not version 1.4).
Results and discussion	Applicant's version is acceptable with the addition of the following information. Section 11.1 For gas-phase reaction with ozone, the atmospheric half-life is 49 days (based on an ozone concentration of 7 x 10^{11} molecules/cm^3 and a 24-hour day). The RMS evaluator has calculated an alternative atmospheric half-life for the gas-phase reaction of permethrin with hydroxyl radicals using the Technical Guidance Document assumptions of a 24-hour day and a hydroxyl radical concentration of 5 x 10^5 radicals/cm^3. This resulted in a value of 0.701 days (16.83 hours).
Conclusion	Applicant's version is acceptable with the addition of the following information. Section 12.2 An atmospheric half-life of 5.6 hours was calculated for the gas-phase reaction of permethrin with hydroxyl radicals, based on a hydroxyl radical concentration of 1.5 x 10^6 radicals/cm^3 and a 12-hour day (5.6 hours = 0.47 days). Using Technical Guidance Document assumptions of a 24-hour day and a hydroxyl radical concentration of 5 x 10^5 radicals/cm^3, an alternative value of 0.701 days (16.83 hours) was obtained.
Reliability	1
Acceptability	acceptable / ~~not acceptable~~

Section A7.3.1	Phototransformation in air (estimation method)
BPD Data set IIIA/ Annex Point VII.5	, including identification of breakdown products

	Key Study
Remarks	Section 8.2 The applicant states that the results are data protected but the study is only a calculation using publicly available software.
	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	

Section A7.3.2 Annex Point IIIA, VII.5	Fate and behaviour in air, further study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure** [X]	**Technically not feasible** [ ] **Scientifically unjustified** [ ] **Other justification** [ ]	
Detailed justification:	In view of the vapour pressure (2 µPa), Saturated Vapour Concentration (2.6×10^{-9} ppm) and Henry's Constant (1.87×10^{-6} atm-m^3/mole) of permethrin, losses to air will be negligible. Any atmospheric permethrin which might occur due to spraying will rapidly deposit onto the terrestrial environment. Permethrin has a high photolytic half-life in soil and water. Combining the low rate of phototransformation observed in other environmental matrices with the extremely low exposure and very short residence time in the atmosphere, a justification for non-submission of data is proposed on the basis that residence time will not be sufficient to allow phototransformation to occur. Furthermore, the technical aspects of designing a methodology which would maintain a suitable atmospheric concentration would make the assessment financially expensive in order to provide a value of no significance to the risk assessment.	X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
Date	**EVALUATION BY RAPPORTEUR MEMBER STATE** *20 April 2005*	
Evaluation of applicant's justification	The RMS considers that rationale of the applicant's justification is acceptable, but that a more detailed justification is required. Furthermore, a number of the values presented for vapour pressure and the Henry's Law constant do not match those in the submitted dossier. Permethrin is known to be resistant to photo-oxidation in the atmosphere, especially when considering the development of permethrin as one of the first photostable pyrethroids. Other reactions in the atmosphere such as dechlorination are considered to be of minor importance. In the context of field use permethrin is considered to be relatively resistant to photodegradation but it subject to facile photoisomerism in which photo-induced molecular species are formed. The vapour pressure of both the *cis*-isomer and *trans*-isomer of permethrin at 25 °C were determined to be 2.88×10^{-6} Pa and $<1.0 \times 10^{-7}$ Pa, respectively. These values indicate permethrin volatility for the pure pesticide to be classified as low or non-volatile according to a range of classification categories. An indicator of the tendancy for volatilisation from dilute aqueous solutions is the Henry's Law constant, which the applicant, using the EPIWIN model, calculated at 2.88×10^{-7} atm m^3 mol for permethrin. This value, also,	

Section A7.3.2 Annex Point IIIA, VII.5	Fate and behaviour in air, further study
Conclusion	indicates low/non-volatile nature of permethrin to the atmosphere, thus, limiting atmospheric exposure and any potential phototransformation. Furthermore, the high adsorption characteristics of permethrin to soil (mean K_{Foc} = 76880) and sediment would tend to reduce volatilisation loss to the atmosphere. Overall, combining the low rate of phototransformation observed in other environmental matrices [e.g. photohydrolysis study (Amos and Donelan, 1987) and soil photolysis study (Brown and Leahey, 1987)] with the extremely low exposure of permethrin to the atmosphere, a justification for non-submission of data is proposed on the basis that residence time will not be sufficient to allow phototransformation to occur. The applicant's justification is considered acceptable, with the following additions to the justification as outlined by the RMS.
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	

Appendix 1 to Doc III-A7

Bayer Environmental Science is a an affiliated company of Bayer CropScience, therefore the studies submitted by Bayer Environmental Science are owned by Bayer CropScience AG.

Reference List Doc. III-A7. sorted by reference no.

Reference No	AUTHOR (S)	Year	Title. Source, Report No. Glp /(Un) Published	Data Protection Claimed (Yes/No)	Owner
7,1,1,1,1(1)	Alvarez, M. & Dziedzic, J.E.	1977	Hydrolysis of FMC 33297. FMC Corporation. Report No. CGP-77-12; Not GLP; Unpublished	Yes	Sumitomo Chemical
7,1,1,1,1(2)	Allsup, T.L. & Russell, K. H.	1976	Hydrolysis of FMC 33297 Insecticide. FMC Corporation. Report No. W-0103; Not GLP; Unpublished	Yes	Sumitomo Chemical
7,1,1,1,2	Amos, R. and Donelan, R. B.	1987	Permethrin: Photolysis in sterile water at pH5. Report No. RJ0577B, 15 June 1987; Not GLP; Unpublished	Yes	Syngenta
7,1,1,1,2	Buerkle, L.W	2007	Calculation of the Environmental Photolysis Half Lives for permethrin, Report number MEF-07/414, 2007-09-21	Yes	Bayer CropScience AG
7,1,1,2,1	Mead, C	2004	Permethrin: Assessment of Ready Biodegradability; Manometric Respirometry Test; 1430/018;July 2004;GLP; Unpublished	Yes	Sumitomo Chemical
7,1,1,2,1	Caplan, J.A., Isbister, J	1979	14C-permethrin (Acid and Alcohol label) Activated Sludge Metabolism; Biospherics Incorporated; 9PL-7-SL; 30 April 1979	Yes	Syngenta
7,1,2,2,2(1)	Robinson, R.A & Ryan, J.E.	1996a	Aerobic aquatic metabolism of [14C]Permethrin. XenoBiotic Laboratories, Inc., Plainsboro, NJ. report Ref. Study No. XBL94092, Report Ref. RPT00220.;GLP; Unpublished	Yes	Bayer CropScience AG
7,1,2,2,2(2)	Robinson, R.A & Ryan, J.E.	1996b	Anaerobic aquatic metabolism of [14C]Permethrin. XenoBiotic Laboratories, Inc., Plainsboro, NJ. report Ref. Study No. XBL94091, Report Ref. RPT00252; GLP; Unpublished	Yes	Bayer CropScience AG
7,1,4	Hatfield, M.W.	1996a	Aquatic dissipation of permethrin in California and North Carolina. American Agricultural Services Report on Study No. AA940907; GLP; Unpublished	Yes	Bayer CropScience AG
7,2,1	Hawkins, D.R.	1992	The aerobic soil metabolism of 14C - Permethrin. Report number HRC/ISN 251/911499; GLP; Unpublished	Yes	Syngenta
7,2,2,1(1)	Hawkins, D.R.	1992	The aerobic soil metabolism of 14C - Permethrin. Report number HRC/ISN 251/911499; GLP; Unpublished	Yes	Syngenta
7,2,2,1(2)	Kaufman, D.D., Clark Haynes; S., Jordan, E.G, Kayser, A.J.	1978	Permethrin Degradation in Soil and Microbial Cultures. In Synthetic Pyrethroids; Not GLP; Published	No	N/A
7,2,2,1(3)	Allen R	2007	Permethrin: calculation of DT50 in aerobic soil. Report No. MEF-07/421 , September, 2007; Not GLP; Unpublished	Yes	Bayer CropScience AG

7,2,2,1(4)	Schäfer, D. and Mikolasch, B.	2004	Kinetic Evaluation of Soil Laboratory Studies with Deltamethrin and its Metabolites D-COOH, Br2CA and mPBacid to Determine Input Parameters for Model Calculations Bayer CropScience AG, Germany Document; C044585 8 October 2004; Unpublished	Yes	Bayer CropScience AG
7,2,2,1(5)	Sakata, S., Mikami, N., Yamada, H.	1992	Degradation of Pyrethroid Optical Isomers in Soil. J. Pesticide. Sci. 17, 169-180; Not GLP; Published	No	N/A
7,2,2,3	Sakata, S., Mikami, N., Yamada, H.	1992	Degradation of Pyrethroid Optical Isomers in Soil. J. Pesticide. Sci. 17, 169-180; Not GLP; Published	No	N/A
7,2,2,4	Brown, P.M and Leahey, J.P.	1987	Permethrin: Photolysis on a soil surface. Report No. RJ0581B, 29 April 1987; Not GLP; Unpublished	Yes	Syngenta
7,2,3,1(1)	Davis, M. L.	1991	Sorption/Desorption of 14C-Permethrin on Soils by the Batch Equilibrium Method. Battelle Memorial Institute. Report No. Sc900199; GLP; Unpublished	Yes	Sumitomo Chemical
7,2,3,1(2)	Reynolds, J.L.	1992	Adsorption and Desorption of 14C-m-Phenoxybenzoic Acid in Four Soils; XenoBiotic Laboratories Inc, USA; Document A71037 ; 18 November 1992; Unpublished	Yes	Bayer CropScience AG
7,2,3,1(3)	Slangen, P	1999	Adsorption/desorption of FCR 1272-permethric acid on soil, NOTOX B.V.Hambakenwetering 3, 5231 DD `s-Hertogenbosch, The Netherlands.Bayer AG, Bayer Report No.: IM 1983, BES Ref: M-015423-01-1.Report date: 30 August 1999Unpublished	Yes	Bayer CropScience AG
7,2,3,2	Smith, S. & Willis, G.H.	1985	Movements of pesticides in soil columns as affected by anhydrous ammonia. Env. Tox. Chem, 4, 425-434; Not GLP; Published	No	N/A
7,3,1	Hellpointer, E.	2007	Permethrin : Calculation of the chemical lifetime in the troposphere. BAYER CropScience AG, Report No.: MEF-07/395 September 17, 2007.unpublished	Yes	Bayer CropScience AG

Competent Authority Report

Programme for Inclusion of Active Substances in Annex I to Council Directive 98/8/EC

Permethrin (PT 8)

CAS-No. 52645-53-1

DOCUMENT IIIA (A7)

Bayer Environmental Science

Sumitomo Chemicals (UK) Plc.

Rapporteur: Ireland

March 2011

Permethrin PT8

Document IIIA (A7) (A7.4 – A7.5.7.1.3)

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Section A7.4 Effect on aquatic organism

Annex Point IIA7.2

Probabilistic risk assessment of cotton pyrethroids: I. Distributional Analyses of laboratory Aquatic Toxicity Data

Additional information

Official use only

		1 REFERENCE
1.1	**Reference**	Soloman, K.R., Giddings, J.M. and Maund, J. 2001 Environmnetal Toxicology and Chemistry ,Vol 20, No.3 652-659. Not GLP
1.2	**Data protection**	Yes
1.2.1	**Data owner**	SETAC
1.2.2	**Criteria for data protection**	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No
2.2	**GLP**	No
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Pyrethroids, with cypermethrin as the representative compound
3.1.1	**Lot/Batch number**	Not reported
3.1.2	**Specification**	Not reported
3.1.3	**Purity**	Not reported
3.1.4	**Composition of Product**	Not applicable
3.2	**Reference substance**	No
3.3	**Testing procedure**	
3.3.1	**Test organisms**	Arthropod invertebrates and fish
3.3.2	**Test parameter**	Toxicity
		4 RESULTS
4.1	**Limit Test**	Not performed
4.2	**Results test substance**	
4.2.1	**Effect data**	Synthetic are highly toxic to insects ads well as some aquatic orgnanisms, especially aquatic insects.
4.3	**Results of controls**	No observed effects
4.4	**Test with reference**	Not performed

Section A7.4 **Effect on aquatic organism**

Annex Point IIA7.2 **Probabilistic risk assessment of cotton pyrethroids: I. Distributional Analyses of laboratory Aquatic Toxicity Data**

		Additional information
	substance	
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	Materials and methods	The method followed no specific test guidelines. The basic approach to characterizing the toxicity of each of the pyrethroids was to compile all of the available data for aquatic species into a cumulative frequency distribution.tor the purposes of characterixing the toxicity profile , the diftribution was described by a linear regression of the log-probability –transformed data. THE acute toxicity data consisted of LD50 and EC50 measuremetns. THE EC values in aquatic arthropods and insects are normally considered equivalent to LC values because the assay endpoint is immobility, an endpoint that either is or will lead to death.
5.2	Results and discussion	Cypermethrin gave a 10^{th} centile intercept of 10 ng/L when all organisms were considered. The arthropods only group gave a 10^{th} centile intercept of 6.4 ng/L , whereas the, for vertebrates the intercept was 380 ng/L . Permethrin toxicity to all organisms and toxicity to and vertebrates gave 10^{th} centrile intercepts of 180, 76, and 1,600 ng/L respectively.
5.3	Conclusion	Toxicity values were fairly similar for the aquatic invertebrates. Fish however displayed a higher tolerance to the pyrethroids.
5.3.1	Reliability	1
5.3.2	Deficiencies	None.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	11/05/05
Materials and Methods	The omission of sample analysis is noted however in general materials and methods were adequate.
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	2
Acceptability	acceptable
Remarks	None
	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	

Section A7.4.1.1 **Acute toxicity to fish**
Annex Point IIA7.1

Key Study

Official use only

1 REFERENCE

1.1 Reference	[redacted]; 1978c; Determination of the Acute Toxicity of Compound 21z (WRL) to Rainbow Trout (Salmo gairdneri) Using Dimethyl Sulphoxide as the Solvent. [redacted]; Not GLP; Unpublished Analytical report : [redacted]; Analysis of test concentrations of. the compound 21Z (Wellcome Research Laboratories) in acute toxicity flow-through tests with Bluegill Sunfish (*Lepomis macrochirus*) and Rainbow Trout (*Salmo gairdneri*) using dimethyl sulphoxide as a solvent aid.. [redacted], unpublished
1.2 Data protection	Yes
1.2.1 Data owner	Sumitomo Chemical (UK) PLC
1.2.2 Companies with letter of access	Bayer Environmental Science
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

1 GUIDELINES AND QUALITY ASSURANCE

Guideline study	No - testing pre-dates guidelines
GLP	No - GLP was not compulsory at the time the study was performed
Deviations	No - No guidelines were followed

2 MATERIALS AND METHODS

Test material	As given in section 2
Lot/Batch number	Ref No. ex lot ZJ
Specification	As given in section 2
Purity	94.5% w/w
Composition of Product	Not applicable
Further relevant properties	Very low water solubility
Method of analysis	Gas chromatography
Preparation of TS solution for poorly soluble or volatile test substances	A 1 mg ml^{-1} solution of permethrin was prepared in dimethylsulphoxide. This was then diluted with water to form stable intermediate stock solutions
Reference substance	No
Method of analysis	Not applicable

Section A7.4.1.1 Annex Point IIA7.1	**Acute toxicity to fish**
for reference substance	
Testing procedure	The test was run in two series to minimise the chance of a single bias in the test design. Series 1 was 6/10/77 to 12/10/77, Series 2 was 17/10/77 to 21/10/77.
Dilution water	see table A7_4_1_1-2
Test organisms	see table A7_4_1_1-3
Test system	see table A7_4_1_1-4
Test conditions	see table A7_4_1_1-5
Duration of the test	96 hours
Test parameter	Mortality
Sampling	24, 48, 72, 96 hours
Monitoring of TS concentration	Yes
Statistics	A dose response graph was plotted and LC50 values with their respective 95% confidence intervals were derived using Probit analysis.
	3 RESULTS
Limit Test	Not performed
Results test substance	
Initial concentrations of test substance	**Series 1 & 2 had a solvent control plus;** **Series 1**: Nominal 0.0010, 0.01, 0.018, 0.032, 0.042, 0.056 mg l^{-1} **Series 2**: Nominal 0.0032, 0.0056, 0.0075 mg l^{-1}
Actual concentrations of test substance	see table A7_4_1_1-8
Effect data (Mortality)	see table A7_4_1_1-6 see table A7_4_1_1-7
Concentration / response curve	See Figure 1
Other effects	**Series 1**: Jaw spasms were observed in the 0.056 mg l^{-1} exposure after 2 hours and 0.032 mg l^{-1} after 5 hours. The 0.001 mg l^{-1} showed no effect after 96 hours exposure. **Series 2**: After 72 hours the 0.0075 mg l^{-1} had intermittent loss of equilibrium, and 1 death occurred after 78 hours. At 96 hours the 0.0075 mg l^{-1} had a total of 3 deaths and the 0.0032 mg l^{-1} showed no effect.
Results of controls	
Number/ percentage of animals showing	None

Section A7.4.1.1 **Acute toxicity to fish**
Annex Point IIA7.1

adverse effects	
Nature of adverse effects	None observed
Test with reference substance	Not performed

4 APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods	No test guidelines were followed. However, the methodology employed a flowthrough system with delivery via a carrier solvent, DMSO. To allow for one-off bias within the methodology, the test was run over two time periods, with the concentration range being spread between the two stages. 20 fish were tested per concentration, and observations were made and recorded at regular intervals. Because the test was performed before current reporting standards, much of the specific data relating to current guidelines have not been reported, for example photoperiod. This lack of data is not thought to affect the overall reliability of the data, since it presents results broadly in line with the existing dataset on permethrin toxicity to aquatic vertebrate species. Test solutions were analysed sporadically.
Results and discussion	The mortality and symptoms indicate a 96 hour LC50 of 0.0090 mg l^{-1} (9 µg l^{-1}) and an LC0 of 0.0056 mg l^{-1}. The NOEC for the test was reported as 0.0032 mg l^{-1}. These results are consistent with the known effects of pyrethroids, in that they display a very steep lethality curve. The analysis of permethrin in the test solutions was not as standard as would be expected in more recent tests. The results presented in Table A7_4_1_1-8 show that the sampling pattern was non-uniform and conclusions may be hard to draw. Where measurements were taken throughout the exposure period indicate stable delivery of the test article, at somewhere between 40 – 72% of nominal concentration. It had been demonstrated in the laboratory that the concentration of permethrin in dilute aqueous solution may diminish significantly by adsorption on to glass surfaces. It might be expected, therefore, that similar adsorptive losses would occur after 48 hours in a static bioassay test situation, and this may be the explanation of the lower percent of nominal figures (see point 7.4.1.2 (2), Analytical report : Bowles F.P.). It is considered appropriate to adjust the derived LC_{50} values by a suitable percentage recovery. 57% recovery is chosen since it is based upon measurements taken at 4 time points during the study at a nominal concentration (0.018 mg/L), near the nominal LC50 (0.009 mg/L). Therefore, the proposed LC_{50} is 5.13 µg/L (9x57%) based on mean

Section A7.4.1.1 Annex Point IIA7.1	**Acute toxicity to fish**
	measured concentration.
LC_0	0.0056 mg l^{-1} (5.6 µg l^{-1})
LC_{50}	0.0090 mg l^{-1} (9.0 µg l^{-1}) mean measured concentration: 0.0051 mg l^{-1} (5.1 µg l^{-1})
LC_{100}	0.032 mg l^{-1} (32 µg l^{-1})
Conclusion	The validity criteria with respect to control organism mortality and DO concentration can be considered achieved. With respect to test concentration delivery, within the strict limits defined, the test can be considered to have failed these quality criteria. However, where analysis was undertaken it can be shown that delivery was fairly consistent (78, 100, 129% of starting concentration, where measured, assuming 24 hour measurement to be 'starting' concentration). It had been demonstrated in the laboratory that the concentration of permethrin in dilute aqueous solution may diminish significantly by adsorption on to glass surfaces. It might be expected, therefore, that similar adsorptive losses would occur after 48 hours in a static bioassay test situation, and this may be the explanation of the lower percent of nominal figures (see point 7.4.1.2 (2), Analytical report : Bowles F.P.). The steep dose-response is typical of those observed for pyrethroid chemicals which tend to have a very defined cut-off concentration for effect. The LC100 is 32 µg l^{-1}, however the 10 µg l^{-1} concentration produced 90% mortality, and the LC0 was 5.6 µg l^{-1}, less than half that concentration which produced 90%.
Other Conclusions	The results of this test show good comparability with the wider data set for permethrin available in the public domain.
Reliability	2
Deficiencies	Yes – analysis of the test solutions appeared to give low but consistent recoveries. A discussion in the analytical report mentions a cloudy appearance in the mixing chambers, implying a true solution was difficult to achieve. However, the consistent low results indicate delivery was stable and achievable. The reporting was not up to the standards expected of more recent reports, and several data, for example photoperiod, are not reported.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21/4/05*
Materials and Methods	Applicant's version is acceptable despite the fact that no sprecific guideline was used in the study. Applicant has addressed any discrepancies above.
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	2
Acceptability	Acceptable – see remarks below.
Remarks	Analysis of the test solutions appeared to give low but consistent recoveries. A discussion in the analytical report mentions a cloudy appearance in the mixing chambers, implying a true solution was difficult to achieve. However, the consistent low results indicate delivery was stable and achievable. Also, the reporting was not up to the standards expected of more recent reports, and several data, for example photoperiod, are not reported. In general, adequate information was provided to assess the quality of the study and the Notifiers have attempted in all cases to address the discrepancies adequately. As such, the study is considered acceptable.
	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_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes - DMSO
Concentration of vehicle	0.1% v/v
Vehicle control performed	Yes
Other procedures	Tests run in two series to minimise the chance of a single bias in the test design.

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	90,000 Reservoir
Alkalinity	Not reported
Hardness	50.5 - 52.5 mg/l as $CaCO_3$
pH	Between 7.60 – 7.70 throughout the duration of the exposure periods (Series 1 & 2)
Oxygen content	86 - 90% of saturation throughout the duration of the exposure periods (Series 1 & 2)
Conductance	Not reported
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	*Oncorhynchus mykiss*
Source	Samaaki trout farms, [redacted]
Wild caught	No
Age/size	Mean weight 3.9g, mean length 63 mm
Kind of food	Not reported
Amount of food	Not reported
Feeding frequency	Not reported
Pretreatment	Test apparatus was run with dosing solutions but without fish exposure for 48 hours prior to the introduction of the test organisms.
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	200ml/min
Volume of test vessels	20L
Volume/animal	1L
Number of animals/vessel	20

Number of vessels/ concentration	One
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	**Details**
Test temperature	At no time did the measured temperature deviate from the nominal 12 ± 0.5°C
Dissolved oxygen	Dissolved oxygen was measured daily in each vessel. At no time did the levels fall below 86%
pH	7.6 - 7.7
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Not reported
Photoperiod	Not reported

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal)[1] [mg l^{-1}]	**Mortality (out of 20)**							
	Number				**Percentage**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
0.056	20	20	20	20	100	100	100	100
0.042	16	20	20	20	80	100	100	100
0.032	15	20	20	20	75	100	100	100
0.018	11	16	17	19	55	80	85	95
0.010	1	10	16	18	5	50	80	90
0.0075	0	0	0	3	0	0	0	15
0.0056	0	0	0	0	0	0	0	0
0.0032	0	0	0	0	0	0	0	0
0.0010	0	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0	0
Temperature [°C]	12 ± 0.5°C	12 ± 0.5°C	12 ± 0.5°C	12 ± 0.5°C				
pH	7.60 – 7.70	7.60 – 7.70	7.55 – 7.65	7.60 – 7.65				
Oxygen [% air sat^{n}]	87 - 96	88 - 91	88 - 91	88 - 91				

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	**95 % c.l.**	**96 h [mg/l][1]**	**95 % c.l.**
LC_0	0.0075 (n)	-	0.0056 (n)	-
LC_{50}	0.0121 (n)	010106 – 010138	0.0090 (n)	0.0073 – 0.0124
C_{100}	0.032 (n)	-	0.032 (n)	-

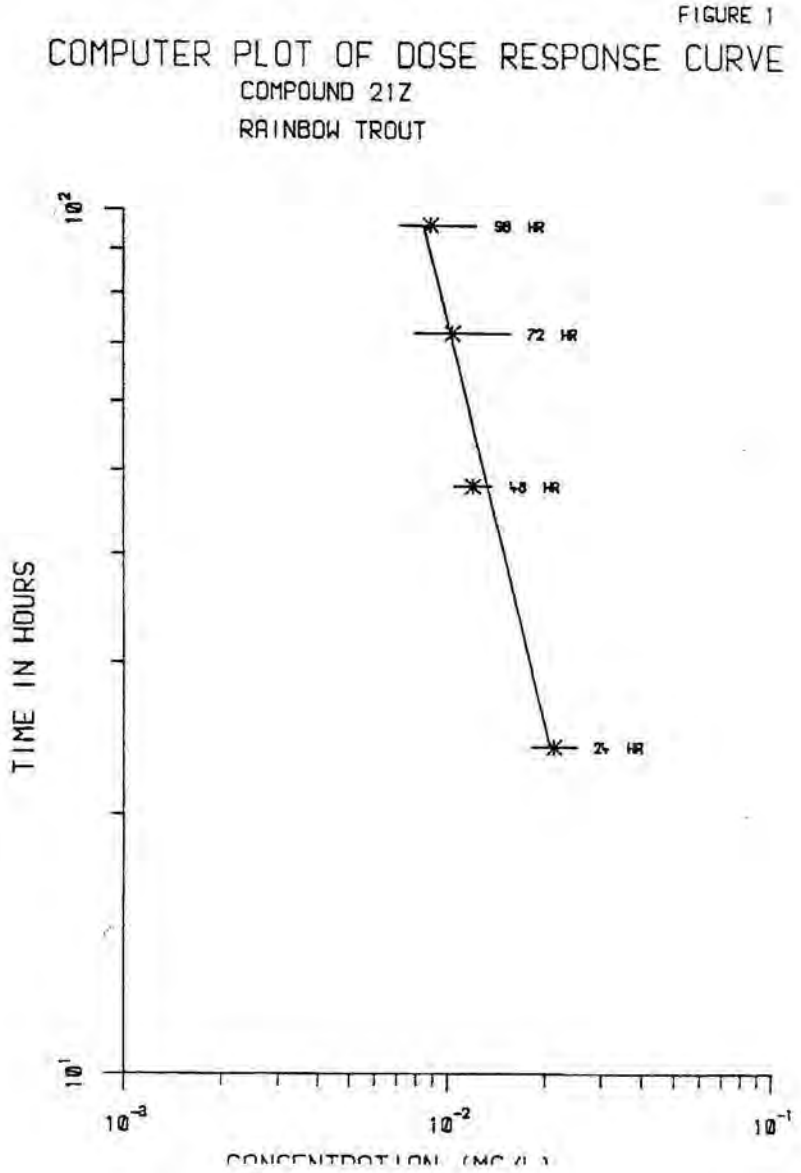
Table A7_4_1_1-8: Measured concentrations

Series	*Nominal conc* ($\mu g\ l^{-1}$)	Measured concentration ($\mu g\ l^{-1}$)					Mean Measured concentration (% nominal)
		24 h	48 h	72 h	96 h	Mean	
1, 2	Sol Con	<0.20	<0.20	<0.20	<0.20	<0.20	-
1	10	-	7.2[a]	-	-	-	72
1	18	11.4[a]	10.0[a]	10.5[a]	8.9[a]	10.2	56
1	32	21.3[a]	-	-	-	21.3	67
1	42	24.8[a]	-	-	-	24.8	59
1	56	-	-	-	-	-	-
2	3.2	2.0[a]	-	2.5[a]	2.0[a]	2.2	63
2	5.6	2.4[a]	1.7[a]	3.2[a]	3.1[a]	3.5	63
2	7.5	2.6	3.3[a]	-	-	3.0	40

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	X	
Concentration of dissolved oxygen in all test vessels > 60% saturation	X	
Concentration of test substance ≥80% of initial concentration during test		X

Figure 1: Dose-response curve



Section A7.4.1.1(2)
Annex Point IIA7.1

Acute toxicity to fish, metabolites

Key Study

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1984), Acute toxicity of dichlorovinylcarboxylic acid to rainbow trout., [redacted]; Report date : 7 September 1984 unpublished
1.2	**Data protection**	Yes
1.2.1	**Data owner**	Bayer CropScience AG
1.2.2	**Companies with letter of access**	
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.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No, in essence method complied to EPA - FIFRA § 72-1, EEC/C.1. and OECD 203
2.2	**GLP**	Yes
2.3	**Deviations**	None
		3 MATERIALS AND METHODS
3.1	**Test material**	Dichlorovinylcarboxylic acid
3.1.1	**Lot/Batch number**	150-6-52
3.1.2	**Specification**	Not relevant, metabolite testing
3.1.3	**Purity**	99.9 %
3.1.4	**Composition of Product**	Not relevant, metabolite testing
3.1.5	**Further relevant properties**	none
3.1.6	**Method of analysis**	Not stated
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Dichlorovinylcarboxylic acid was dissolved in dimethyl formamide (DMF). See table A7.4.1.1(2)-1
3.3	**Reference substance**	**None.**
3.3.1	**Method of analysis for reference substance**	**Not applicable.**
3.4	**Testing procedure**	
3.4.1	**Dilution water**	dechlorinated tap water: see table A7.4.1.1(2)-2

Section A7.4.1.1(2)
Annex Point IIA7.1

Acute toxicity to fish, metabolites

Key Study

3.4.2	**Test organisms**	Rainbow trout obtained from [redacted]. see table A7.4.1.1(2)-3
3.4.3	**Test system**	see table A7.4.1.1(2)-4
3.4.4	**Test conditions**	Under static conditions, see table A7.4.1.1(2)-5
3.4.5	**Duration of the test**	**96 h.**
3.4.6	**Test parameter**	**Mortality and signs of intoxication**
3.4.7	**Sampling**	The fish were observed daily for mortality and signs of intoxication.
3.4.8	**Monitoring of TS concentration**	No
3.4.9	**Statistics**	Limit test with no observed effects, no statistics performed
		4 RESULTS
4.1	**Limit Test**	Performed
4.1.1	**Concentration**	14.7 ppm
4.1.2	**Number/ percentage of animals showing adverse effects**	0
4.1.3	**Nature of adverse effects**	no adverse effects observed
4.2	**Results test substance**	
4.2.1	**Initial concentrations of test substance**	control, 14.7 mg test substance/1.(nominal concentration) *(*Range finding concentration: 0.001,0.01, 0.1, 1.0, 10.0 mg test substance/1*)*
4.2.2	**Actual concentrations of test substance**	no analytical confirmation of the test concentration
4.2.3	**Effect data (Mortality)**	No mortality were recorded. See table A7.4.1.1(2)-6 and 7
4.2.4	**Concentration / response curve**	none
4.2.5	**Other effects**	none
4.3	**Results of controls**	
4.3.1	**Number/ percentage of animals showing adverse effects**	No mortality or signs of intoxication were recorded.
4.3.2	**Nature of adverse effects**	Not relevant
4.4	**Test with reference substance**	Not performed

Section A7.4.1.1(2)
Annex Point IIA7.1

Acute toxicity to fish, metabolites

Key Study

4.4.1	**Concentrations**	Not relevant
4.4.2	**Results**	Not relevant
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	An acute toxicity study was performed with dichlorovinylcarboxylic acid (DCVA) on Rainbow Trout under static conditions. A single concentration of 14.7 ppm plus a water control group were used after a pilot study showed no toxicity at up to 10 mg/l
5.2	**Results and discussion**	The single 14.7 ppm concentration of dichlorovinylcarboxylic acid produced no signs or mortality in the test species. Therefore, the 96-hour LC and the lowest lethal concentration were greater than 14.7 mg/l. The no-observed effect concentration was 14.7 mg/l.
5.2.1	**LC_0**	14.7 mg/l
5.2.2	**LC_{50}**	14.7 mg/l
5.2.3	**LC_{100}**	
5.3	**Conclusion**	Up to a tested concentration of 14.7 mg/l no signs of intoxication were observed
5.3.1	**Other Conclusions**	Although with deficiencies to nowadays technical standard the study is considered meaningful within the context of the risk of permethrin to the aquatic environment. DCVA is by far less toxic than the parent compound permethrin.
5.3.2	**Reliability**	2-3
5.3.3	**Deficiencies**	Concentration higher than 14.7 mg/l, eg. 100 mg/l, indicating absence of toxicity , was not tested. Nominal concentrations were not analytically confirmed.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*12/1/09*
Materials and Methods	*Applicants version is acceptable.*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	*2-3*
Acceptability	*acceptable as a limit test*
Remarks	*Only a single dose (14.7 mg/L) was tested which did not induce any toxicity, and nominal concentrations were not analytically confirmed.*
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A7.4.1.1(2) Annex Point IIA7.1	**Acute toxicity to fish, metabolites**
	Key Study
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	No
Vehicle	dimethyl formamide (DMF)
Concentration of vehicle	2.2 ml of DMF in 19 litres jar
Vehicle control performed	No
Other procedures	None

Table A7.4.1.1(2)-2: Dilution water

Criteria	Details
Source	Johnson County Rural Water District No. 2
Alkalinity	64 mg/l, as $CaCO_3$
Hardness	170 mg/l, as $CaCO_3$
pH	7.3 to 7.9
Oxygen content	0.5 and 9.1 mg/l
Conductance	none
Holding water different from dilution water	No

Table A7.4.1.1(2)-3: Test organisms

Criteria	Details
Species/strain	Rainbow Trout (*Oncorhynchus mykiss*)
Source	[redacted]
Wild caught	No
Age/size	mean body weight was 2.1 g.
Kind of food	Ralston Purina trout chow
Amount of food	no feeding
Feeding frequency	none
Pretreatment	Acclimation to the test temperature for at least five days
Feeding of animals during test	No

Table A7.4.1.1(2)-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	Not stated
Volume of test vessels	19 liters glass jar
Volume/animal	1.5 l per animal
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.1(2)-5: Test conditions

Criteria	Details
Test temperature	11 to 11.9°C
Dissolved oxygen	0.5 to 9.1 mg/l
pH	7.3 to 7.9
Adjustment of pH	No
Aeration of dilution water	Not stated
Intensity of irradiation	Not stated
Photoperiod	16 hours light daily

Table A7.4.1.1(2)-6: Mortality Data: main test

Test-Substance Concentration (nominal) [mg/l]	**Mortality**							
	Number				**Percentage**			
	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	0/0	0/0	0/0	0/0
14.7	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

Table A7.4.1.1(2)-7: Effect data

nominal	48 h [mg/l][1]	96 h [mg/l][1]
LC_0	>14.7 (n)	>14.7 (n)
LC_{50}	>14.7 (n)	>14.7 (n)
LC_{100}	none	none

[1] indicates if effect data are based on nominal (n) or measured (m) concentrations

Table A7.4.1.1(2)-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	**Y**	
Concentration of dissolved oxygen in all test vessels > 60% saturation		

Concentration of test substance ≥80% of initial concentration during test		**x**

The test substance is soluble.

Criteria for poorly soluble test substances		

Section A7.4.1.2(1) Acute toxicity to invertebrates – *Daphnia magna*

Annex Point IIA7.2

Key study

		1 REFERENCE	Official use only
1.1	Reference	Thompson R.S. and Williams T.D; 1978; Determination of the acute toxicity of compound 21Z (WRL) to daphnia magna using acetonr as the solvent. Wellcome . Report No. HEFG 78-10; Unpublished Analytical report : Bowles F.P.; Analysis of the test compound Z21 (Wellcome Research Laboratories) in acute toxicity study static tests with daphnia magna. ICI Brixham Laboratory BL/B/1897, unpublished.	
1.2	Data protection	Yes	
1.2.1	Data owner	Sumitomo Chemical (UK) PLC	
1.2.2	Companies with letter of access	Bayer Environmental Science	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	No	
2.2	GLP	No	
2.3	Deviations	Yes – Analysis was performed but not reported	
		3 MATERIALS AND METHODS	
3.1	Test material	As given in section 2	
3.1.1	Lot/Batch number	Ex Lot ZJ	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	94, 5 % w/w	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Low water solubility	
3.1.6	Method of analysis	Fresh water sample were analysed by GC equipped with electron capture detection system after extraction with hexane	
3.2	Preparation of TS solution for poorly soluble or volatile test substances	see table A7.4.1.2(1)-1	
3.3	Reference substance	No	
3.4	Testing procedure		

Section A7.4.1.2(1) Acute toxicity to invertebrates – *Daphnia magna*

Annex Point IIA7.2

		Key study
3.4.1	**Dilution water**	Reconstituted dilution water, see table A7.4.1.2(1)-2
3.4.2	**Test organisms**	Daphnia magna, see table A7.4.1.2(1)-3
3.4.3	**Test system**	see table A7.4.1.2(1)-4
3.4.4	**Test conditions**	see table A7.4.1.2(1)-5
3.4.5	**Duration of the test**	48 hours
3.4.6	**Test parameter**	Immobility
3.4.7	**Sampling**	Water samples were taken at 0 and 48 hours
3.4.8	**Monitoring of TS concentration**	Yes
3.4.9	**Statistics**	Probit analysis
		4 RESULTS
4.1	**Limit Test**	Not performed
4.2	**Results test substance**	
4.2.1	**Initial concentrations of test substance**	nominal concentrations : control, solvent-control, 0.00032, 0.00056, 0.0010, 0.0018, 0.0032, 0.0056, 0.010, 0.018, 0.032 and 0.056 mg permethrin/L
4.2.2	**Actual concentrations of test substance**	Mean measured at 0 hours : 0.00039, 0.00053, 0.0008, 0.00157, 0.00245, 0.00416, 0.00874, 0.0154, 0.0322, 0.0623 mg permethrin/L Control, solvent below the limit of determination. Mean measured at t 48 hours : 0.0004, 0.00058, 0.00044, 0.00075, 0.00125, 0.00226, 0.00438, 0.01192, 0.0182, 0.035 mg permethrin/L. see table A7.4.1.2(1)-6
4.2.3	**Effect data (Immobilisation)**	The mortality data as numbers of immobile Daphnia and as percent of exposed animals are given in Table A7.4.1.2(1)-7 The EC50 values for 24 and LC50 for 48 h are given in Table A7.4.1.2(1)-8 based on calculation provided in annex I.
4.2.4	**Concentration / response curve**	See Figure 1 and 2
4.2.5	**Other effects**	No other effects observed
4.3	**Results of controls**	No observed effects
4.4	**Test with reference substance**	Not performed
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and**	Daphnia (<24 hours old) were exposed to permethrin for 48 hours at

Section A7.4.1.2(1) Acute toxicity to invertebrates – *Daphnia magna*

Annex Point IIA7.2

		Key study
	methods	the following nominal test concentrations range from 0.00056 to 0.010 mg/l. A dilution water control and solvent control were also included in the study. The concentration of the test substance was analysed at day 0 and at the termination of the study.
5.2	**Results and discussion**	The immobilisation data indicate a 48 hour EC_{50} of 0.002 mg /L (nominal) corresponding to EC_{50} of 0.00127 mg /L (mean measured concentrations). There were no mortalities or adverse effects observed with the solvent control or control (water only) Daphnia. Mean concentrations were between 39 and 125% of nominal values. The overall recovery was 62%
5.2.1	**EC_0**	0.000018 mg l^{-1} (0.018 µg l^{-1}) based on mean measured concentrations
5.2.2	**EC_{50}**	0.00127 mg l^{-1} (1.27 µg l^{-1}) based on mean measured concentrations
5.2.3	**EC_{100}**	0.9895 mg l^{-1} (989.5 µg l^{-1}) based on mean measured concentrations
5.3	**Conclusion**	The estimated 48 hour LC_{50} value was 0.00127 mg l^{-1} (1.27 µg l^{-1}) based on mean measured concentrations for Daphnia exposed to permethrin
5.3.1	**Reliability**	1
5.3.2	**Deficiencies**	Validity criteria : See table A7.4.1.2(1)-9 Justification on the concentration It had previously been demonstrated in the laboratory that the concentration of permethrin in dilute aqueous solution may diminish significantly by adsorption on to glass surfaces. It might be expected, therefore, that similar adsorptive losses would occur after 48 hours in a static bioassay test situation, and this may be the explanation of the lower percent of nominal figures in table A7_4_1_2-6.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*12/1/09*
Materials and Methods	*Applicants version is acceptable.*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	*1-2*
Acceptability	*acceptable*
Remarks	*The test concentration was not maintained at >80% (test validity criteria not met) but the Notifiers acknowledged and addressed this issue, providing a reasonable explanation (poor solubility and adsorption of permethrin onto glass surfaces).*
	COMMENTS FROM ...

Section A7.4.1.2(1) Acute toxicity to invertebrates – *Daphnia magna*

Annex Point IIA7.2

	Key study
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)-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	**Details**
Dispersion	No
Vehicle	Yes - Acetone
Concentration of vehicle	Serie 1 : 12,65 mg acetone/L Serie 2, 3 and 4 : 3,95 mg/L Serie 5 : 22,14 mg/L
Vehicle control performed	yes
Other procedures	None

Table A7.4.1.2(1)-2: Dilution water

Criteria	**Details**
Source	The reconstituted dilution water was made up according to the formula for soft water recommended by the US EPA (1975)
NaHCO3	48.0 mg/L
CaSO4.2H2O	30.0 mg/L
MgSO4	30.0 mg/L
KCL	2.0 mg/L
Oxygen content	>98% at 0 hours and 92 to 99% at 48 hours (% air saturation)
Conductance	none
Holding water different from dilution water	No

Table A7.4.1.2(1)-3: Test organisms

Criteria	**Details**
Strain	*Daphina magna (straus culture)*
Source	In house cultures. These culture commenced in October 1975, using Daphnia obtained from IRCHA laboratory, France.
Age	12 ± 12 hours old
Breeding method	Diploid parthenogenesis
Kind of food	Sediment algae (*chlorella vulgaris*)
Amount of food	Not reported
Feeding frequency	The daphnia were not fed during the course of the test
Pretreatment	e.g. acclimation
Feeding of animals during test	No

Table A7.4.1.2(1)-4: Test system

Criteria	Details
Renewal of test solution	No
Volume of test vessels	400 ml beakers containing 200 ml of test solution
Volume/animal	40 ml
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2(1)-5: Test conditions

Criteria	Details
Test temperature	The temperature was maintained at 17.0±0.5°C by immersion of test vessel in a constant temperature water bath
Dissolved oxygen	>98% at 0 hours and 92 to 99% at 48 hours (% air saturation)
pH	7.45-7.75
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Not reported
Photoperiod	16 hours light: 8 hours dark provided by fluorescent lights

Table A7.4.1.2(1)-6: analytical results at 0 and 48 hours

nominal [mg l^{-1}]	Mean measured at 0 hours	Mean as % of nominal	Mean measured at 48 hours	Mean as % of nominal	over all geometric mean (mg/L)	over all mean as % of nominal
Control	**<0.0002***	-	**<0.0002***	-	-	-
Solvent control	**<0.0002***	-	**<0.0002***	-	-	-
0.00032	0.00039	**122%**	**0.0004**	**125%**	**0.00039**	**123%**
0.00056	0.00053	**94%**	**0.00058**	**104%**	**0.00055**	**99%**
0.0010	0.0008	**80%**	**0.00044**	**44%**	**0.00059**	**59%**
0.0018	0.00157	**87%**	**0.00075**	**42%**	**0.00109**	**60%**
0.0032	0.00245	**77%**	**0.00125**	**39%**	**0.00175**	**55%**
0.0056	0.00416	**74%**	**0.00226**	**40%**	**0.00307**	**55%**
0.010	0.00874	**87%**	**0.00438**	**44%**	**0.00619**	**62%**
0.018	0.0154	**86%**	**0.01192**	**66%**	**0.01355**	**75%**
0.032	0.0322	**101%**	**0.0182**	**57%**	**0.02421**	**76%**
0.056	0.0623	**111%**	**0.035**	**62%**	**0.04670**	**83%**

*** limit of analytical determination**

Table A7.4.1.2(1)-7: Immobilisation data

Test-Substance Concentration [mg l^{-1}]		**Immobile *Daphnia***				**Oxygen [% air saturation]**	**pH**
nominal	**mean measured**	**Number**		**Percentage**			
		24 h	**48 h**	**24 h**	**48 h**	**48 h**	**48 h**
control		0/20[1]	0/20	0	0	97	7.70
Solvent control		0/100	0/100	0	0	93-98	7.45-7.70
0.00032	0.0004	1/20	2/20	5	10	93-94	7.60-7.70
0.00056	0.00058	1/60	3/60	1.7	5	93-96	7.50-7.65
0.0010	0.00044	0/60	27/60	0	45	93-97	7.55-7.70
0.0018	0.00075	3/60	33/60	5	55	93.5-97	7.50-7.70
0.0032	0.00125	7/100	66/100	7	66	93-97.5	7.45-7.60
0.0056	0.00226	14/100	70/100	14	70	92.5-99	7.50-7.70
0.010	0.00438	19/100	84/100	19	84	92-97	7.50-7.70
0.018	0.01192	20/40	38/40	50	95	95-97	7.50-7.65
0.032	0.0182	30/40	40/40	75	100	94.5-97	7.50-7.70
0.056	0.035	14/20	20/20	70	100	96.5-97	7.45-7.5

1. a/b : total of immobile daphnia/ total of daphnia tested

Table A7.4.1.2(1)-8: Effect data

	EC_{50}	95 % c.l.	EC_0	EC_{100}
24 h [µg l^{-1}]	1.3 (n)[1]	1.1 – 1.7	0.32 µg l^{-1} (1)	5.6 µg l^{-1} (1)
48 h [µg l^{-1}]	0.64 (n)[1]	0.51 – 0.79	0.10 µg l^{-1} (1)	1.8 µg l^{-1} (1)
48 h [µg l^{-1}]	1.27(m)[2]	0.795-1.846	0.018µg l^{-1} (2)	989.5µg l^{-1} (2)

1 effect data are based on nominal (n) concentrations

2 effect data are based on mean measured concentration

Table A7.4.1.2(1)-9: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	Not fullfilled
Immobilisation of control animals <10%	X	
Control animals not staying at the surface	X	
Concentration of dissolved oxygen in all test vessels >3 mg/l	X	
Concentration of test substance ≥80% of initial concentration during test		X

Criteria for poorly soluble test substances ergänzen		

Annex I : Calculation of LC50 based on mean measured concentration

Statistical Evaluation of a Quantal Response: Date from Thompson Williams (1978)

General:

Test identification/project no:	Date from Thompson & Williams (1978)
Test item:	Permethrin
Unit of test item concentration:	µg/L
Start of experiment on day:	
Date and time of the evaluation:	2007 09 27, 09:11:12
Raw data filename:	Quantal Response Data_daphnia_mean_m xls

Test design

Number of treatments (incl. control(s)):	11
Duration of the test:	48 h
Test system:	Daphnia

Mortality as Dependent on Concentration and Time

Tab. 1: Cumulative mortality of Daphnia as dependent on concentration of the test item and time (from InputRawData)

Treatm. [µg/L]	Control	46.690	24.200	13.540	6.180	3.060	1.750	1.080	0.590	0.550	0.390
0 h:	0	0	0	0	0	0	0	0	0	0	0
48 h:	0	20	40	38	84	70	66	33	27	3	2

Overview over the effects on mobility at 48 h

Tab. 2: % Immobility caused by the test item at 48 h.

Treatm.[µg/L]	Introduced	Mobile	Immobile	% Immobility
Control	100	100	0	0.0
46.690	20	0	20	100.0
24.200	40	0	40	100.0
13.540	40	2	38	95.0
6.180	100	16	84	84.0
3.060	100	30	70	70.0
1.750	100	34	66	66.0
1.080	60	27	33	55.0
0.590	60	33	27	45.0
0.550	60	57	3	5.0
0.390	20	18	2	10.0

Effective Concentrations (ECx) with Mobility at 48 h

Probit analysis using linear max. likelihood regression

Tab. 3: Probit analysis using linear max. likelihood regression: Determination of the concentration/response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of organisms; Emp. Probit: empirical probits; reg. probit: calculated probits for the final function.

Treatm. [µg/L]	Log(x)	% Immobility	n	Emp. Probit	Weight	Reg. Probit
Control		0.10	100			excluded
46.690	1.669	99.90	20	8.0926	0.762	7.620
24.200	1.384	99.90	40	8.0926	4.102	7.141
13.540	1.132	95.00	40	6.6445	8.108	6.717
6.180	0.791	84.00	100	5.9922	38.869	6.145
3.060	0.486	70.00	100	5.5218	54.899	5.632
1.750	0.243	66.00	100	5.4099	62.465	5.226
1.080	0.033	55.00	60	5.1244	37.988	4.879
0.590	-0.229	45.00	60	4.8756	34.009	4.440
0.550	-0.260	5.00	60	3.3555	33.261	4.389
0.390	-0.409	10.00	20	3.7199	9.855	4.138

excluded: value not in line with the chosen function

Inhibitions lower equal 0% or greater equal 100% were replaced by 0.100 and 99.900, respectively.

Parameters of the probit analysis

Tab. 4: Parameters of the probit analysis: Results of the regression analysis

Parameter	Value

Figure

Computation runs:	4
Slope b:	1.67600
Intercept a:	4.82100
Variance of b:	0.02002
Goodness of Fit	
Chi²:	30.48263
Degrees of freedom:	8
p(Chi²):	< 0.001
Log EC50:	0.10600
s Log EC50:	0.15049
F:	36.825
p(F) (df: 1,8):	0.000

Chi² is a goodness of fit measure. If the probability, p(Chi²), is lower or equal than 0.100, data is much scattering round the computed dose/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).

Results of the probit analysis

Tab. 6: Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits

Parameter	EC0	EC50	EC100
Value [µg/L]	0.018	1.279	89.544
lower 95%-cl	0.001	0.795	29.017
upper 95%-cl	0.067	1.846	1041.422
lower 99%-cl	0.000	0.657	12.852
upper 99%-cl	0.171	2.236	2351.282

n.d.: not determined due to mathematical reasons

Inhibitions lower equal 0% or greater equal 100% were replaced by 0.100 and 99.900, respectively. Slope function after Litchfield and Wilcoxon: 3.951

(The slope function is derived from the slope, b, of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

Figure 1: 48 hour dose response curve based on mean measured concentrations

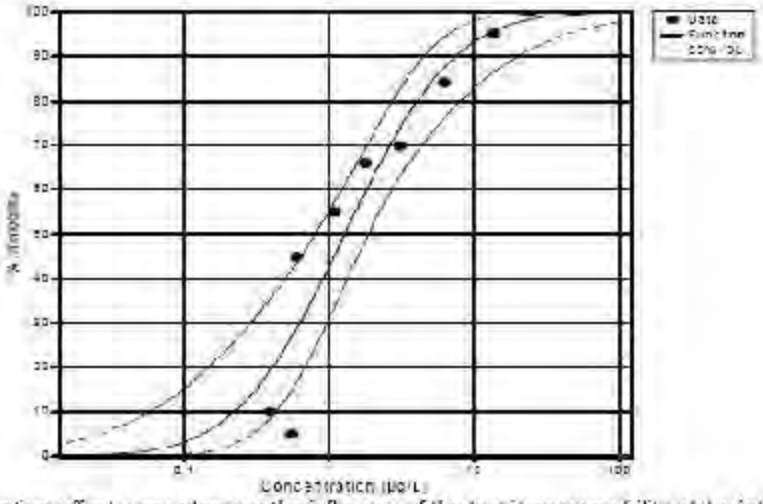
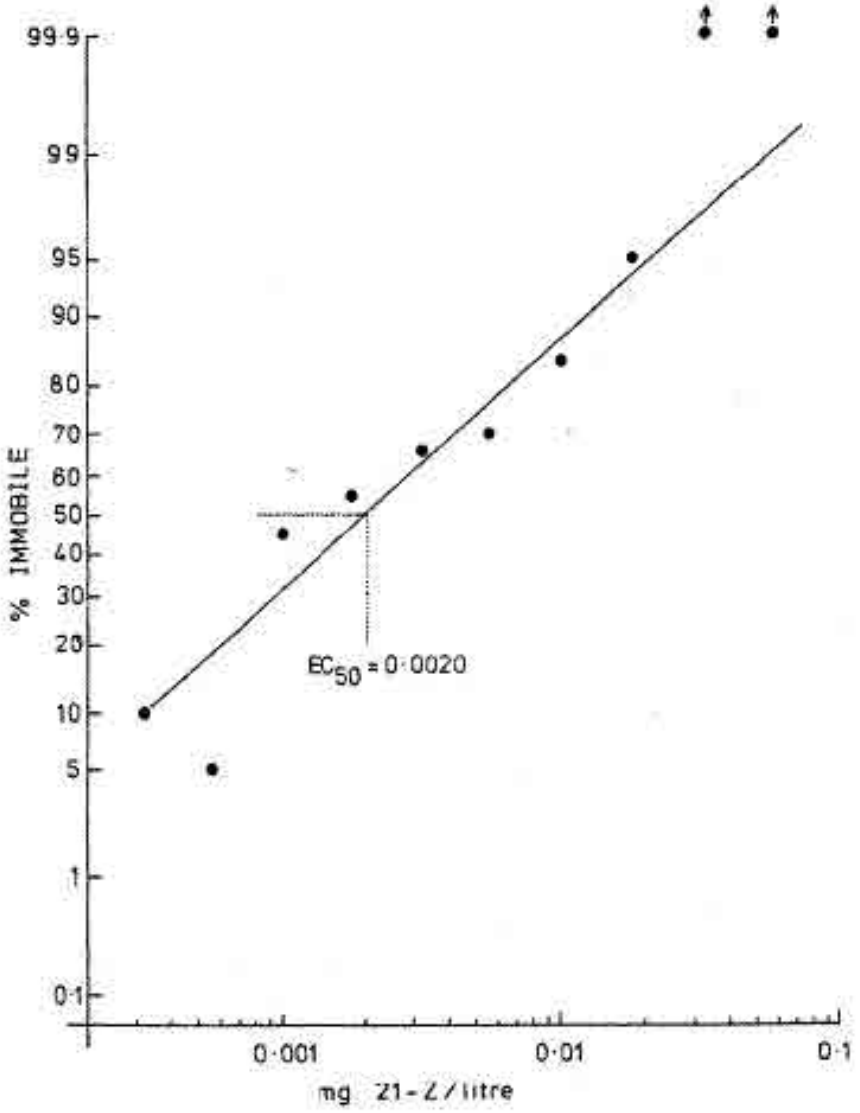


Fig. 1 Concentration-effect curve showing the influence of the test item on mobility of the invertebrate Daphnia as observed after 48 h.

Figure 2: 48 hour dose response curve based on nominal



Section A7.4.1.2(3)
Annex Point IIA7.2

Acute toxicity to invertebrates, metabolites

Daphnia magna

Key Study

			Official use only
		1 REFERENCE	
1.1	**Reference**	A.D. Forbis; Burgess, D (1984); Static Acute toxicity report : Acute toxicity of DCVA to Daphnia magna. Mobay chemical corporation, Stillwel, Kansas; Report N°505 BES Ref : M-034747-01-1;Report date : 25 June 1984:unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS before 14 May 2000 on existing a.s for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No but in essence method complied to EPA - FIFRA § 72-2, EEC/C.2. and OECD 202	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Dichlorovinylcarboxylic acid	
3.1.1	Lot/Batch number	150-6-52	
3.1.2	Specification	Not relevant,metabolite testing	
3.1.3	Purity	99.9%	
3.1.4	Composition of Product	Not relevant, metabolite testing	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	No analytical confirmation of the test concentration	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Dichlorovinylcarboxylic acid was dissolved in acetone, see table A7.4.1.2(3)-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not relevant	
3.4	**Testing procedure**		

Section A7.4.1.2(3)
Annex Point IIA7.2

Acute toxicity to invertebrates, metabolites

Daphnia magna

3.4.1	Dilution water	Aged well water, see table A7.4.1.2(3)-2
3.4.2	Test organisms	Daphnia magna from ABC laboratories (see table A7.4.1.2(3)-3)
3.4.3	Test system	(see table A7.4.1.2(3)-4)
3.4.4	Test conditions	Static condition (see table A7.4.1.2(3)-5)
3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Mortality and abnormal effects such as quiescence, erratic movement and daphnids lying on the bottom of test chambers.
3.4.7	Sampling	Observations were made every 24 hours
3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	The 24 and 48-hour LC50 values and corresponding 95% confidence limits were determined by an LC_{50} computer program developed by Stephan et al. (4). This program calculated the LC_{50} statistic and its 95 percent confidence limits using the binomial, moving average angle and probit methods because no one method is appropriate for all possible sets of data.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Control, solvent control, 3.2, 5.6, 10,18, 32 mg test substance/ L
4.2.2	Actual concentrations of test substance	No analytical confirmation of the test concentration, results are based upon nominal values
4.2.3	Effect data (Immobilisation)	Abnormal behaviour as quiescence, erratic movement and daphnids lying on the bottom were observed in the 10, 18 and 32 mg/1 test concentrations, mortality was reported for 32 mg/L after 48 h. see table A7.4.1.2(3)-6 and A7.4.1.2(3)-7
4.2.4	Concentration / response curve	not reported
4.2.5	Other effects	No other effects than those given under 4.2.3 were reported.

Section A7.4.1.2(3) Annex Point IIA7.2		Acute toxicity to invertebrates, metabolites *Daphnia magna*
4.3	**Results of controls**	No abnormal effects of mortality, quiescence, erratic movement and daphnids lying on the bottom were observed in control.
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Dichlorovinylcarboxylic acid (DCVA) was tested for acute toxicity to water fleas (Daphnia magna) according to a method complying with OECD guideline No. 202. 10 young daphnid (24 hours old) per duplicate were exposed to 3.2, 5.6, 10, 18 and 32 mg/l. A solvent control and a negative control were included. Observations were made 24h and 48 h past test initiation.
5.2	**Results and discussion**	The abnormal effects of mortality, quiescence, erratic movement and daphnies lying on the bottom were observed in the 10, 18 and 32mg/l test concentration. The no-effect concentration based on the lack of mortality and abnormal effects was 5.6 mg/l after 48 hours. The 24 and 48 hour LC50 values for DCVA were > 32 and 25 mg/l, respectively.
5.2.1	EC_0	5.6 mg/l after 48 hours
5.2.2	EC_{50}	25 mg/l after 48 hours
5.2.3	EC_{100}	not calculated
5.3	**Conclusion**	
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes, the concentration of DCVA was not measured in the test water.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*12/1/09*
Materials and Methods	*Applicants version is acceptable.*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	*2*
Acceptability	*acceptable*
Remarks	Actual concentration of the test substance (DCVA) was not measured in the test water.

Section A7.4.1.2(3)
Annex Point IIA7.2

Acute toxicity to invertebrates, metabolites

Daphnia magna

	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(3)-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Acetone
Concentration of vehicle	Up to 0.32 ml acetone in 200 ml well water
Vehicle control performed	Yes, 0.32 ml acetone in 200 ml well water
Other procedures	none

Table A7.4.1.2(3)-2: Dilution water

Criteria	Details
Source	Aged well water
Alkalinity	325-375 ppm
Hardness	225-275 ppm
pH	7.8-8.3
Oxygen content	9.2-10.2 ppm
Conductance	700 µmhos/cm
Holding water different from dilution water	No

Table A7.4.1.2(3)-3: Test organisms

Criteria	Details
Strain	Daphnia magna
Source	Cultured at the ABC facilities.
Age	First-instar daphnids (<24 hours old)
Breeding method	In house
Kind of food	Algae *(seleneastrum capriornutum)* and supplemented with a suspension of trout chow.
Amount of food	Not given

Feeding frequency	Every three days prior the testing
Pretreatment	acclimation
Feeding of animals during test	No

Table A7.4.1.2(3)-4: Test system

Criteria	Details
Renewal of test solution	Static test design without renewal
Volume of test vessels	250 ml
Volume/animal	20 ml
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2(3)-5: Test conditions

Criteria	Details
Test temperature	20°C
Dissolved oxygen	7.5-7.9 mg/l
pH	8.6-8.7
Adjustment of pH	No
Aeration of dilution water	Not stated
Quality/Intensity of irradiation	50-70 foot-candles on a 16 hours daylight photoperiod
Photoperiod	16 hours daylight photoperiod

Table A7.4.1.2(3)-6: Immobilisation data

Test-Substance Concentration (nominal) [mg/l]	**Immobile *Daphnia***						
	Number		**Percentage**		**Oxygen [mg/l]**	**pH**	**Tempera-ture [°C]**
	24 h	**48 h**	**24 h**	**48 h**	**48 h**	**48 h**	**48 h**
Control	0	0	0	0	7.9	8.6	20
Solvent control	0	0	0	0			
3.2	0	0	0	0	7.8	8.7	20
5.6	0	0	0	0			
10	0	0	0	0	7.8	8.7	20
18	0	0	0	0			
32	0	19	0	95	7.5	8.6	20

Table A7.4.1.2(3)-7: Effect data

	EC_{50}	**95 % c.l.**	**EC_0**	**EC_{100}**
24 h [mg/l][1]	>32 (n)			
48 h [mg/l][1]	25(n)	18-32 (n)	5.6 (n)	not determined

[1] **indicate if effect data are based on nominal (n) or measured (m) concentrate**

Table A7.4.1.2(3)-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	**Not fullfilled**
Immobilisation of control animals <10%	**Y**	
Control animals not staying at the surface	**Y**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**Y**	
Concentration of test substance ≥80% of initial concentration during test		**x**

Criteria for poorly soluble test substances		**x**

Section A7.4.1.3(1) Growth inhibition test on algae

Annex Point IIA7.3

Key Study

Official use only

		1 REFERENCE
1.1	**Reference**	Dorgerloh, M; 2008; *Pseudokirchneriella subcapitata* growth inhibition test with permethrin (techn.).Report No. E323 3265-4; GLP; Unpublished
1.2	**Data protection**	Yes
1.2.1	**Data owner**	Bayer CropScience
1.2.2	**Companies with letter of access**	Sumitomo Chemical (UK) PLC
1.2.3	**Criteria for data protection**	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes – OECD 201
2.2	**GLP**	Yes
2.3	**Deviations**	none
		3 MATERIALS AND METHODS
3.1	**Test material**	Permethrin 25/75
3.1.1	**Lot/Batch number**	102000001397
3.1.2	**Specification**	As given in section 2
3.1.3	**Purity**	97.3 % w/w
3.1.4	**Composition of Product**	Not applicable
3.1.5	**Further relevant properties**	Very low water solubility
3.1.6	**Method of analysis**	No analysis
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	309.5 mg of permethrin in 10 mL acetone was prepared immediately prior to test initiation. The stock solution was well agitated on a magnetic stirrer for 20 minutes before further use. An adequate amount of the stock solution was transferred to a dilution series. To obtain the concentration levels used in the study 100 µL of these dilutions were transferred to nutrient medium (see Table A7.4.1.3(1)-1).
3.3	**Reference substance**	No. Strain material of defined sensitivity was used, as shown by reference substance testing with 3,5-dichlorophenol or potassium dichromate. Such reference tests are done event driven (i.e. in case of receiving new strains, introduction of new test conditions, apparatus, etc.). Such work is documented and archived together with strain protocols.
3.4	**Testing**	

Section A7.4.1.3(1) Growth inhibition test on algae

Annex Point IIA7.3

Key Study

	procedure	
3.4.1	**Culture medium**	
3.4.2	**Test organisms**	see Table A7.4.1.3(1)-2
3.4.3	**Test system**	see Table A7.4.1.3(1)-3
3.4.4	**Test conditions**	see Table A7.4.1.3(1)-4
3.4.5	**Duration of the test**	72 hours
3.4.6	**Test parameter**	Cell numbers per volume (as a surrogate for biomass per volume) and possible alterations in algae cells such as unusual cell size were estimated by direct algae cell counting under a microscope at a magnification of 400 times.
3.4.7	**Sampling**	24, 48, 72 hours
3.4.8	**Monitoring of TS concentration**	Samples were analysed for the actual concentration of permethrin present in the test medium of all treatment levels and the controls on day 0 and 3.
3.4.9	**Statistics**	ECx values (e.g. x = 50) and confidence intervals were calculated for the stated exposure period, using a commercial program. The LOEC determinations from the appropriate parameter (inhibition) were done, using the ANOVA procedure (p = 0.05, one sided) and properly selected multiple t-tests of a commercial program.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.2	**Results test substance**	
4.2.1	**Initial concentrations of test substance**	0 (control), 0 (solvent control), 0.0286, 0.0916, 0.293, 0.938, and 3.00 mg a.s./L nominal concentrations
4.2.2	**Actual concentrations of test substance**	0 (control), 0 (solvent control), 0.0131, 0.0631, 0.175, 0.395, and 1.13 mg a.s./L geometric mean measured concentrations (day 3)
4.2.3	**Growth curves**	Cell number as influenced by the test item and time in Figure 1
4.2.4	**Concentration / response curve**	See Figure 2
4.2.5	**Cell concentration data**	see Table A7.4.1.3(1)-5
4.2.6	**Effect data (cell multiplication inhibition)**	**ErC10** = 0.0023 mg a.s/L **ErC50** > 1.13 mg a.s./L **LOE_rC** ≤ 0.0131 mg a.s./L **NOE_rC** < 0.0131 mg a.s./L

Section A7.4.1.3(1) Growth inhibition test on algae

Annex Point IIA7.3

Key Study

4.2.7	**Other observed effects**	None
4.3	**Results of controls**	see Table A7.4.1.3(1)-5
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aim of the study was to determine the influence of permethrin technical (97,3% w/w of purity) on exponentially growing *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate of algal biomass (cells per volume). *Pseudokirchneriella subcapitata* (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to nominal (geometric mean measured) concentrations of 0.0286 (0.0131), 0.0916 (0.0631), 0.293 (0.175), 0.938 (0.395) and 3.00 (1.13) mg a.s./L in comparison to controls. The pH values ranged from 8.1 to 9.5 in the water controls and the incubation temperature ranged from 22.4°C to 23.2°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6903 lux. Quantitative amounts of permethrin were measured in all treatment groups and in the controls on day 0 and day 3 of the exposure period.
5.2	**Results and discussion**	The analytical findings of permethrin in the treatment levels found on day 0 were 33 % to 76 % of nominal (average 51 %). On day 3 analytical findings of 30 % to 63 % of nominal (average 52 %) were found. Because of the expected low analytical findings (based on low solubility of the test item in water and the high adsorptivity of the test item to glass surfaces) all results are based on geometric mean measured test concentrations. The static 72 hour algae growth inhibition test provided the following effects:
5.2.1	**NOE_rC**	< 0.0131 mg a.s./L
5.2.2	**E_rC_{50}**	> 1.13 mg a.s./L
5.2.3	**E_rC_{10}**	0.0023 mg a.s./L
5.3	**Conclusion**	The toxicity of permethrin to algae is considerably greater than its water solubility, therefore is never likely to manifest under environmental conditions
5.3.1	**Reliability**	1
5.3.2	**Deficiencies**	None

Section A7.4.1.3(1) Growth inhibition test on algae

Annex Point IIA7.3

Key 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	*12/1/09*
Materials and Methods	*Applicants version is acceptable.*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	1
Acceptability	acceptable
Remarks	*E_bC_{50} values were not estimated - cell numbers per volume were measured as a surrogate for biomass per volume.* *The test concentration was not maintained at >80% (test validity criteria not met) but the Notifiers acknowledged and addressed this issue, providing a reasonable explanation (poor solubility and adsorption of permethrin onto glass surfaces).*
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.3(1)-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes -Acetone
Concentration of vehicle	Not reported
Vehicle control performed	Yes
Other procedures	None

Table A7.4.1.3(1)-2: Test organisms

Criteria	Details
Species	*Pseudokirchneriella subcapitata* (freshwater microalgae, formerly known as *Selenastrum capricornutum*)
Strain	SAG 61.81
Source	Collection of Algal Cultures, Inst. for Plant Physiology, University of Göttingen, Nikolausberger Weg 18, 37077 Göttingen, Germany
Laboratory culture	JRF in-house cultures
Method of cultivation	200 µL of a 7-9 days old stock culture was transferred into a 250 mL cotton plugged Erlenmeyer flask containing 50 mL of nutrient medium (Bringmann and Kühn, 1980) once every week. Stock cultures of algae were kept at 23 ± 2°C with 16 h light. All operations were conducted under sterile conditions to handle an axenic[1] algae culture
Pretreatment	To ensure that the algae used as inoculum were exponentially growing, a pre-culture was prepared 4 days before the start of the test and cultivated under the same conditions as in the main test. In order to reach the appropriate cell denisty in the test medium at the beginning of the 72 hours exposure period of the main test, an adequate dilution of the pre-culture was done with nutrient medium.
Initial cell concentration	10,000 cells/mL

Table A7.4.1.3(1)-3: Test system

Criteria	Details
Volume of culture flasks	300 mL-Erlenmeyer flasks
Culturing apparatus	Multitron (Infors GmbH) incubator
Light quality	The exposure of individual flasks to permanent light[2] was made more uniform by randomised repositioning after each observation day mean: 4,440 – 8,880 lux; variation within the test: ± 15 % lux [3]

[1] Axenic cultures are cultures of a single species.

[2] The uniform overhead illumination was provided by a bank light containing cool white fluorescent lamps attached to the ceiling. The inner walls were covered with reflective metal.

[3] Light intensity was determined with a RadioLux 111, PRC Krochmann GmbH.

Procedure for suspending algae	Test vessels were placed on a tablet rotating 100 rpm to prevent sedimentation of the cells without additional aeration.
Number of vessels/ concentration	3 replicate vessels per test level and 6 replicate vessels per control
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.3(1)-4: Test conditions

Criteria	**Details**
Test temperature	22 ± 2°C (actual: 22.4 -23.2°C)
pH	8.1 to 9.5 (water controls)
Aeration of dilution water	No
Light intensity	Mean 6903 lux.
Photoperiod	Continuous

Table A7.4.1.3(1)-5: The static 72 hour algae growth inhibition test provided the following effects:

Geometric mean measured concentration [mg a.s./L]	**Cell Number after 72 h (means) per mL**	**(0-72h)-Average Specific Growth Rates [days^{-1}]**	**Inhibition of Average Specific Growth Rate [%]**	**Doubling Time of Algae Cells [days]**
Water control	898,000	1.498	--	0.463
Solvent control	875,000	1.489	--	0.466
Pooled controls	886,000	1.494	--	0.464
0.0131	535,000	1.323	11.4	0.524
0.0631	395,000	1.225	18.0	0.566
0.175	368,000	1.201	19.6	0.577
0.395	347,000	1.180	21.0	0.587
1.13	322,000	1.154	22.8	0.601

A7.4.1.3(1)-6 Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	**Not fullfilled**
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**X**	
Concentration of test substance ≥80% of initial concentration during test		**X**

Criteria for poorly soluble test substances	**X**	

Figure 1: Cell Number as Influenced by the Test Item and Time

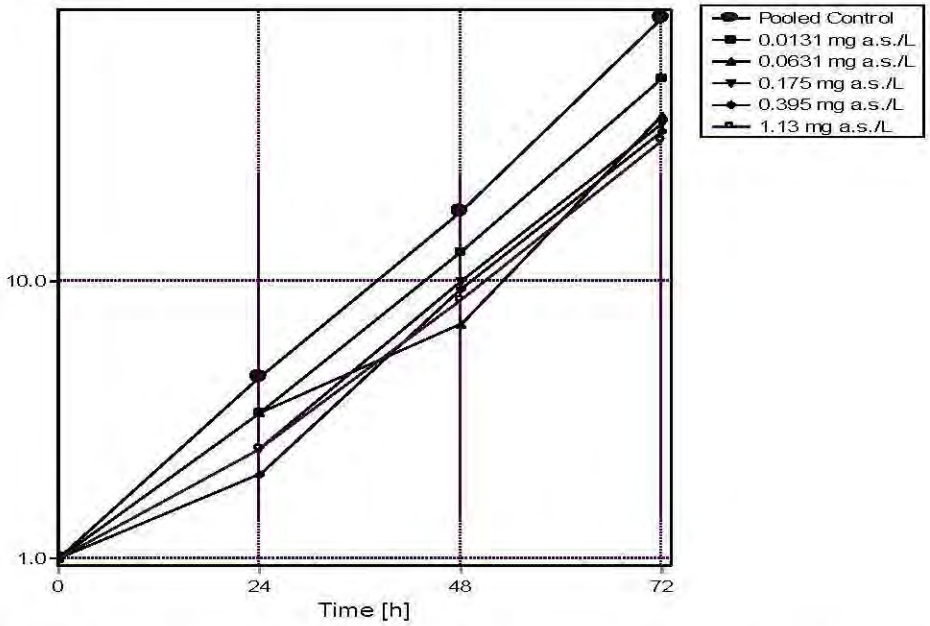
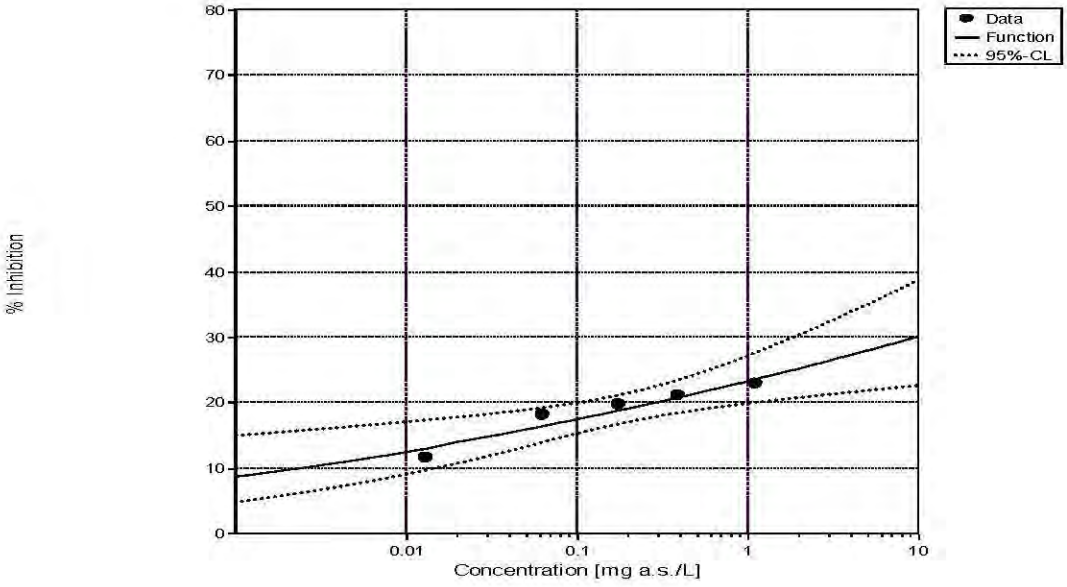


Figure 2: Concentration / Effect Relationship on the Growth Rate over the Test Period (0 – 72 h)



Section A7.4.1.3(2) **Growth inhibition test on algae**

Annex Point IIA7.3

Supportive Data

Official use only

	1 REFERENCE	
1.1	**Reference**	Satheesh, V.K.; 1997; Alga (*Selenastrum capricornutum*), Growth Inhibition Test for Permethrin Technical. Jai Research Foundation. Report No. 1015/JRF/BTC/97; GLP; Unpublished
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer Environmental Science Bayer CropScience AG
1.2.2	Companies with letter of access	Sumitomo Chemical (UK) PLC
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes – OECD 201
2.2	**GLP**	Yes
2.3	**Deviations**	Yes – No analysis was performed
	3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	002/96
3.1.2	Specification	As given in section 2
3.1.3	Purity	94%
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	Very low water solubility
3.1.6	Method of analysis	No analysis
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	61.44 mg of permethrin was dissolved in 3 ml acetone and dilted to 20 ml with algal media to provide a stock solution of 3072 mg/l. Aliquots of this were serially diluted with algal media to 1536, 768, 384, 192, 96, 48, 24, 12, 6, 3 mg/l. These stocks were diluted with algal media to provide test solutions. (see table A7.4.1.3(2)-1)
3.3	**Reference substance**	No
3.4	**Testing procedure**	
3.4.1	Culture medium	Culture medium was as described in OECD testing guideline 201. The pH was adjusted to pH 8.0 +/- 1 and sterilised in an autoclave. Measurements at the start and end of the study indicated the pH did not exceed these limits.
3.4.2	Test organisms	see table A7.4.1.3(2)-2

Section A7.4.1.3(2) **Growth inhibition test on algae**

Annex Point IIA7.3

Supportive Data

3.4.3	Test system	see table A7.4.1.3(2)-3
3.4.4	Test conditions	see table A7.4.1.3(2)-4
3.4.5	Duration of the test	72 hours
3.4.6	Test parameter	Cell density, measure by haemocytometry
3.4.7	Sampling	24, 48, 72 hours
3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	The EbC50 value was determined by probit analysis using JRF software. The ErC50 value was calculated by linear regression as described in OECD testing guideline 201.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal concentrations of 0.03, 0.06, 0.12, 0.24, 0.48, 0.96, 1.92 mg l^{-1}
4.2.2	Actual concentrations of test substance	Not performed
4.2.3	Growth curves	See Figure 1
4.2.4	Concentration / response curve	See Figure 2
4.2.5	Cell concentration data	see table A7.4.1.3(2)-5
4.2.6	Effect data (cell multiplication inhibition)	**EbC50** 0.497 mg l^{-1} (95% CI 0.223 – 1.11 mg l^{-1}) **ErC50** 2.348 mg l^{-1} (95% CI 0.972 – 24.0 mg l^{-1}) **NOECbiomass** <0.03 mg l^{-1} **NOECrate** 0.12 mg l^{-1}
4.2.7	Other observed effects	None
4.3	**Results of controls**	see table A7.4.1.3(2)-5
4.4	**Test with reference substance**	Not performed
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The test follows OECD 201 guidelines, and exposes algal cells to concentrations of permethrin. The test is undertaken in flasks which

Section A7.4.1.3(2) **Growth inhibition test on algae**

Annex Point IIA7.3

Supportive Data

		are static, but shaken twice daily and exposed to constant fluorescent light. Cell density is determined by haemocytometry at 24 hourly intervals for 72 hours. Concentration responses over 72 hours in the exposure vessels are compared to the pooled data from the solvent and aqueous controls.
5.2	**Results and discussion**	
5.2.1	NOE_rC	0.12 mg l^{-1}
5.2.2	E_{r50}	2.348 mg l^{-1} (95% CI 0.972 – 24.0 mg l^{-1})
5.2.3	E_bC_{50}	0.497 mg l^{-1} (95% CI 0.223 – 1.11 mg l^{-1})
5.3	**Conclusion**	The observed toxicity is occurring from 6× to several orders of magnitude above the reported solubility of permethrin. This may account for the observed toxicities across the concentration ranges investigated (30 to 1920 µg l^{-1}), which would be confounded by the supersaturated concentrations which were employed. The main conclusions which can be drawn from the test are that; • The toxicity of permethrin to algae is considerably greater than its water solubility, therefore is never likely to manifest under environmental conditions • For the aquatic compartment, primary producers are the least sensitive of the trophic levels
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes The study has three main deficiencies: The effects were observed at concentrations higher than the solubility limit of permethrin. The analytical data are not presented, which would support the maintenance of test substance concentration in a static system over 72 hours. The study failed to achieve a NOEC for biomass. However, since current recommendations are that the rate values be used, this is not considered to seriously affect the validity of the study.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	11/05/05
Materials and Methods	State if the applicants version is acceptable
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	2
Acceptability	acceptable
Remarks	The study has three main deficiencies: (1) The effects were observed at concentrations higher than the solubility limit of permethrin. (2) The analytical data are not presented, which would support the maintenance of test substance concentration in a static system over 72 hours. (3) The study failed to achieve a NOEC for biomass. However, since current recommendations are that the rate values be used, this is not considered to seriously affect the validity of the study.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.3(2)-4: Test conditions

Criteria	Details
Test temperature	21 (min) to 24 (max) ± 0.5°C
pH	8.0 +/- 1
Aeration of dilution water	No
Light intensity	7373 +/- 117.6 lux
Photoperiod	Continuous

Table A7.4.1.3(2)-5: Cell concentration data

Test Substance Concentration (nominal) [mg/l]	**Cell concentrations (mean values) [cells/ml]**							
	measured				**Percent of solvent control**			
	0 h	**24 h**	**48 h**	**72 h**	**0 h**	**24 h**	**48 h**	**72 h**
Control	10808	37500	153333	262500	-	-	-	-
Solvent control	10808	36667	142500	244167	-	-	-	-
0.03	10808	35417	131250	222500	-	96.6	92.1	91.1
0.06	10808	33750	122917	214167	-	92.0	86.3	87.7
0.12	10808	32083	116250	191667	-	87.5	81.6	78.5
0.24	10808	31667	105417	169167	-	86.4	74.0	69.3
0.48	10808	23333	85000	127083	-	63.6	59.6	52.0
0.96	10808	22083	64167	63333	-	60.2	45.0	25.9
1.92	10808	20417	45417	43750	-	55.7	31.9	17.9
Temperature [°C]	21 – 24 ± 0.5°C							
pH	8.0 ± 1							

[1] specify, if TS concentrations were nominal or measured

A7.4.1.3(2)-6 Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	**Not fullfilled**
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥80% of initial concentration during test		X

Criteria for poorly soluble test substances		

Figure 1 Growth curves

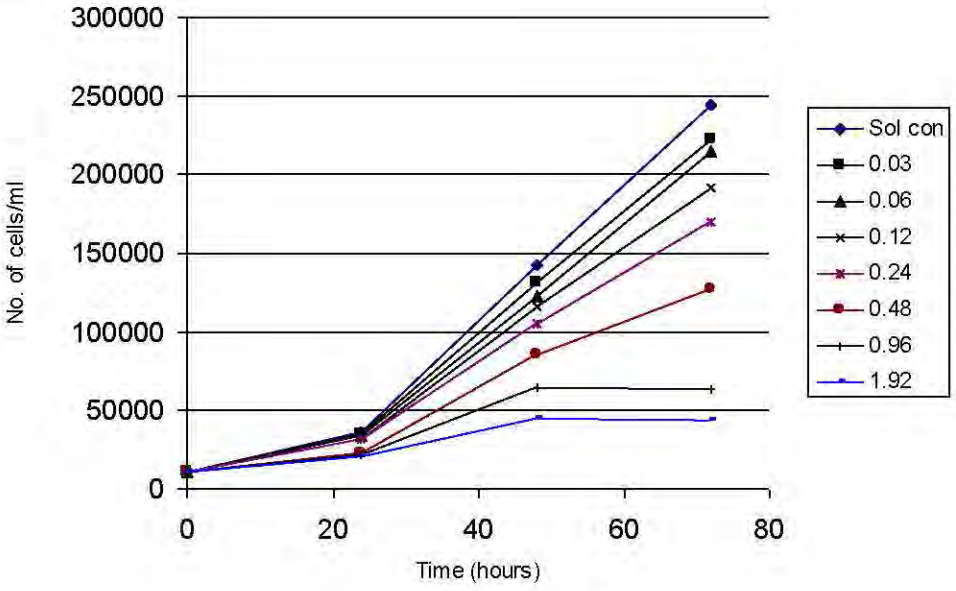
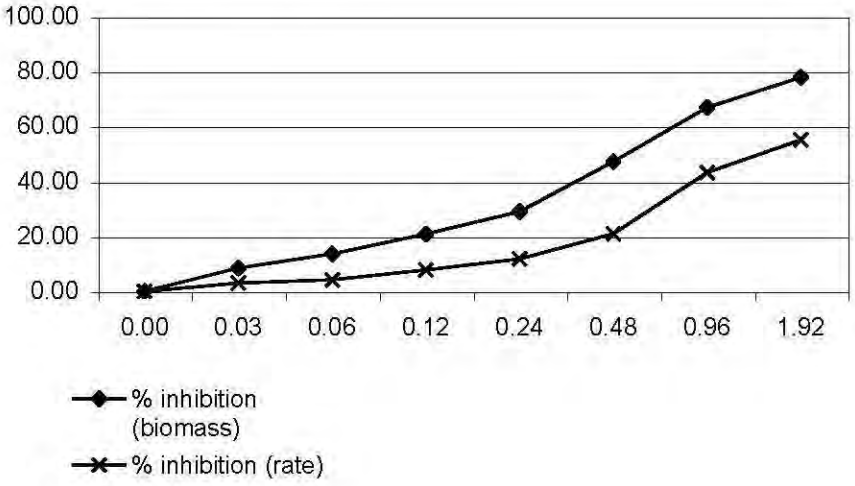


Figure 2 Dose – response curves



Section A7.4.1.3(3)
Annex Point IIA7.1

Acute toxicity to algae, metabolites

Key study

Official use only

1 REFERENCE

1.1	**Reference**	Stratton, G.W. and C.T. Corke. 1982. Toxicity of the insecticide permethrin and some degradation products towards algae and cyanobacteria. *Environ. Pollut., Ser. A 29:71-80.*
		Published
1.2	**Data protection**	No
1.2.1	**Data owner**	Published
1.2.2		
1.2.3	**Criteria for data protection**	

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No,
2.2	**GLP**	No
2.3	**Deviations**	No, although method complied in essence to EPA - FIFRA § 72-1, EEC/C.1. and OECD 201

3 MATERIALS AND METHODS

3.1	**Test material**	Permethrin and 10 metabolites including 3-phenoxybenzoic acid
		The products tested are listed in table 1
3.1.1	**Lot/Batch number**	Not reported
3.1.2	**Specification**	Not relevant, metabolite testing
3.1.3	**Purity**	>95%
3.1.4	**Composition of Product**	Not relevant , metabolite testing
3.1.5	**Further relevant properties**	none
3.1.6	**Method of analysis**	no analytical confirmation of the test concentration
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Stock solutions of permethrin and the phenoxylated series of degradation products were prepared in pesticide grade acetone (Caledon Laboratories, Georgetown, Ontario, Canada). The other test compounds were soluble in water. Level of acetone 0.1% v/v
3.3	**Reference substance**	**None.**
3.3.1	**Method of analysis for reference substance**	**Not applicable.**
3.4	**Testing procedure**	
3.4.1	**Dilution water**	Not reported
3.4.2	**Test organisms**	The cyanobacteria (blue-green algae) *Anabaena inaequalis, A. cylindrica.* and *A. variabilis*, and the green algae *Chlorella*

Section A7.4.1.3(3) **Acute toxicity to algae, metabolites**
Annex Point IIA7.1

		Key study
		pyrenoidosa and *Scenedesmus quadricauda* were used as test organisms *A. cylindrica* and *A. variabilis* were obtained from the American Type Culture Collection, Rockville, Maryland, USA (ATCC 27899 and 27892, respectively) while the other cultures were provided by the Department of Botany and Genetics, University of Guelph, Guelph, Ontario,Canada Only tests with green algae are regarded valid because these test organisms are requested in current guidelines.
3.4.3	**Test system**	see table A7.4.1.3(2)-1
3.4.4	**Test conditions**	Under static conditions, see table A7.4.1.3(2)-2
3.4.5	**Duration of the test**	12 to 14 days
3.4.6	**Test parameter**	Photosynthesis was assayed by following the uptake of $^{14}CO_2$ from $NaH^{14}CO_3$ (Amersham/Searle, Oakville, Ontario, Canada). Nitrogenase activity was determined only for A. inaequalis and A. cylindrical using the acetylene reduction technique (Hardy et al., 1973). Growth was assessed by measuring the absorbance of cultures with time, using a Bausch and Lomb Spectronic 20 spectrophotometer. For each culture there was a linear relationship (correlation coefficient ≥ 0.999) between cell concentrations and the absorbance.
3.4.7	**Sampling**	Photosynthesis: 3h Acetylene reduction : 5h Growth : 12 to 14 days
3.4.8	**Monitoring of TS concentration**	No
3.4.9	**Statistics**	EC50 values determined by probit (Finney, 1971) or regression analysis (Bliss, 1967), where applicable. Analyses for significant differences (p = 0-05) were performed using Dunnett's test (Winer, 1971) and Duncan's multiple range test (Bliss, 1967).
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	**Concentration**	Not relevant
4.1.2	**Number/ percentage of animals showing adverse effects**	Not relevant
4.1.3	**Nature of adverse effects**	Not relevant
4.2	**Results test substance**	
4.2.1	**Initial concentrations of**	Photosynthesis: Each compound was assayed at a minimum of five concentrations ranging from 0 to l0 mg/L and all treatments were

Section A7.4.1.3(3)
Annex Point IIA7.1

Acute toxicity to algae, metabolites

Key study

	test substance	replicated five times. Acetylene reduction : Each chemical was tested at a minimum of five concentrations ranging from 0 to l00 mg/L and all treatments were replicated five times Growth : Each compound was assayed at a minimum of five concentrations ranging from 0 to l0 mg/L and all treatments were replicated five times
4.2.2	**Actual concentrations of test substance**	no analytical confirmation of the test concentration
4.2.3	**Growth curves**	none
4.2.4	**Concentration / response curve**	none
4.2.5	**Effect data**	Photosynthesis : Permethrin was relatively non-toxic towards photosynthesis and all EC50 values were >100 mg/L. The same results were obtained for the degradation products HBAlc, HBAld, BAlc and BAld. However, the remaining metabolites were significantly more toxic than permethrin towards at least four of the organisms (Table 2). The EC_{50} of 3-phenoxybenzoic acid range from 20 mg/L to >100 mg/L. Inhibition of nitrogenase activity : . Permethrin and the degradation products HBAlc,HBAld, BAlc and BAld had EC50 values > l00 mg/L. The other metabolites were significantly more toxic than permethrin. 3-phenoxybenzoic acid yielded EC50 values of 35 to 95 mg/L (Table 2). Growth : Growth is generally more sensitive to toxicants than either photosynthesis or acetylene reduction (Stratton *et al.,* 1979) and, consequently, chemical concentrations > l0 mg/L were not tested in growth experiments. Permethrin and the compounds HBAlc, HBAld, BAlcand Bald did not affect the growth yield and growth rate of test cultures at l0 mg/L. PBAc, HBAc and BAc, as well as C1CA, were also relatively non-toxic for most cultures (Table 2). With growth experiments the pH of test systems was unaltered by the concentrations of chemicals used.
4.3	**Results of controls**	none
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Toxic effects of permethrin and ten of its degradation products were determined for the growth., photosynthesis and acetylene-reducing ability of two species of green algae and three species of cyanobacteria (blue-green algae). Each compound was assayed at a

Section A7.4.1.3(3)
Annex Point IIA7.1

Acute toxicity to algae, metabolites

Key study

		minimum of five concentrations ranging from 0 to l0 mg/L and all treatments were replicated five times except for acetylene reduction where concentration ranged from 0 to 100 mg/L .
5.2	**Results and discussion**	Permethrin was relatively non-toxic in all systems. The most toxic metabolites were 3-phenoxybenzaldehyde and 3-phenoxybenzyl alcohol, followed by benzoic acid, 3-hydroxybenzoic acid and 3-phenoxybenzoic acid. The photosynthesis $EC_{50}$of 3-phenoxybenzoic acid range from 20 mg/L to >100 mg/L. 3-phenoxybenzoic acid was relatively non-toxic for most cultures towards the growth yield and the growth rate with an EC_{50} value of >10.0 mg/L
5.2.1	**EC_0**	
5.2.2	**EC_{50}**	> 10.0 mg/L based on the growth yield and *growth rate for , C.pyrenoidosa and S. quadricaula.*
5.2.3	**EC_{100}**	
5.3	**Conclusion**	
5.3.1	**Other Conclusions**	
5.3.2	**Reliability**	2
5.3.3	**Deficiencies**	No analytical confirmation of the test concentration

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*12/1/09*
Materials and Methods	*Applicants version is acceptable.*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	2
Acceptability	*acceptable*
Remarks	*No analytical confirmation of the test concentrations*
	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*

Section A7.4.1.3(3) Annex Point IIA7.1	**Acute toxicity to algae, metabolites**
	Key study
Remarks	

Table A7.4.1.3(2)-1: Test organisms

Criteria	Details
Species	*A. inaequalis* *A.cylindrica* *A. variabilis* *C.pyrenoidosa* *S. quadricaula*
Method of cultivation	*A. inaequalis* and *A. cylindrica* were maintained in a liquid nitrogen-free medium (Stratton et *al.*, 1979) at a temperature of 20°C and a light intensity of 7000lux on a 12 h light-dark cycle. The other cultures were maintained under the same conditions except that the growth medium was supplemented with 1.5g $NaNO_3$ $litre^{-1}$.
Initial cell concentration	6.5×10^4 cyanobacterial or 1.0×10^5 green algal cells ml^{-1}

Table A7.4.1.3(2)-2: Test system

Criteria	Details
Volume of culture flasks	Photosynthesis/Acethylene reduction; Plastic tissue culture flasks with a total internal volume of 74ml Growth : sidearm flask of 500 ml
Culturing apparatus	none
Light quality	a light intensity of 7000lux on a 12 h light-dark cycle

Table 1 : description of chemicals :

Chemical name of test compound	*Designated abbreviation*	*Conversion factor[a]*
3-phenoxybenzyl-(1RS)-*cis,trans*-3-(2,2-dichlorovinyl) 2,2-dimethylcyclopropanecarboxylate	Permethrin[b]	2·56
3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	ClCA[c]	4·78
3-phenoxybenzyl alcohol	PBAlc[d]	4·99
3-phenoxybenzaldehyde	PBAld[d]	5·05
3-phenoxybenzoic acid	PBAc[d]	4·67
3-hydroxybenzyl alcohol	HBAlc[d]	8·06
3-hydroxybenzaldehyde	HBAld[d]	8·19
3-hydroxybenzoic acid	HBAc[d]	7·24
benzyl alcohol	BAlc[d]	9·25
benzaldehyde	BAld[d]	9·42
benzoic acid	BAc[d]	8·19

Table 2 : Effect of selected permethrin degradation product on algae

Test chemical/ toxicity criterion	*Test culture* A. inaequalis	A. cylindrica	A. variabilis	C. pyrenoidosa	S. quadricauda
Photosynthesis					
PBAIc	30 (3)[a]	90 (5)[a]	35 (5)	95 (8)[a]	79 (5)[a]
PBAId	2 (1)[b,c]	50 (6)[b,c]	50 (8)[a]	70 (10)[b]	35 (7)
PBAc	20 (2)	> 100	100 (9)	> 100	> 100
HBAc	80 (8)[d]	65 (4)[d]	60 (3)	100 (8)[a]	90 (5)
BAc	5 (4)[c,e]	60 (2)[d]	55 (8)[a]	60 (10)[b]	75 (5)[a]
Acetylene reduction					
PBAIc	48 (5)	60 (10)[d,e]	—[1]	—	—
PBAId	12 (5)[f]	46 (10)[b,e,f]	—	—	—
PBAc	35 (7)[g]	95 (15)[g]	—	—	—
HBAc	68 (10)	85 (11)[g]	—	—	—
BAc	90 (9)[d]	30 (10)[f]	—	—	—
Growth yield[3]					
PBAIc	2·5 (0·5)[b]	2·2 (0·4)[a]	1·4 (0·4)	2·8 (0·6)	4·3 (0·7)
PBAId	> 10	2·4 (0·3)[a]	2·3 (0·4)	> 10	6·6 (0·6)
PBAc	> 10	10·0 (1·5)[b]	> 10	> 10	> 10
HBAc	> 10	> 10	> 10	> 10	> 10
BAc	9·0 (1·0)[c]	> 10	> 10	> 10	> 10
Growth rate[4]					
PBAIc	4·5 (0·6)[a]	8·0 (1·0)[b,c]	5·5 (0·5)[b]	5·2 (0·3)	4·5 (0·4)
PBAId	> 10	7·6 (0·9)[c]	5·5 (0·7)[b]	10·0 (2·0)	5·8 (0·5)
PBAc	> 10	> 10	> 10	> 10	> 10
HBAc	> 10	> 10	> 10	> 10	> 10
BAc	> 10	> 10	> 10	> 10	> 10

[1] Table entries are mean EC_{10} values (ppm) with the standard deviation in parentheses. Those entries in each column that are followed by the same letter do not differ significantly at $p = 0.05$.
[2] Test not performed due to the lack of nitrogenase activity.
[3] Growth yield refers to the culture's absorbance, which is correlated to cell numbers.
[4] Growth rate was measured according to the method of Sorokin (1973).

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**
Annex Point IIA7.4

Official use only

Key Study

1 REFERENCE

1.1	**Reference**	Dengler, D; 1999; Testing of Toxic Effects of Permethrin Technical Insecticide on Activated Sludge with the Respiration Inhibition Test. GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH. Report No. 99385/01-AAHT; GLP; Unpublished
1.2	**Data protection**	Yes
1.2.1	**Data owner**	Sumitomo Chemical (UK) PLC
1.2.2	**Companies with letter of access**	Bayer Environmental Science
1.2.3	**Criteria for data protection**	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes – OECD 209
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	**Lot/Batch number**	PL 98-0934
3.1.2	**Specification**	As given in section 2
3.1.3	**Purity**	96.1%
3.1.4	**Composition of Product**	Not applicable
3.1.5	**Further relevant properties**	Low water solubility
3.1.6	**Method of analysis**	Yes – permethrin concentration in the saturated stock solution was determined by solvent extraction followed by gas chromatography
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A saturated stock solution of permethrin was prepared by mixing approximately 0.5 ml of permethrin technical with 1 L of water and stirred overnight. After stirring, the solutions were filtered through glass wool to remove particulate materials observed in the range-finding test.
3.3	**Reference substance**	Yes; 3,5-Dichlorophenol
3.3.1	**Method of analysis for reference substance**	No analysis was performed

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**
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3.4	**Testing procedure**	
3.4.1	**Culture medium**	The test was performed in synthetic wastewater prepared by dissolving the following amounts of substances in 1L water; Peptone 16.0g Meat extract 11.0g Urea 3.0g NaCl 0.7g CaCl2.2H2O 0.4g MgSO4.7H2O 0.2g K2HPO4 2.8g
3.4.2	**Inoculum / test organism**	see table A7.4.1.4-2
3.4.3	**Test system**	see table A7.4.1.4-3
3.4.4	**Test conditions**	see table A7.4.1.4-4
3.4.5	**Duration of the test**	3 hours (also measured at 30 minutes)
3.4.6	**Test parameter**	Respiration inhibition
3.4.7	**Analytical parameter**	Oxygen measurement
3.4.8	**Sampling**	Test assays were prepared at 15 minute intervals
3.4.9	**Monitoring of TS concentration**	Yes – permethrin concentration in the saturated stock solution was determined by solvent extraction followed by gas chromatography
3.4.10	**Controls**	Control without test substance (×2); Toxicity controls (5, 15, 30 mg/l 3,5-DCP)
3.4.11	**Statistics**	Deviation of both controls from the mean was determined statistically to ensure validity criteria. The EC50 value for the reference compound was determined by Probit analysis.
		4 RESULTS
4.1	**Preliminary test**	Performed
4.1.1	**Concentration**	Five concentrations were tested, saturated concentration (sc) and 0.1×sc, 0.01×sc, 0.001×sc, 0.0001×sc
4.1.2	**Effect data**	No effects observed
4.2	**Results test substance**	
4.2.1	**Initial concentrations of test substance**	740 µg l^{-1} in saturated solution
4.2.2	**Actual concentrations of**	Not measured *in situ*

	test substance	
4.2.3	**Growth curves**	No effects observed
4.2.4	**Cell concentration data**	Not performed
4.2.5	**Concentration/ response curve**	No effects observed
4.2.6	**Effect data**	No effects observed
4.2.7	**Other observed effects**	No effects observed
4.3	**Results of controls**	See Table A7.4.1.4-6. The deviation of both controls from the mean was <15%.
4.4	**Test with reference substance**	Performed
4.4.1	**Concentrations**	5, 15, 30 mg l^{-1}
4.4.2	**Results**	The EC50 value for the reference compound was determined by Probit analysis at 10 mg l^{-1} (30 min) and 7 mg l^{-1} (3 hour)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The test followed OECD 209 guidelines, and measured the respiration inhibition of a saturated solution of permethrin upon sewage sludge microorganisms. Standard laboratory equipment was employed, and oxygen consumption of the microorganisms after 30 mins and 3 hours exposure was measured using potentiometric methods.
5.2	**Results and discussion**	The saturated solution had a measured concentration of 740 µg l^{-1}. This was tested at two levels; 284 ml diluted to 500 ml (nominal 420 µg l^{-1}) and 30 ml diluted to 500 ml (nominal 44 µg l^{-1}). Both concentrations are above the solubility limit of permethrin (<5 µg l^{-1}), implying a large amount of adsorption to the organisms, or similar exposure to bioavailable material. No effects were observed at either concentration. The response of the microorganisms to 3,5-DCP indicated the organisms were responding within expected quality standards.
5.2.1	**EC_{20}**	No effects observed, >420 µg l^{-1}
5.2.2	**EC_{50}**	No effects observed >420 µg l^{-1}
5.2.3	**EC_{80}**	No effects observed >420 µg l^{-1}
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. Analysis of the saturated solution ensured organisms were exposed. No effects were observed at levels considerably above the solubility, and hence expected environmental concentrations.
5.3.1	**Reliability**	1

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**
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5.3.2	**Deficiencies**	Yes – pH data was not reported. However, since the organisms responded to DCP within guideline limits, this is not considered to have affected the validity of the findings.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	12/05/05
Materials and Methods	State if the applicants version is 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	