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

Criteria	**Details**
Dispersion	Yes
Vehicle	No
Concentration of vehicle	No vehicle used
Vehicle control performed	No vehicle used
Other procedures	Test performed on saturated solutions of permethrin

Table A7.4.1.4-2: Inoculum / Test organism

Criteria	**Details**
Nature	Activated sludge
Species	Not applicable
Strain	Not applicable
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	Pforzheim, Germany
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	1L sludge (initial MLSS 8 g l^{-1}) was collected on the dat of the test. It was washed twice with tapwater by centrifugation, resuspended in 2L tap water and aerated with an air pump. The MLSS was adjusted to 1.6 g l^{-1} in the test medium.
Pretreatment	No pretreatment
Initial cell concentration	The MLSS was adjusted to 1.6 g l^{-1} in the test medium.

Table A7.4.1.4-3: Test system

Criteria	**Details**
Culturing apparatus	BOD flasks
Number of culture flasks/concentration	2 × Controls, 3 × 3,5-DCP controls, 6 × permethrin technical sat'd solution diluted (see experimental design: Table A7.4.1.4-5), 1 × permethrin technical sat'd solution
Aeration device	WISA air pump
Measuring equipment	Potentiometric recorder
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.4-4: Test conditions

Criteria	Details
Test temperature	20 ± 2°C
pH	No pH data was reported
Aeration of dilution water	Yes – 0.5 to 1.0 l min^{-1}
Suspended solids concentration	4 g l^{-1} dry mass

Table A7.4.1.4-5: Experimental design

Study group	Synthestic waste water (ml)	Tap water (ml)	Permethrin sat'd stock solution (ml)	DCP stock solution (ml)	Activated sludge (ml)
Control 1	16	284	0	0	200
Permethrin technical	16	254	30	0	200
Permethrin technical	16	0	284	0	200
DCP 5 mg l^{-1}	16	279	0	5	200
DCP 15 mg l^{-1}	16	269	0	15	200
DCP 30 mg l^{-1}	16	254	0	30	200
Control 2	16	284	0	0	200

Table A7.4.1.4-6: Results

30 min Test vessel	mg O_2/L Start	mg O_2/L End	Δ mg O_2/L	Time min	mg O_2/L/min	mg O_2/L/h	R_{C1} + R_{C2}	% Inhibition
Control 1	6.95	2.60	4.35	14.5	0.30	18.0	35.4	-1.7
Ref. 5 mg/L	6.20	3.40	2.80	12.9	0.22	13.2	35.4	25.4
Ref. 15 mg/L	6.75	5.50	1.25	11.2	0.11	6.6	35.4	62.7
Ref 30 mg/L	7.70	7.20	0.50	11.0	0.05	3.0	35.4	83.1
sat.1	6.90	2.80	4.10	13.0	0.32	19.2	35.4	-8.5
sat.2	5.40	0.95	4.45	12.2	0.36	21.6	35.4	-22.0
sat.3	5.70	1.80	3.90	11.2	0.35	21.0	35.4	-18.6
sat.4	6.00	1.05	4.95	14.9	0.33	19.8	35.4	-11.9
sat.5	5.70	1.50	4.20	13.0	0.32	19.2	35.4	-8.5
sat.6	6.20	1.80	4.40	11.5	0.38	22.8	35.4	-28.8
sat. 248 mL	5.10	1.45	3.65	11.8	0.31	18.6	35.4	-5.1
Control 2	4.45	0.70	3.75	12.9	0.29	17.4	35.4	1.7
3h								
Control 1	7.20	3.95	3.25	12.7	0.26	15.6	31.8	1.9
Ref. 5 mg/L	7.55	5.60	1.95	11.3	0.17	10.2	31.8	35.8
Ref. 15 mg/L	6.30	5.60	0.70	11.4	0.06	3.6	31.8	77.4
Ref 30 mg/L	8.40	7.75	0.65	11.4	0.06	3.6	31.8	77.4
sat.1	5.20	2.40	2.80	10.6	0.26	15.6	31.8	1.9
sat.2	6.30	3.20	3.10	11.3	0.27	16.2	31.8	-1.9
sat.3	4.70	1.20	3.50	13.1	0.27	16.2	31.8	-1.9
sat.4	6.30	2.80	3.50	12.7	0.28	16.8	31.8	-5.7
sat.5	5.90	2.60	3.30	12.4	0.27	16.2	31.8	-1.9
sat.6	6.70	2.60	4.10	11.8	0.35	21.0	31.8	-32.1
sat.284 mL	6.60	3.10	3.50	12.6	0.28	16.8	31.8	-5.7
Control 2	5.80	2.00	3.80	14.1	0.27	16.2	31.8	-1.9

Section A7.4.2(1) Bioconcentration

Annex Point IIA7.5

Key Study

			Official use only
		1 REFERENCE	
1.1	**Reference**	Burgess, D.; 1989; Uptake, depuration and bioconcentration of carbon-14-permethrin by Bluegill Sunfish *(Lepomis macrochirus)*; ABC Lab Project No PC-0117; FMC No. 138E5489E1; ABC Final report 37676.; GLP; Unpublished study prepared by Analytical Biochemistry Laboratories, Inc. in cooperation with FMC Corp.	
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; EPA-FIFRA 165-4	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Individual radiolabelled *cis* and *trans* isomers of permethrin	
3.1.1	**Lot/Batch number**	Acid ^{14}C-labelled: M184:105-107 Alcohol ^{14}C-labelled: No number	
3.1.2	**Specification**	Acid ^{14}C-labelled: Labelled in the acid moiety Alcohol ^{14}C-labelled: Labelled in the alcohol moiety	
3.1.3	**Purity**	Acid ^{14}C-labelled: No data Alcohol ^{14}C-labelled: No data	
3.1.4	**Further relevant properties**	Acid ^{14}C-labelled: 0.55mCi in 5 ml DMF Alcohol ^{14}C-labelled: 24 µCi µmol^{-1} Spec Act.	
3.1.5	**Radiolabelling**	As above	
3.1.6	**Method of analysis**	Samples were analysed for total radioactivity by liquid scintillation counting.	
3.2	**Reference substance**	No	
3.3	**Testing/estimation procedure**		
3.3.1	**Test system/ performance**	**Test Water**: The test water used is from a deep well source, and is summarised in Table A7.4.2(1)-1 **Test Fish**: The test fish were Bluegill Sunfish (*Lepomis machrochirus*). They were obtained from Osage fisheries, Missouri. They were held for at least 14 days prior to testing. Prior to and	

Section A7.4.2(1) Bioconcentration
Annex Point IIA7.5

Key Study

during testing fish were fed a commercial fish food ad libitum. At test initiation, fish had a mean weight of 6.18 g and a mean length of 72 mm. At the end of the study, control fish were weighed and measured. Fish had a mean weight of 9.8 g and a mean length of 84 mm

Exposure system: Fish were exposed in a flowthrough system with test substance delivered by a modified proportional diluter delivering ^{14}C-permethrin in DMF (82 µl l^{-1}) to water at a concentration of 0.5 µg l^{-1}. There were 5 aquaria in total, 2 × acid radiolabelled permethrin (1 radioanalysis, one metabolite analysis), 2 × alcohol radiolabelled permethrin (1 radioanalysis, one metabolite analysis) and 1 × control tank. The test aquaria were immersed in a water bath set at 22 ± 2°C. Aerated well water was delivered at an average rate of 320 – 350 ml/min/aquarium, equivalent to 6.6 to 7.1 volume changes per day (working volume 100L).

Test procedure - exposure: Approximately 610 fish were impartially transferred in groups of 20 into the control and test aquaria, which had previously been dosed with the appropriate dosing solutions for a 4 day equilibration period. Loading was 120 fish per aquaria, equivalent to an initial loading of 7.4 g l^{-1}. Water and fish were sampled throughout the exposure period according to the regime described in Table A7.4.2(1)-2. Fish were dissected into edible and non-edible portions and frozen. Water samples were stored in refrigerators prior to analysis. Water and tissue samples were analysed for total radioactivity and sent to the sponsor for metabolite analysis. These data are not available for review.

Test procedure – depuration: On day 28, water was drained from the tanks to a depth of 8 cm and replaced with fresh well water. The process was repeated and the fish exposed to flowing, uncontaminated well water for 14 days. During the depuration phase fish and water were samples as described in Table A7.4.2(1)-2. Fish were dissected into edible and non-edible portions and frozen. Water samples were stored in refrigerators prior to analysis. Water and tissue samples were analysed for total radioactivity and sent to the sponsor for metabolite analysis. These data are not available for review.

Water Quality Parameters: Parameters (temp, pH, DO) were taken initially and as given in Table A7.4.2(1)-2.

3.3.2 Estimation of bioconcentration

Bioconcentration was measured as the ratio of radioactivity in the whole fish compared to the concentration in the water.

The uptake rate and depuration rate were determined by the Dow BIOFAC computer programme, a non-linear kinetic modelling program estimating k1 (uptake) k2 (depuration), BCF (steady state), time to 90% of steady state and time to reach 50% clearance were also calculated.

4 RESULTS

4.1 Experimental

Section A7.4.2(1) Bioconcentration
Annex Point IIA7.5

Key Study

	data			
4.1.1	**Mortality/behaviour**	No mortality or behavioural anomalies were observed.		
4.1.2	**Lipid content**	Not reported		
4.1.3	**Concentrations of test material during test**	See Table A7.4.2(1)-3		
4.1.4	**Bioconcentration factor (BCF)**	Acid ^{14}C-labelled:	570 ± 81	
		Alcohol ^{14}C-labelled:	500 ± 120	
4.1.5	**Uptake and depuration rate constants**	Acid ^{14}C-labelled:	k1	83 ± 10
			k2	0.15 ± 0.011
		Alcohol ^{14}C-labelled:	k1	76 ± 12
			k2	0.15 ± 0.029
4.1.6	**Depuration time**	Acid ^{14}C-labelled:	4.7 ± 0.34 days	
		Alcohol ^{14}C-labelled:	4.6 ± 0.86 days	
4.1.7	**Metabolites**	No metabolite analysis available for review		
4.1.8	**Other Observations**	No other observations		

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Bluegill sunfish were exposed to a flowthrough steady state concentration of 0.5 µg l^{-1} ^{14}C-radiolabelled permethrin (labelled individually in the acid- and alcohol- positions) for 28 days as described in EPA-FIFRA guidelines 165-4. Water and fish samples were taken at intervals and analysed for total radioactivity by LSC.		
5.2	**Results and discussion**	There was no measurable difference between acid and alcohol labelled permethrin, as would be expected.		
		BCF		
		Acid ^{14}C-labelled:	570 ± 81	
		Alcohol ^{14}C-labelled:	500 ± 120	
		Uptake rate constant		
		Acid ^{14}C-labelled:	k1	83 ± 10
		Alcohol ^{14}C-labelled:	k1	76 ± 12
		Depuration rate constant		
		Acid ^{14}C-labelled:	k2	0.15 ± 0.011
		Alcohol ^{14}C-labelled:	k2	0.15 ± 0.029
		DT_{50}		
		Acid ^{14}C-labelled:	4.7 ± 0.34 days	
		Alcohol ^{14}C-labelled:	4.6 ± 0.86 days	
5.3	**Conclusion**	Validity criteria can be considered as fulfilled.		

Section A7.4.2(1) Bioconcentration

Annex Point IIA7.5

		Key Study
		In fresh water the half life for depuration of tissue residues was approximately 4/5 days with approximately 80% of the accumulated residues depurated within 14 days. This data would indicate that bioconcentration in fish tissues would not significantly occur and any residues accumulated are readily eliminated, so biomagnification through the food chain through predation is unlikely.
5.3.1	**Reliability**	1
5.3.2	**Deficiencies**	Yes – samples were taken for metabolite analysis, but these data are not available for review.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*14/1/09*
Materials and Methods	*Applicant's version is acceptable, however, it should be noted that only one concentration was tested (Guidelines recommend at least two test concentrations).*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	*1-2*
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.2(1)-1 – Test water parameters

FMC Corporation
138E5489E1

PC-0117
ABC LABS NO. 37676-30 of 54

TABLE II

Chemical Characteristics of Well Water Used By
ABC's Aquatic Toxicology Division

Monthly/Daily Screens[a]	
Hardness	266-279 mg/l as $CaCO_3$
Alkalinity	306-320 mg/l as $CaCO_3$
pH	7.8-8.2
Conductivity	272-540 µMhos/cm
Total Organic Carbon	<1.0 ppm
Suspended Solids	0.50-0.70 ppm
Un-ionized Ammonia	<0.00262 ppm
Chlorine (TRC)	<0.05 ppm

Quarterly Screens[b]

Elements		Chlorinated Hydrocarbons	
Aluminum	<0.20 ppm	DDE	<0.05 ppb
Arsenic	<1.0 ppb	DDD	<0.05 ppb
Boron	0.312 ppm	DDT	<0.05 ppb
Cadmium	<0.005 ppm	Dieldrin	<0.05 ppb
Chromium	<0.050 ppm	α-BHC	<0.01 ppb
Cobalt	<0.050 ppm	β-BHC	<0.01 ppb
Copper	<0.025 ppm	γ-BHC	<0.01 ppb
Fluoride	0.98 ppm	Δ-BHC	<0.01 ppb
Iron	0.10 ppm	HCB	<0.01 ppb
Lead	<0.003 ppm	Endrin	<0.05 ppb
Mercury	<0.0004 ppm	H.E.	<0.01 ppb
Nickel	<0.040 ppm	Mirex	<0.1 ppb
Silver	<1.0 ppb	Methoxychlor	<0.1 ppb
Zinc	0.021 ppm	Toxaphene	<1.0 ppb
		PCB	
Organophosphate Insecticides		PCB	<0.50 ppb
Vapona	<0.5 ppb		
Thimet	<0.90 ppb		
Diazinon	<0.5 ppb		
Methyl Parathion	<0.5 ppb		
Ethyl Parathion	<0.5 ppb		
Ronnel	<0.5 ppb		
Malathion	<0.5 ppb		

[a]Represents the values measured during the testing period.

[b]Represents the values obtained from the screen of January, 1989.

Note: All raw data to support these values is on file at ABC Laboratories.

Table A7.4.2(1)-2 – Experimental design

FMC Corporation
138E5489E1

PC-0117
ABC LABS NO. 37676-31 of 56

TABLE III

Sampling Schedule for Radioanalysis, Metabolite Characterization and Water Quality Determinations During the Bioconcentration Study with Bluegill Sunfish (*Lepomis macrochirus*) Exposed to ^{14}C-Permethrin

Sample	Uptake Phase (Day) 0	1	3	7	14	21	28	Depuration Phase (Day) 1	3	7	10	14
Fish (number collected)												
Total ^{14}C-Residues in Fillet & Viscera												
Radioassay Treatment Chamber	0	3	3	3	3	15	15	3	3	3	3	3
Control Chamber	0	3	3	3	3	3	3	3	3	3	3	3
Total ^{14}C-Residues in Whole Fish												
Radioassay Treatment Chamber	0	3	3	3	3	5	5	3	3	3	3	3
Control Chamber	0	3	3	3	3	3	3	3	3	3	3	3
Metabolite Characterization												
Metabolite Treatment Chamber						60	60					
Control Chamber						20	20					
Total												
Radioassay Treatment Chamber	0	6	6	6	6	20	20	6	6	6	6	6
Metabolite Treatment Chamber	0	0	0	0	0	60	60	0	0	0	0	0
Control Chamber	0	6	6	6	6	20	20	6	6	6	6	6
Remaining Fish												
Radioassay Treatment Chamber	120	114	108	102	96	76	56	50	44	38	32	26
Metabolite Treatment Chamber	120	120	120	120	120	60	0	0	0	0	0	0
Control Chamber	120	114	108	102	96	76	56	50	44	38	32	26
Water												
Total ^{14}C-Residues (ml)												
Radioassay Treatment Chamber	20	20	20	20	20	20	20	20	20	20	20	20
Metabolite Treatment Chamber	20	20	20	20	20	20	20	20	20	20	20	20
Control Chamber	20	20	20	20	20	20	20	20	20	20	20	20
Metabolite Assay (ml)												
Radioassay Treatment Chamber	500	500	500	500	500	500	500	500	500	500	500	500
Metabolite Treatment Chamber	500	2000	500	500	2000	2000	2000	500	500	500	500	500
Mixing Box Chamber	500	1000	500	500	1000	1000	1000					
Control Chamber	500	500	500	500	500	500	500					
Water Quality	X	X	X	X	X	X	X	X	X	X	X	X

Table A7.4.2(1)-3 – Total radioactivity (Acid ^{14}C-radiolabelled permethrin)

FMC Corporation
138E5489E1

PC-0117
ABC LABS NO. 37676-32 of 54

TABLE IV

Total ^{14}C-Radioactivity Calculated as ^{14}C-Permethrin (Acid-Labelled) in Test Water and Fish Tissue During 28 Days Exposure and 14 Days Depuration with Bluegill Sunfish (*Lepomis macrochirus*)

	Total ^{14}C Concentration as ^{14}C-Permethrin (Acid-Labelled)[a]							
	Water		Fillet		Whole Fish		Viscera	
Day	Actual µg/l	Running Mean	µg/kg	BCF[b]	µg/kg	BCF[b]	µg/kg	BCF[b]
Uptake								
0[c]	0.44	—	—	—	—	—	—	—
1	0.30	0.37	13	35X	80	220X	130	350X
3	0.29	0.34	21	62X	82	240X	150	440X
7	0.35	0.35	43	120X	170	490X	200	570X
14	0.38	0.35	40	110X	150	430X	250	710X
21	0.34	0.35	59	170X	190	540X	300	860X
28	0.41	0.36	83	230X	220	610X	390	1100X
Depuration								
1	0.097	—	42	—	170	—	270	—
3	0.023	—	33	—	110	—	170	—
7	<MQL[d]	—	28	—	80	—	180	—
10	<MQL[d]	—	28	—	78	—	120	—
14	<MQL[d]	—	15	—	46	—	68	—

[a] All values have been rounded to represent two significant figures.

[b] Daily bioconcentration factor (BCF) obtained by dividing the tissue concentration by the mean measured water concentration up to and including the respective sampling day (running mean).

[c] Samples taken immediately prior to addition of fish.

[d] Below minimum quantifiable limit of 0.0218 µg/l for water.

Table A7.4.2(1)-4 – Total radioactivity (Alcohol ^{14}C-radiolabelled permethrin)

FMC Corporation
138E5489E1

PC-0117
ABC LABS NO. 37676-33 of 54

TABLE V

Total ^{14}C-Radioactivity Calculated as ^{14}C-Permethrin (Alcohol-Labelled) in Test Water and Fish Tissue During 28 Days Exposure and 14 Days Depuration with Bluegill Sunfish (Lepomis macrochirus)

	Total ^{14}C Concentration as ^{14}C-Permethrin (Alcohol-Labelled)[a]							
	Water		Fillet		Whole Fish		Viscera	
Day	Actual µg/l	Running Mean	µg/kg	BCF[b]	µg/kg	BCF[b]	µg/kg	BCF[b]
Uptake								
0[c]	0.25	—	—	—	—	—	—	—
1	0.26	0.26	7.6	29X	58	220X	73	280X
3	0.29	0.27	13	48X	62	230X	110	410X
7	0.35	0.29	27	93X	160	550X	230	790X
14	0.55	0.34	43	130X	170	500X	290	850X
21	0.49	0.37	59	160X	210	570X	300	810X
28	0.70	0.41	72	180X	210	510X	390	950X
Depuration								
1	0.096	—	43	—	140	—	220	—
3	<MQL[d]	—	53	—	110	—	220	—
7	<MQL[d]	—	47	—	95	—	160	—
10	<MQL[d]	—	24	—	54	—	92	—
14	<MQL[d]	—	14	—	56	—	68	—

[a] All values have been rounded to represent two significant figures.

[b] Daily bioconcentration factor (BCF) obtained by dividing the tissue concentration by the mean measured water concentration up to and including the respective sampling day (running mean).

[c] Samples taken immediately prior to addition of fish.

[d] Below minimum quantifiable limit of 0.0218 µg/l for water.

Section A7.4.2(2)
Annex Point IIIA, XIII.2.3

Bioaccumulation in an appropriate invertebrate species

		Additional information	Official use only
		1 REFERENCE	
1.1	**Reference**	Muir, D.C.G., Rawn, G.P, Townsend, B.E., Lockhart, W.L., and Greenhalgh, R.;1985; Bioconcentration of cypermethrin, deltamethrin, fenvalerate, and permethrin by Chironomus tentans larvae in sediment and water. Environmental Toxicology and Chemistry. 4:51-61; Not GLP; Published	
1.2	**Data protection**	No	
1.1.1	Data owner	No data protection claimed	
1.1.2	Companies with letter of access	No data protection claimed	
1.1.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No:	
2.3	**Deviations**	No: Protocol was not to any guidelines	
		3 MATERIALS AND METHODS	
3.1	**Test material**	^{14}C-Permethrin	
3.1.1	**Lot/Batch number**	No data	
3.1.2	**Specification**	No data	
3.1.3	**Purity**	No data, but reference states samples was purified by TLC prior to use	
3.1.4	**Further relevant properties**	No data	
3.1.5	**Radiolabelling**	Carbon-14 (cyclopropyl ring labelled) racemic mix of *cis*- and *trans*-Permethrin. No information on specific activity are provided	
3.1.6	**Specific chemical analysis**	**Water**: At 0, 12, 24 and 48 hours, 50 ml of water was extracted with dichloromethane and chromatographed by TLC. The proportion of unchanged (parent) compound was determined by scraping the plate, followed by LSC. 4 ml of water was assayed by LSC for total radioactivity in solution, following centrifugation at 20,000 g. **Sediment**: Sediment samples were extracted by refluxing with 1:1 acetone:hexane. The extracts were diluted with water, and the hexane fraction recovered. The aqueous phase was further extracted with DCM, and the hexane and DCM combined for LSC and TLC analysis. **Larvae tissue**:Individual animals were placed in a LSC vial and dissolved in 1.0 ml of tissue solubiliser. After 24 hours the solution	

Section A7.4.2(2) Annex Point IIIA, XIII.2.3	Bioaccumulation in an appropriate invertebrate species	
		was analysed for total radioactivity. **Analysis**: Analysis was via TLC – no data supplied
3.2	Reference substance	No
3.3	Test ing procedure	
3.3.1	test species	*Chironomus tentans* fourth-instar larvae (mean weight approximately 20 mg) were obtained from laboratory cultures.
3.3.2	Test system	Fifty grams of each sediment (sand, silty-clay river sediment [2.3% OC], pond bottom clay [3.7% OC] – no further details supplied) were treated with 0.01 and 0.1 ml solutions of permethrin to give 5 and 50 ng/g sediment concentrations. Sediments were then flooded with 25o ml dechlorinated water and equilibrated for 24 hours prior to addition of larvae. Larvae were added to a nylon screened glass container suspended in the water above the sediment, or directly to the water, where they established themselves in the sediment. All exposures were in duplicate, and larvae were sampled (3 per replicate) after 3, 6, 12, 24, 48 hours (water exposure) or 24 hours (sediment exposure). Following exposure, all remaining larvae were transferred to clean water/silica sand systems and sampled after 12, 24, 48 and 96 hours.
3.3.3	Initial TS concentration	5 and 50 ng g^{-1}
3.3.4	Duration of test	Bioaccumulation (water): 48 hours Bioaccumulation (sediment): 24 hours Depuration (water and sediment): 96 hours
3.3.5	Analytical parameter	Permethrin quantification by GC-ECD
3.3.6	Sampling	Bioaccumulation: 3, 6, 12, 24, 48 hours (water exposure) or 24 hours (sediment exposure) Depuration (water and sediment): 12, 24, 48 and 96 hours
3.3.7	Statistics	LSC results (dpm/g larvae) were converted to ng/g via specific activity calculation. BCFs (water exposure) were calculated from the mean water concentration (total ^{14}C). BCFs (sediment exposure) were calculated based on porewater or overlying water concentrations.
		4 RESULTS
4.1	Degradation of test substance	
4.2	Experimental data	

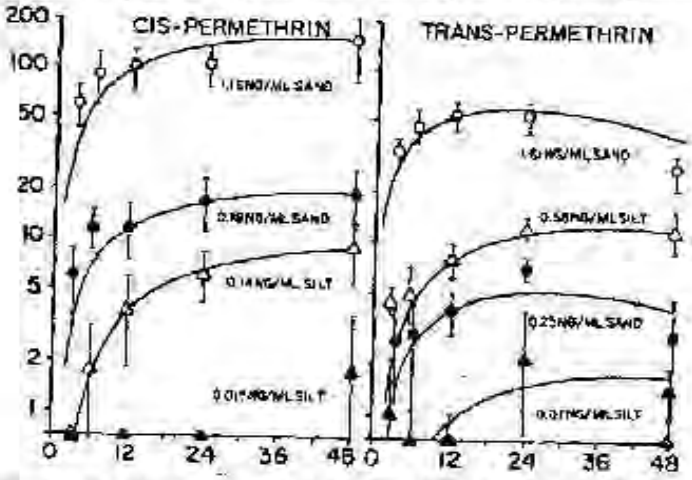
Section A7.4.2(2) Annex Point IIIA, XIII.2.3 — **Bioaccumulation in an appropriate invertebrate species**

4.2.1 Mortality/behaviour — No mortality was reported

4.2.2 Concentrations of test material during test — See Table A7.4.2(3)_1

Organism data are not presented.

BCF data are presented graphically, Log[concentration] (ng/g) against time (hours). Concentrations in larvae above silt did not differ significantly from clay, therefore the clay data were not presented.



4.2.3 Bioconcentration factor (BCF) — Levels of radioactivity reached a steady state in 12 to 24 hours in water exposure larvae. BCF data are presented in Table A7.4.2(3)_2

4.2.4 Uptake and depuration rate constants and Depuration time — Depuration data are presented in Table A7.4.2(3)_3

4.3 Estimation of bioconcentration — Very hydrophobic chemicals that are rapidly metabolized may not have very high BCFs (at least not as high as would be predicted on the basis of hydrophobicity alone) because the body may rapidly eliminate what it has absorbed. Highly lipophilic synthetic pyrethroid insecticides are rapidly metabolized by esterases; therefore these compounds have very low bioconcentration factors.

The observed BCF (logBCF 1 to 2) and the rapid loss from the organism once exposure had terminated indicate permethrin is unlikely to bioaccumulate in the environment.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods — The accumulation of ^{14}C permethrin in chironomids in water and sediment was measured by comparison of total activity in the water column or sediment to the total body concentration in the organisms.

The test was performed in glass vessels with water exposure animals being suspended above the sediment in nylon mesh. Because of the rapid kinetics of the adsorption of permethrin to sediment, the non-specific adsorption onto the nylon is not considered to have

Section A7.4.2(2) Annex Point IIIA, XIII.2.3	**Bioaccumulation in an appropriate invertebrate species**
	adversely affected the test.
5.2 Results and discussion	The results indicate that; • Permethrin rapidly bioaccumulates (Log BCF 1 to 2). • Depuration is rapid following end of exposure period
5.3 Conclusion	The authors present no numerical data on the body concentrations to support the calculated BCF values. However, the methodology appears robust, and the inclusion of ^{14}C material adds credibility to the derived BCF values. No mass balance data are presented.
5.3.1 Reliability	2
5.3.2 Deficiencies	Not applicable

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*14/1/09*
Materials and Methods	*Specific data on BCF not provided, however the Materials and Methods can be accepted when considered in concert with the Discussion section.*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	*2-3*
Acceptability	*acceptable*
Remarks	*The authors present no numerical data on the body concentrations to support the calculated BCF values and no mass balance data are presented. Such non-inclusions are typical for articles in peer-reviewed journals. However, it should not detract from the overall assessment of the quality of the endpoint as the methodology appears robust and the results are considered to be useful as supplementary data.*
	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 A7.4.2(2)_1: Concentration measured during test

	Sediment	Conc (ng g^{-1})	Water (ng ml^{-1})				Porewater @ 24 hours
			0	12	24	48	
Trans-permethrin	Sand	50	1.61	0.96	0.57	0.20	5.12
		5	0.25	0.15	0.140	0.04	0.92
	Silt	50	0.58	0.41	0.29	0.14	0.55
		5	0.07	0.05	0.04	0.02	0.08
	Clay	50	0.99	0.47	0.22	0.05	0.32
		5	0.07	0.06	0.05	0.03	0.07
Cis-permethrin	Sand	50	1.16	0.80	0.55	0.26	2.70
		5	0.18	0.14	0.11	0.06	0.53
	Silt	50	0.14	0.13	0.12	0.11	0.19
		5	0.02	0.02	0.01	0.01	0.02
	Clay	50	0.14	0.13	0.11	0.09	0.14
		5	0.04	0.04	0.04	0.04	0.07

Table A7.4.2(2)_2: Bioconcentration factors (24 hour) ng g^{-1} larvae:ng ml^{-1} water

	Sediment	Conc (ng g^{-1})	Water	Sediment	Sediment/porewater
Trans-permethrin	Sand	50	67 ± 13	134 ± 24	21 ± 4
		5	49 ± 9	136 ± 33	21 ± 5
	Silt	50	26 ± 13	65 ± 20	50 ± 16
		5	25 ± 24	25 ± 27	19 ± 21
	Clay	50	51 ± 14	67 ± 30	50 ± 22
		5	69 ± 23	12 ± 17	9 ± 13
Cis-permethrin	Sand	50	166 ± 49	298 ± 122	71 ± 29
		5	135 ± 44	333 ± 71	79 ± 17
	Silt	50	47 ± 16	415 ± 86	296 ± 61
		5	83 ± 81	38 ± 60	27 ± 43
	Clay	50	71 ± 33	113 ± 31	82 ± 23
		5	8 ± 16	29 ± 48	21 ± 35

Table A7.4.2(2)_3: Depuration rates x10^2 (h^{-1}) and half-lives (h)

	Sediment	Conc (ng g^{-1})	Water	Sediment	Mean DT50
Trans-permethrin	Sand	50	3.47 ± 0.28	2.69 ± 0.30	23
	Silt	50	1.75 ± 0.57	3.50 ± 0.52	26
	Clay	50	1.02 ± 0.57	2.94 ± 0.57	35
Cis-permethrin	Sand	50	NA	3.16 ± 0.51	22

	Silt	50	4.54 ± 1.14	3.45 ± 0.52	17
	Clay	50	4.61 ± 0.86	0.78 ± 0.18	26

Section 7.4.3.1	Prolonged toxicity to an appropriate species of fish	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure** [X]	**Technically not feasible** [ ] **Scientifically unjustified** [ ] **Other justification** [X]	
Detailed justification:	• Permethrin has a water solubility of <5 µg l^{-1}. (White D.F, Mullee, D.M; 2004). • Permethrin rapidly adsorbs to sediment in aquatic systems (Hatfield, M.W., 1996) • The Technical Guidance on Data Requirements for Active Substances states that usually this test is not required. Based on the physical-chemical parameters of permethrin, prolonged toxicity is not likely to be a concern for permethrin. Therefore a justification for non-submission is suggested based upon the limited exposure to permethrin in wood-preservative use.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	21/04/05	
Evaluation of applicant's justification	Applicant's justification is deemed acceptable	
Conclusion	Adopt applicant's justification	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	Give date of comments submitted	
Evaluation of applicant's justification	Discuss if deviating from view of rapporteur member state	
Conclusion	Discuss if deviating from view of rapporteur member state	
Remarks		

Section 7.4.3.2(1) Effects on reproduction and growth rate of fish

Annex Point IIIA XIII 2.2

Key Study

Official use only

		1 REFERENCE
1.1	**Reference**	Spehar, R.L, Tanner, D.K., Nordling, B.R.; 1983; Toxicity of the synthetic pyrethroids, permethrin and AC222,705 and their accumulation in early life stages of fathead minnows and snails; Aquatic toxicology; 3; 171-182; not GLP; Published
1.2	**Data protection**	No
1.2.1	**Data owner**	No data protection claimed
1.2.2	**Companies with letter of access**	No data protection claimed
1.2.3	**Criteria for data protection**	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No – peer reviewed journal.
2.2	**GLP**	No – US EPA Research laboratory, Duluth
2.3	**Deviations**	No – No guidelines followed
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	**Lot/Batch number**	Not specified
3.1.2	**Specification**	Supplied by ICI America – no detail supplied
3.1.3	**Purity**	92%
3.1.4	**Composition of Product**	Not applicable
3.1.5	**Further relevant properties**	Low water solubility.
3.1.6	**Method of analysis**	Water samples were extracted with hexane and analysed on a packed bed gas chromatography system employing an electron capture detector
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Because of the extremely low water solubility, and to avoid the use of carrier solvents, a saturated toxicant solution was prepared and maintained in a saturator system. A concentration of 16 $\mu g\ l^{-1}$ was maintained in the saturator.
3.3	**Reference substance**	No
3.4	**Testing procedure**	
3.4.1	**Dilution water**	see table A7.4.3.2(1)-2

Section 7.4.3.2(1) Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

3.4.2	**Test organisms**	see table AA7.4.3.2(1)-3
3.4.3	**Handling of embryos and larvae (OECD 210/212)**	25 embryos (<1 day old) were transferred into 120 ml glass jars with 40 mesh nytex screen bottoms that were oscillated in the chambers for hatchability studies. After all embryos hatched (4 to 5 days), 15 larvae were distributed to randomised replicate chambers to determine effects on larval survival for a period of 28 days. All animals were fed 3 to 5 ml brine shrimp nauplii 3 times per day (once per day on weekends). After 32 days total exposure, fish were killed in ice water, blotted dry and weighed to the nearest mg.
3.4.4	**Test system**	see table AA7.4.3.2(1)-4
3.4.5	**Test conditions**	see table AA7.4.3.2(1)-5
3.4.6	**Duration of the test**	4 days embryo to hatching, 28 days larval exposure
3.4.7	**Test parameter(s)**	Embryo hatchabilty, normal larvae at hatch, larval survival and larval growth (weight)
3.4.8	**Examination / Sampling**	Visual observations were made daily
3.4.9	**Monitoring of TS concentration**	Samples were taken for analysis twice weekly. Volumes were extracted in hexane and analysed on the following system; Column: 1.0m × 2mmid glass Packing: 3% OV7 on 80/100 mesh GasChromQ Column. Temp: 220°C Injector Temp: No data Detector Temp: No data Carrier gas: 5% methane in Argon The limit of detection of this procedure was 0.01 $\mu g\ l^{-1}$ and the mean percentage and SD recovery for 13 spiked samples was 91 ± 8%
3.4.10	**Statistics**	Survival and hatchability were transformed to arcsin and individual weights pooled before data were subjected to one way analysis of variance and Dunnetts one-sided comparison of treatment means to the control means.

4 RESULTS

	Range finding test	Not performed
	Results test substance	
4.1.1	**Initial concentrations of test substance**	Not reported
4.1.2	**Actual concentrations of test substance**	See table A7.4.3.2(1)-8

Section 7.4.3.2(1) Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

4.1.3	**Effect data**	Give the mortality/survival data at embryo, larval and juvenile stages as well as overall mortality/survival and report: **See table A7.4.3.2(1)-8** Time to start of hatching and end of hatching: **All hatched between 4-5 days** Numbers of larvae hatching each day: **Not reported** Length and weight of surviving animals: **Only weight reported, See table A7.4.3.2(1)-8** Numbers of deformed larvae: **See table A7.4.3.2(1)-8** Numbers of fish exhibiting abnormal behaviour: **Most of the surviving larvae at 1.4 µg l^{-1} were convulsing for short periods of time. Four days after hatch, only one larva remained alive.** NOEC Embryo hatchability: **1.4 µg l^{-1}** NOEC normal larvae at hatch: **1.4 µg l^{-1}** NOEC larval survival: **0.66 µg l^{-1}** NOEC larval growth (weight): **0.66 µg l^{-1}**
4.1.4	**Concentration / response curve**	Not applicable
4.1.5	**Other effects**	Most of the surviving larvae at 1.4 µg l^{-1} were convulsing for short periods of time. Four days after hatch, only one larva remained alive.
	Results of controls	
4.1.6	**Number/ percentage of animals showing adverse effects**	See table A7.4.3.2(1)-8
4.1.7	**Nature of adverse effects**	See table A7.4.3.2(1)-8
	Test with reference substance	Not performed

Section 7.4.3.2(1) Effects on reproduction and growth rate of fish
Annex Point IIIA XIII 2.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	Materials and methods	The methodology, although not to any specific guidelines, follows closely the requirements of OECD210. Fathead minnow embryos from in-house cultures were exposed to steady state concentrations of permethrin in a flowthrough system until hatching when they were transferred to holding vessels and exposed to the same flowthrough concentrations for a further 28 days. Stable concentrations were delivered via a saturation system and steady flow micro pumps. Observations on health and survival were made daily. At the end of the exposure period, surviving embryos were sacrificed and weighed for assessment of growth effects.
5.2	Results and discussion	Test concentrations were maintained throughout the duration of the exposure period. The results are presented in Table A7.4.3.2(1)-8, and indicate larvae are more sensitive than embryos. The results also show a very severe cut-off for toxic action, and that if exposed larvae are not killed, then no other observable growth effects are likely to occur. The sole surviving larvae in the top concentration appeared to have achieved more growth, although SD for growth in the controls indicate this length may fall within the normal growth curve.
5.2.1	NOEC	0.66 $\mu g\ l^{-1}$
5.2.2	LOEC	1.4 $\mu g\ l^{-1}$
5.3	Conclusion	In respect to the validity criteria: • The dissolved oxygen was maintained at greater than 60%. • There is no reported information to show that the difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species, but the authors maintain the temperature was between 25 ± 2°C for the duration of the exposure period, and this is within the recommended range for fathead minnow. • Overall survival of fertilized eggs in controls was within the criteria >66%, and overall larval survival was >70% • Not enough data are presented to indicate whether test substance concentrations maintained within ± 20% of mean measured values, however the maximum SD at the critical concentration around the LOEC-NOEC were <25%.
5.3.1	Other Conclusions	None
5.3.2	Reliability	2
5.3.3	Deficiencies	Yes – Full data on individual animals were not presented, and not enough detail on exposure concentrations were presented. These non-inclusions are typical for articles in peer-reviewed journals. It

Section 7.4.3.2(1) **Effects on reproduction and growth rate of fish**
Annex Point IIIA XIII 2.2

should not detract from the overall assessment of the quality of the endpoint.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*21/04/05*
Materials and Methods	*Data on exposure concentrations and full data on individual organisms are lacking applicants version is acceptable*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	*2*
Acceptability	acceptable
Remarks	Full data on individual animals were not presented, and not enough detail on exposure concentrations were presented. These non-inclusions are typical for articles in peer-reviewed journals. It should not detract from the overall assessment of the quality of the endpoint.
	COMMENTS FROM ... ***(specify)***
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	No
Other procedures	Because of the extremely low water solubility, and to avoid the use of carrier solvents, a saturated toxicant solution was prepared and maintained in a saturator system (Phipps & Holcombe; 1982; Prog. Fish cult., **44**, 115-116). A concentration of 16 µg l^{-1} was maintained in the saturator.

Table A7.4.3.2(1)-2: Dilution water

Criteria	Details
Source	Lake superior water, filtered through sand and sterilised with ultraviolet light.
Salinity	Not applicable
Hardness	Hardness (mg l^{-1} $CaCO_3$) 34 – 38 Alkalinity (mg l^{-1} $CaCO_3$) 37 – 46 Acidity (mg l^{-1} $CaCO_3$) 1.0 – 4.4
pH	7.4 – 7.9
Oxygen content	5.5 – 7.8 mg l^{-1}
Conductance	Not reported
Holding water different from dilution water	No

Table A7.4.3.2(1)-3: Test organisms

Criteria	Details
Species/strain	Fathead minnow (Pimephales promelas)
Source	In-house culture unit
Wild caught	No
Age/size	Embryos, <1 day old
Kind of food	On hatching, larvae were fed brine shrimp nauplii
Amount of food	3- 5 ml
Feeding frequency	3 times per day (Once on weekends)
Post-hatch transfer time	Not reported
Time to first feeding	Not reported
Feeding of animals during test	Yes – On hatching, larvae were fed brine shrimp nauplii, 3- 5 ml, 3 times per day (once on weekends)
Treatment for disease within 2 weeks preceeding test	Not applicable

Table A7.4.3.2(1)-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	Tests were conducted with a continuous flow mini-diluter exposure system which delivered five concentrations and a control to 4 replicate exposure chambers per concentration. The flow rate to each chamber was 12.5 ± 1 ml min^{-1}.
Volume of test vessels	Glass chambers measure 7 × 19 × 9 cm with a working volume of 600 ml.
Volume/animal	40 ml (hatchlings)
Number of animals/vessel	25 embryos, 15 hatchlings
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.2(1)-5: Test conditions

Criteria	Details
Test temperature	25 ± 2°C
Dissolved oxygen	5.5 – 7.8 mg l^{-1} (65 – 100%)
pH	7.4 – 7.9
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Sylvania cool-white fluorescent bulbs were used, providing an intensity of 2 to 6 lux at the water surface
Photoperiod	16:8 hour light:dark photoperiod.

Table A7.4.3.2(1)-6: Validity criteria for fish tests according to OECD Guidelines 210/212

	fulfilled	Not fullfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	X	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	(X)	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	X	
Test substance concentrations maintained within ± 20% of mean measured values	(X)	
No effect on survival nor any other adverse effect found in solvent control	(X)	
Further criteria for poorly soluble test substances	(X)	

Table A7.4.3.2(1)-8: Results

Measured concentration ($\mu g\ l^{-1}$)	Embryo hatchability[a] (%)	Normal larvae at hatch[a] (%)	Survival[b] (%)	Mean weight (mg)
<0.01[c] (control)	95 ± 3.8	94 ± 2.3	92 ± 13.0	96 ± 25 (55)[e]
0.11 ± 0.04[d]	99 ± 2.0	98 ± 2.3	97 ± 6.0	89 ± 24 (59)
0.18 ± 0.03	96 ± 3.3	96 ± 3.3	97 ± 6.5	96 ± 27 (57)
0.33 ± 0.08	95 ± 3.8	95 ± 3.8	97 ± 4.0	91 ± 26 (58)
0.66 ± 0.16	94 ± 2.3	92 ± 5.7	93 ± 9.4	93 ± 26 (56)
1.40 ± 0.12	95 ± 5.0	95 ± 5.0	2 ± 3.5[f]	110 (1)

a) Hatchability based on 100 embryos per concentration
b) Survival based on 60 larvae per concentration
c) Detection limit of analyses
d) Mean ± SD of 10 analyses
e) Number of fish weighed in parentheses
f) Values significantly less than controls

Section 7.4.3.2 (2)
Annex Point IIIA XIII 2.2

Effects on reproduction and growth rate of fish

Supportive data

Official use only

		1 REFERENCE
1.1	**Reference**	Hansen D.J., Goodman L.R, Moore J.C., Higdon P.K.; 1983; Effect of the synthetic pyrethroids AC222,705, permethrin and Fenvalerate on Sheepshead minnows in early life stages toxicity test ; Environmental Toxicology and Chemistry; 2; pp 251-258; not GLP; Published
1.2	**Data protection**	No
1.2.1	**Data owner**	No data protection claimed
1.2.2	**Companies with letter of access**	No data protection claimed
1.2.3	**Criteria for data protection**	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No – peer reviewed journal.
2.2	**GLP**	No – US EPA Research laboratory
2.3	**Deviations**	No – No guidelines followed
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	**Lot/Batch number**	Not specified
3.1.2	**Specification**	Supplied by ICI America – no detail supplied
3.1.3	**Purity**	93%
3.1.4	**Composition of Product**	Not applicable
3.1.5	**Further relevant properties**	Low water solubility.
3.1.6	**Method of analysis**	Seawater extracted twice with petroleum ether. The petroleum ether was dried over glass wool and reduced in volume by kuderna-danish. Analysis was via GC-ECD
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Test material was dissolved in triethylene glycol. Solvent concentration was constant at 9 mg/L.
3.3	**Reference substance**	No
3.4	**Testing procedure**	
3.4.1	**Dilution water**	see table A7.4.3.2(2)-2
3.4.2	**Test organisms**	Sheepshead minnows; see table A7.4.3.2(2)-3
3.4.3	**Handling of embryos and larvae**	20 embryos were randomly assigned to each of the four incubation cups per treatment. Embryos and hatched fish were examined for

Section 7.4.3.2 (2)
Annex Point IIIA XIII 2.2

Effects on reproduction and growth rate of fish

	(OECD 210/212)	survival and fish were fed *artemia nauphii* twice daily (once daily on week-end)
3.4.4	**Test system**	see table A7.4.3.2(2)-4
3.4.5	**Test conditions**	see table A7.4.3.2(2)-5
3.4.6	**Duration of the test**	4 days embryo to hatching, 28 days larval exposure
3.4.7	**Test parameter(s)**	Embryonic development, hatching success and survival and growth of hatched fish (length)
3.4.8	**Examination / Sampling**	Visual observations were made daily
3.4.9	**Monitoring of TS concentration**	Samples were taken for analysis at least weekly
3.4.10	**Statistics**	Analysis of variance and duncan's multiple range test were used to determine treatment differences in embryos hatching, juvenile survival and effects on length (α=0.05)
		4 RESULTS
4.1	**Range finding test**	Not performed
4.2	**Results test substance**	
4.2.1	**Initial concentrations of test substance**	Not reported
4.2.2	**Actual concentrations of test substance**	See table A7.4.3.2(2)-7
4.2.3	**Effect data**	In the 42 µg/L concentration, 64% of the fish were dead on day 5; all were dead on day 6. Fish in the 22 µg/L concentration began to die on day 5(13% mortality) and all but two (98% mortality) were dead by day 9. Survival in concentrations < 10 µg/L was unaffected (Table A7.4.3.2(2)-8). Time-to hatching, survival of embryos to hatching and standard lengths of fish surviving exposure to permethrin were not different from those of the control. Fifty-three percent of the fish had hatched by the third day of exposure, but none appeared affected. On the fourth day, 99% had hatched and fish in 22 and 42 µg/L concentrations were visibly affected. Affected fish swam abnormally, their heads moving laterally far in excess of normal. Severely affected fish were lethargic, and some were flexed as much as 90° at mid body.
4.2.4	**Concentration / response curve**	Not applicable
4.2.5	**Other effects**	Fish in 22 and 42 µg/L concentrations swam abnormally, their heads moving laterally far in excess of normal. Severely affected fish were lethargic, and some were flexed as much as 90° at mid body.

Section 7.4.3.2 (2)
Annex Point IIIA XIII 2.2

Effects on reproduction and growth rate of fish

4.3	**Results of controls**	
4.2.6	**Number/ percentage of animals showing adverse effects**	See table A7.4.3.2(2)-7
4.2.7	**Nature of adverse effects**	See table A7.4.3.2(2)-7
4.4	**Test with reference substance**	Not performed
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	An early fish toxicity study was conducted with permethrin on sheephead minnows for 28 days. Fish were exposed to a range concentration of permethrin from 1.6 to 42 µg/L (mean measured) in an intermittent flow system. Embryonic development, hatching success and survival and growth of hatched fish (length) was recorded daily.
5.2	**Results and discussion**	Survival of newly hatched sheepshead minnows is reduced at and above 22 µm/L, but not affected below at and below 10 µm/L. Time-to hatching, survival of embryos to hatching and standard lengths of fish surviving exposure to permethrin were not different from those of the control. Fifty-three percent of the fish had hatched by the third day of exposure, but none appeared affected. On the fourth day, 99% had hatched and fish in 22 and 42 µg/L concentrations were visibly affected. Affected fish swam abnormally, their heads moving laterally far in excess of normal. Severely affected fish were lethargic, and some were flexed as much as 90° at mid body.
5.2.1	**NOEC**	10 µg/L
5.2.2	**LOEC**	20 µg/L
5.3	**Conclusion**	Based on the measured concentrations, the no-observed-effect concentration (NOEC) was 10 µg/l.
5.3.1	**Other Conclusions**	Validity criteria, see table A7.4.3.2(2)-6
5.3.2	**Reliability**	2
5.3.3	**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	*14/1/09*
Materials and Methods	*Applicant's version is acceptable.*
Results and discussion	*Adopt applicant's version*

Section 7.4.3.2 (2)
Annex Point IIIA XIII 2.2

Effects on reproduction and growth rate of fish

Conclusion	*Adopt applicant's version*
Reliability	*2*
Acceptability	acceptable
Remarks	*It was not reported whether or not test substance concentrations were maintained within ± 20% of mean measured values (validity criteria).*
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.3.2(2)-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes
Concentration of vehicle	9 mg/L
Vehicle control performed	No
Other procedures	No

Table A7.4.3.2(2)-2: Dilution water

Criteria	Details
Source	Not reported
Salinity	22 to 32%
Hardness	Not reported
pH	Not reported
Oxygen content	3.8 – 6.6 mg l^{-1}
Conductance	Not reported
Holding water different from dilution water	No

Table A7.4.3.2(2)-3: Test organisms

Criteria	Details
Species/strain	Sheepshead minnows
Source	Eggs obtained from hormone-injected female and fertilized by using 5 or more male.
Wild caught	No
Age/size	Embryos, <1 day old
Kind of food	*Artemia salina*
Amount of food	
Feeding frequency	2 times per day (Once daily on weekends)
Post-hatch transfer time	Not reported
Time to first feeding	Not reported
Feeding of animals during test	Yes
Treatment for disease within 2 weeks preceeding test	Not applicable

Table A7.4.3.2(2)-4: Test system

Criteria	Details
Test type	Intermittent flow system
Renewal of test solution	The diluter delivered 0.5 litres of test solution during each cycle, 130 to 260 cycles/day to each of the two replicate aquaria per treatment.
Volume of test vessels	Aquaria contained 2.5 litres of solution when full and 1.0 litre of solution following cycling of self starting siphon, which ensured water exchange in the two incubation cups in each aquarium.
Volume/animal	40 embryos per aquarium
Number of animals/vessel	20 embryos per cups (consisting of 9 cm i.d. Petri dish bottom to which a 10 cm high, 450 µm nylon mesh cylinder was attached).
Number of vessels/ concentration	Four incubation cups /treatment
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.2(2)-5: Test conditions

Criteria	Details
Test temperature	30 ± 1.5°C
Dissolved oxygen	3.8 – 6.6 mg l^{-1} (58 – 100%)
pH	none
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Not reported
Photoperiod	12 h

Table A7.4.3.2(2)-6: Validity criteria for fish tests according to OECD Guidelines 210/212

	fulfilled	Not fullfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	X	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	X	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	X	

Test substance concentrations maintained within ± 20% of mean measured values	**not reported**	
No effect on survival nor any other adverse effect found in solvent control	X	
Further criteria for poorly soluble test substances		

Table A7.4.3.2(2)-7: Results

Nominal	Measured concentration ($\mu g\ l^{-1}$)	Embryo survival (%)	Fry survival (%)	Combined Survival[b] (%)	Average standard length (mm)
control	ND[a]	95	97	92	9.8
1.25	1.6 ± 0.13	99	96	95	10.0
2.5	2.4 ± 0.36	99	99	98	10.2
5.0	5.6 ± 0.93	99	80	79	10.0
10.0	10.0 ± 2.6	98	99	96	9.8
20.0	22 ± 2.9	96	1[b]	1[b]	15.5[c]
40.0	42 ± 2.1	99	0[b]	0[b]	-

a)ND not detected <0.25µg/L

b)Significantly different from control (α =0.05)

c) Excluded from statistical analysis because high mortality and feeding bias

Section A7.4.3.2(3) Annex Point IIIA, XIII.2.3.	Effects on reproduction and growth rate of fish	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [X]	
Limited exposure [ ]	Other justification [ ]	
Detailed justification:	**Introduction** Within the evaluation of permethrin as a biocide the RMS raised the question of potential endocrine effects of permethrin, which will be addressed by BCS in this statement. **Absence of endocrine activity of permethrin** There is no evidence from studies performed in toxicology for human safety assessment that permethrin has any endocrine activity. The conclusion from the relevant studies is: Permethrin has no effects on reproductive indices nor fertility nor reproductive tissues and organs as shown in the multi-generation study in rats. No effects on any endocrine organs or reproductive tissues were observed in rats or mice in long term studies. These results support the conclusion that permethrin is not a reproductive toxin or endocrine disrupter in mammals. In the area of ecotoxicology studies on fish have been performed that allow an assessment as to whether permethrin may exert endocrine activity in fish. The acute LC50(96h) to Rainbow Trout (Salmo gairdneri,Maddock, B.G.; 1978) was determined as 9.0 µg/L. In an Early Life Stage Test (ELS, Spehar, R.L, Tanner, D.K., Nordling, B.R.; 1983) on fathead minnows the NOEC was 0.66 µg/L based on larval survival. Endocrine disrupting compounds are characterized by an acute-to-chronic ratio (ACR, acute LC50 divided by NOEC) in the range of several hundreds to thousands. For permethrin this ACR is 13 based on the ELS study and the acute toxicity study thus within the typical range of compounds showing general toxicity. The Acute to Chronic ratio around 10 and the relevance of lethality as endpoint adds further evidence to the conclusion that permethrin is lacking endocrine activity in fish. Taking all these data together there is no evidence that permethrin may be an endocrine disrupter in animals and man. **References** Maddock, B.G.; 1978; Determination of the Acute Toxicity of Compound 21z (WRL) to Rainbow Trout (Salmo gairdneri) Using Dimethyl Sulphoxide as the Solvent. The Wellcome Foundation Ltd. Report No. HEFG 78-11; Not GLP; Unpublished	

Section A7.4.3.2(3) Annex Point IIIA, XIII.2.3.	**Effects on reproduction and growth rate of fish**
	Spehar, R.L, Tanner, D.K., Nordling, B.R.; 1983; Toxicity of the synthetic pyrethroids, permethrin and AC222,705 and their accumulation in early life stages of fathead minnows and snails; Aquatic toxicology; 3; 171-182; not GLP; Published
Undertaking of intended data submission [ ]	
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	21/04/05
Evaluation of applicant's justification	Accept applicant's justification
Conclusion	Adopt applicant's justification
Remarks	
	COMMENTS FROM OTHER MEMBER STATE (specify)
Date	Give date of comments submitted
Evaluation of applicant's justification	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Remarks	

Section A7.4.3.3.1 Annex Point IIIA, XIII.2.3.	Bioaccumulation in an appropriate species of fish	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [X] Other justification [ ]	
Detailed justification:	The Technical Guidance on Data Requirements for Active Substances states that this is required where there is a risk of secondary poisoning. The risk to the fish-eating predators (mammals and/or birds) is calculated as the ratio between the concentration in their food ($PEC_{oral, predator}$) and the no-effect-concentration for oral intake ($PNEC_{oral}$). The concentration in fish is a result of uptake from the aqueous phase and intake of contaminated food (aquatic organisms). Thus, $PEC_{oral, predator}$ is calculated from the bioconcentration factor (BCF) and a biomagnification factor (BMF). The concentration of contaminant in food (fish) of fish-eating predators ($PEC_{oral, predator}$) is calculated from the PEC for surface water (Tier 2 worst case 2.55E-04 mg/L relates to Bridge Dip/Spray – Treated Wood, Table 13.1.3.-3, Doc IIC – no *in situ* treatment of bridges is envisaged), the measured or estimated BCF for fish (570 measured) and the BMF (1 based on measured BCF). $PEC_{oral, predator} = PEC_{water} * BCF_{fish} * BMF$ $PEC_{oral, predator} = 2.55E-04 * 570 * 1$ $PEC_{oral, predator} = 0.145$ mg/kg $_{wet\ fish}$ A predicted no effect oral concentration ($PNEC_{oral}$) can be calculated based on the results of the mammalian and avian repeat dose toxicity tests. The result of this calculation gives a predicted no-effect concentration in food that should be protective to other mammalian and avian species. According to the Technical Guidance Document on Risk Assessment Part II (page 128), Secondary poisoning effects on bird populations rarely occurs over the short-term. Therefore, results from long-term studies are strongly preferred, such as NOECs for mortality, reproduction or growth. Considering a one-generation study with the Northern Bobwhite (*Colinus virginianus*) ([redacted]; 1992) performed to GLP standards according to FIFRA guideline 71-4, the lowest NOEC exceeds 500 ppm. Taking into account a safety factor of 30 (as indicated in Table 23 of the TGD on Risk Assessment Part II, page 130), a $PNEC_{bird}$ of 16.7 mg/kg food is obtained. According to the Technical Guidance Document on Risk Assessment Part II (page 128), Secondary poisoning effects on mammal	

Section A7.4.3.3.1 Annex Point IIIA, XIII.2.3.	Bioaccumulation in an appropriate species of fish
	populations rarely occurs over the short-term. Therefore, results from long-term studies are strongly preferred, such as NOECs for mortality, reproduction or growth. Considering the reproduction study conducted in rats with permethrin (3 generation study;), the NOAEL was set at 180 mg/kg bw/d . For the assessment of secondary poisoning, the results always have to be expressed as the concentration in food. Where toxicity data are presented only as NOAELs only, these NOAELs can be converted to NOECs with the following two formulae: $NOEC_{mammal\_food\_chr} = NOAEL_{mammal\_oral\_chr} \cdot CONV_{mammal}$ A conversion factor ($CONV_{mammal}$) of 20 is selected from table 22 p 129 as the 3 generation study was conducted with rats aged 6 weeks at the initiation of the study. The $NOEC_{mamalfood\_chr}$ is hence calculated to be 3600 ppm. Taking into account a safety factor of 30 (as indicated in Table 23 of the TGD on Risk Assessment Part II, page 130), a $PNEC_{small\ mammal}$ of 120 mg/kg food is obtained. Comparing these values to the calculated $PEC_{oral,predator}$ 0.145 mg/kg wet fish it can be determined that there is no unacceptable risk for fish-eating birds and mammals. By comparing the $PEC_{oral\ predator}$ with the respective PNECs, PEC/PNEC ratios of 0.01 and 0.001 are obtained for birds and mammals respectively, indicating no unacceptable risk for fish-eating birds and mammals. Therefore, a bioaccumulation study in an appropriate species of fish is not required.
Undertaking of intended data submission [ ]	
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	21/04/05
Evaluation of applicant's justification	Accept applicant's justification
Conclusion	Adopt applicant's justification

Section A7.4.3.3.1 Annex Point IIIA, XIII.2.3.	Bioaccumulation in an appropriate species of fish
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	

Sections 7.4.3.3.2 Annex Point XIII.2.3	**Bioaccumulation in an appropriate invertebrate species.**
	Justification for non-submission of data

Other existing data [ ]	**Technically not feasible [ ]**	**Scientifically unjustified [✓]**
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The field of use of permethrin (Product type 8) is in the biological Hazard Classes 1, 2, and 3 (also referred to as Use Classes) see Document I.1, Section 5.1. As permethrin has been not classified for use as a wood preservative in Hazard Class 5 (saltwater) defined in the standard EN 335-1 (CEN, 1992), then a bioaccumulation study in an appropriate invertebrate species conducted in seawater and covering brackish water is not required for permethrin.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*14/1/09*
Evaluation of applicant's justification	*Applicant's justification accepatable. In any case, a review of a published study which investigated the bioconcentration of cypermethrin, deltamethrin, fenvalerate, and permethrin by Chironomus tentans larvae in a freshwater sediment system (Muir et al., (1985), Environmental Toxicology and Chemistry. 4:51-61), is presented in section A7.4.2(2) above.* *The results indicated that;* • *Permethrin rapidly bioaccumulates (Log BCF 1 to 2).* • *Depuration is rapid following end of exposure period*
Conclusion	*Applicant's justification is acceptable – new study in saltwater system not required*
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*

Sections 7.4.3.3.2	Bioaccumulation in an appropriate invertebrate species.
Annex Point XIII.2.3	

	Justification for non-submission of data

Other existing data [ ]	**Technically not feasible** [ ]	**Scientifically unjustified** [✓]
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	The field of use of permethrin (Product type 8) is in the biological Hazard Classes 1, 2, and 3 (also referred to as Use Classes) see Document I.1, Section 5.1. As permethrin has been not classified for use as a wood preservative in Hazard Class 5 (saltwater) defined in the standard EN 335-1 (CEN, 1992), then a bioaccumulation study in an appropriate invertebrate species conducted in seawater and covering brackish water is not required for permethrin.	
Undertaking of intended data submission [ ]		

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

Section 7.4.3.4 Annex Point IIIA XIII 2.4 Effects on reproduction and growth rate with an invertebrate species

Key Study

			Official use only
		1 REFERENCE	
1.1	**Reference**	Kent, S; Williams, N.; Gillings, E.; Morris D. S.; 1995; Permethrin: Chronic toxicity to Daphnia magna; Zeneca Brixham Environmental Lab; Project No. BL5443/B; 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 – ASTM Standard guide for conducting Daphnia magna life cycle toxicity tests	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	**Lot/Batch number**	^{14}C-radiolabelled permethrin: Ref 94-20, D9970/144-152 Permethrin technical: CDD0502	
3.1.2	**Specification**	^{14}C-radiolabelled permethrin: Specific activity 6 MBq mg^{-1}	
3.1.3	**Purity**	^{14}C-radiolabelled permethrin: (radiochemical purity) >98.6% Permethrin technical: 94.8%	
3.1.4	**Composition of Product**	Not applicable	
3.1.5	**Further relevant properties**	Radiolabel in the phenyl position	
3.1.6	**Method of analysis**	^{14}C analysis by liquid scintillation counting ^{14}C permethrin by radio TLC using permethrin technical as a confirmatory marker	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A stock concentration of ^{14}C permethrin was prepared by dissolving a known amount of radioactivity in triethylene glycol. Stock solutions were prepared at 0.40, 0.80, 1.6, 3.2 and 6.4 mg l^{-1} in triethylene glycol. Concentrations in these solutions were determined by LSC. see table A7.4.3.4-1	
3.3	**Reference substance**	No	
3.4	**Testing**		

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

		Key Study
	procedure	
3.4.1	**Dilution water**	see table A7.4.3.4-2
3.4.2	**Test organisms**	see table A7.4.3.4-3
3.4.3	**Handling of offspring**	The test vessels were cleaned every Monday, Wednesday and Friday to remove algal growth. During this process the live Po *Daphnia* were transferred to a spare test vessel containing a small amount of the appropriate test solution. The numbers of live and dead (if observed) F_1 generation *Daphnia* were removed by pouring through a fine nylon mesh, counted using a fine Pasteur pipette and recorded.
3.4.4	**Test system**	see table A7.4.3.4-4
3.4.5	**Test conditions**	see table A7.4.3.4-5
3.4.6	**Duration of the test**	21 days
3.4.7	**Test parameter**	Mortality, reproductivity, growth (length and weight)
3.4.8	**Examination / Sampling**	Observations of mortality were performed daily Offspring were counted daily Weight and length of P0 were recorded at the end of the exposure period
3.4.9	**Monitoring of TS concentration**	Yes – Days 0, 1, 2, 4, 7, 11, 14, 18, 21
3.4.10	**Statistics**	Mortality – not required Reproduction, length and Weight – Analysis of variance of solvent and dilution water control, compared using Dunnetts procedure. Exposure concentrations compared to the pooled control data.
		4 RESULTS
4.1	**Range finding test**	Not performed
4.2	**Results test substance**	
4.2.1	**Initial concentrations of test substance**	Nominal concentrations of Dilution water control, solvent control, 40, 80, 160, 320, 640 ng l^{-1}
4.2.2	**Actual concentrations of test substance**	see Table A7.4.3.4-10
4.2.3	**Effect data**	see Table A7.4.3.4-11 Mortality data: NOEC: 190 ng l^{-1} LOEC: 340 ng l^{-1} EC50:>340 ng l^{-1}

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

Key Study

		Reproduction data: NOEC: 39 ng l^{-1} LOEC: 84 ng l^{-1} Growth (length) data: NOEC: 39 ng l^{-1} LOEC: 84 ng l^{-1} Growth (weight) data: NOEC: 340 ng l^{-1} LOEC: >340 ng l^{-1}
4.2.4	**Concentration / response curve**	See Figure 1
4.2.5	**Other effects**	No other effects observed
4.3	**Results of controls**	see Table A7.4.3.4-11
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The Draft ASTM Standard guide for conducting Daphnia magna life cycle toxicity tests was followed using ^{14}C-radiolabelled permethrin in a flowthrough system. There were no deviations from the reported guideline.
5.2	**Results and discussion**	Mortality was only observed in the highest tested concentration. A range of effects were observed, the most sensitive parameter being the reproductivity, which was reduced significantly from the control animals at concentrations of 84 ng l^{-1} and above. Length was a more sensitive growth parameter than weight. The method employed a flowthrough system from stock solutions in triethylene glycol. Although the mean measure concentrations were on average approximately 50% of the nominal dosing concentrations, a thorough analytical regime indicated that delivery was constant throughout the exposure period. Results are based on mean measured concentrations. This is not considered to effect the validity of the study.
5.2.1	**NOEC**	39 ng l^{-1}
5.2.2	**LOEC**	84 ng l^{-1}
5.2.3	**EC_{50} (EC_x)**	(Mortality) >340 ng l^{-1}
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. This well conducted and well reported study showed a range of effects, the most sensitive parameter being *Daphnia magna*

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA XIII 2.4

Key Study

reproductivity, which was reduced significantly from the control animals at concentrations of 84 ng l^{-1} and above. Length was a more sensitive growth parameter than weight. Mortlaity was observed to be the least sensitive parameter.

5.3.1	**Reliability**	1
5.3.2	**Deficiencies**	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	12/05/05
Materials and Methods	Applicant's version is acceptable
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ... ***(specify)***
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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

Criteria	Details
Dispersion	No
Vehicle	Yes – triethylene glycol
Concentration of vehicle	100 µl l^{-1} (% v/v)
Vehicle control performed	Yes – at 100 µl l^{-1}
Other procedures	None

Table A7.4.3.4-2: Dilution water

Criteria	Details
Source	Dechlorinated water from 100 m^3 reservoir with an average retention of 24 hours was used to prepare *Daphnia* media as described below
Salinity	Not applicable
Hardness	189 ± 12.7 mg l^{-1} $CaCO_3$
pH	8.5 ± 0.1
Ca / Mg ratio	Modified EPA media containing; $NaHCO_3$ 192 mg l^{-1} $CaSO_4.2H_2O$ 120 mg l^{-1} $MgSO_4.7H_2O$ 245 mg l^{-1} KCl 8 mg l^{-1} Na_2SeO_3 2 µg l^{-1} Thiamine hydrochloride 75 µg l^{-1} Cyanocobalamine (B_{12}) 1 µg l^{-1} Biotin 0.75 µg l^{-1}
Na / K ratio	See above
Oxygen content	8.4 – 9.4 mg l^{-1}
Conductance	685 ± 14.5 µS cm^{-1}
TOC	(NPOC) 0.5 mg l^{-1}
Holding water different from dilution water	No

Table A7.4.3.4-3: Test organisms

Criteria	Details
Strain / Clone	*Daphnia magna* straus
Source	In laboratory cultures
Age	<24 hours old at initiation
Breeding method	Diploid parthenogenesis
Kind of food	Algae (Chlorella vulgaris) and a microencapsulated diet "Frippak booster®"
Amount of food	57 µg "Frippak booster®" Chlorella vulgaris stock (1.2×10^8 cells ml^{-1}) Days 0 to 4; 1.25 ml Days 5 to 13; 1.5 ml Days 14 to 21; 1.75 ml
Feeding frequency	Twice per day
Pretreatment	None
Feeding of animals during test	Yes

Table A7.4.3.4-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	3 ml min^{-1}
Volume of test vessels	1000 ml (working capacity 800 ml)
Volume/animal	80 ml
Number of animals/vessel	10
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.4-5: Test conditions

Criteria	Details
Test temperature	See table A7.4.3.4-7
Dissolved oxygen	See table A7.4.3.4-8
pH	See table A7.4.3.4-9
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	"Daylight" fluorescent tubes average 600 lux
Photoperiod	16 hours light: 8 hours dark with 20 min dawn:dusk transition periods

Table A7.4.3.4-6: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

	fulfilled	Not fullfilled
Mortality of parent animals < 20% at test termination	X	

Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	**X**	

Table A7.4.3.4-7: Test temperature

APPENDIX 5

WATER QUALITY
(A) TEMPERATURE OF SOLUTIONS (°C)

Sponsor:	ZENECA Agrochemicals
Test substance:	[14C] Permethrin
Test organism:	*Daphnia magna*
Test water:	Dilution water (see Appendix 1)

Mean measured concn of [14C] permethrin equivalents (ng l-1)	Replicate	Day (date)						
		0 (21.4.95)	4 (25.4.95)	7 (28.4.95)	10 (1.5.95)	14 (5.5.95)	17 (8.5.95)	21 (12.5.95)
Dilution water control	A	19.7	19.8	19.6	19.8	19.7	19.8	19.4
	B	19.8	19.7	19.5	19.8	19.7	19.9	19.4
	C	19.9	19.8	19.6	19.9	19.9	20.0	19.5
	D	19.8	19.8	19.6	19.9	19.8	20.0	19.4
Solvent control	A	20.1	20.1	20.1	20.0	20.0	20.1	19.8
	B	20.3	20.3	20.2	20.2	20.2	20.2	19.8
	C	20.3	20.4	20.2	20.3	20.4	20.3	20.0
	D	20.3	20.3	20.2	20.2	20.3	20.3	20.0
19	A	20.2	20.1	19.9	20.0	20.1	20.2	19.6
	B	20.3	20.1	20.0	20.2	20.2	20.2	19.7
	C	20.3	20.1	20.2	20.4	20.2	20.4	20.0
	D	20.2	20.2	20.1	20.3	20.3	20.4	19.9

Table A7.4.3.4-7: Test temperature (cont'd)

APPENDIX 5 contd

WATER QUALITY
(A) TEMPERATURE OF SOLUTIONS (°C)

Mean measured concn of [^{14}C] permethrin equivalents (ng l^{-1})	Replicate	Day (date)						
		0 (21.4.95)	4 (25.4.95)	7 (28.4.95)	10 (1.5.95)	14 (5.5.95)	17 (8.5.95)	21 (12.5.95)
39	A	20.3	19.9	19.8	19.8	19.9	19.9	19.4
	B	20.1	19.8	19.7	19.9	20.0	19.9	19.5
	C	20.1	19.9	19.7	20.0	20.1	20.0	19.6
	D	20.1	19.9	19.7	20.0	20.1	20.0	19.6
84	A	20.4	20.3	20.2	20.3	20.1	20.4	20.0
	B	20.5	20.4	20.3	20.4	20.3	20.4	20.0
	C	20.5	20.5	20.4	20.5	20.5	20.4	20.2
	D	20.4	20.4	20.3	20.3	20.5	20.5	20.1
190	A	20.3	20.4	20.1	20.3	20.2	20.4	20.0
	B	20.4	20.4	20.3	20.4	20.2	20.4	20.2
	C	20.4	20.5	20.3	20.6	20.4	20.5	20.2
	D	20.4	20.5	20.4	20.5	20.5	20.4	20.2
340	A	20.1	19.9	19.8	20.0	19.9	19.9	19.7
	B	20.2	20.0	19.8	19.8	19.8	20.0	19.7
	C	20.1	20.1	19.9	20.0	20.2	20.1	19.8
	D	20.1	20.1	19.9	20.1	20.1	20.0	19.7

Table A7.4.3.4-8: Dissolved oxygen

APPENDIX 5 contd

WATER QUALITY
(C) DISSOLVED OXYGEN CONCENTRATIONS OF TEST SOLUTIONS (mg l^{-1})

Sponsor:	ZENECA Agrochemicals
Test substance:	[^{14}C] Permethrin
Test organism:	*Daphnia magna*
Test water:	Dilution water (see Appendix 1)

Mean measured concn of [^{14}C] permethrin equivalents (ng l^{-1})	Replicate	Day (date)						
		0 (21.4.95)	4 (25.4.95)	7 (28.4.95)	10 (1.5.95)	14 (5.5.95)	17 (8.5.95)	21 (12.5.95)
Dilution water control	A	9.1	9.0	-	9.4	-	9.2	-
	B	9.1	9.1	-	9.4	-	9.1	-
	C	9.1	-	8.4	-	9.0	-	9.0
	D	9.2	-	8.9	-	8.8	-	8.8
Solvent control	A	9.1	9.0	-	9.0	-	8.9	-
	B	9.1	9.2	-	9.2	-	9.0	-
	C	9.2	-	8.6	-	9.0	-	8.9
	D	9.1	-	8.7	-	9.0	-	9.0
19	A	9.1	9.0	-	9.2	-	9.0	-
	B	9.1	9.1	-	9.0	-	8.8	-
	C	9.0	-	8.8	-	9.0	-	8.8
	D	9.1	-	8.7	-	9.0	-	8.9

\- = Not determined

Table A7.4.3.4-8: Dissolved oxygen (cont'd)

APPENDIX 5 contd

WATER QUALITY
(C) DISSOLVED OXYGEN CONCENTRATIONS OF TEST SOLUTIONS (mg l^{-1})

Mean measured concn of [^{14}C] permethrin equivalents (ng l^{-1})	Replicate	Day (date)						
		0 (21.4.95)	4 (25.4.95)	7 (28.4.95)	10 (1.5.95)	14 (5.5.95)	17 (8.5.95)	21 (12.5.95)
39	A	9.1	9.1	-	9.2	-	9.0	-
	B	9.1	9.1	-	9.2	-	9.1	-
	C	9.1	-	8.6	-	9.0	-	9.0
	D	9	-	8.6	-	8.9	-	8.8
84	A	9.1	9.0	-	9.0	-	8.9	-
	B	9	9.0	-	9.1	-	9.0	-
	C	9.1	-	8.5	-	9.0	-	9.0
	D	9	-	8.4	-	9.0	-	9.0
190	A	9	9.0	-	9.1	-	8.8	-
	B	9	8.9	-	9.0	-	8.6	-
	C	9	-	8.5	-	8.9	-	8.6
	D	9.1	-	8.4	-	9.0	-	8.8
340	A	9.1	9.1	-	9.1	-	9.0	-
	B	9	9.1	-	9.1	-	8.9	-
	C	9.2	-	8.5	-	9.1	-	8.8
	D	9	-	8.4	-	9.0	-	8.8

- = Not determined

Table A7.4.3.4-9: pH

APPENDIX 5 contd

WATER QUALITY
(B) pH OF TEST SOLUTIONS

Sponsor:	ZENECA Agrochemicals
Test substance:	[^{14}C] Permethrin
Test organism:	*Daphnia magna*
Test water:	Dilution water (see Appendix 1)

Mean measured concn of [^{14}C] permethrin equivalents (ng l^{-1})	Replicate	Day (date)						
		0 (21.4.95)	4 (25.4.95)	7 (28.4.95)	10 (1.5.95)	14 (5.5.95)	17 (8.5.95)	21 (12.5.95)
Dilution water control	A	8.4	8.5	-	8.4	-	8.4	-
	B	8.5	8.5	-	8.4	-	8.4	-
	C	8.5	-	8.4	-	8.4	-	8.5
	D	8.5	-	8.5	-	8.4	-	8.5
Solvent control	A	8.5	8.5	-	8.4	-	8.5	-
	B	8.5	8.5	-	8.4	-	8.5	-
	C	8.5	-	8.5	-	8.4	-	8.5
	D	8.5	-	8.5	-	8.4	-	8.5
19	A	8.5	8.5	-	8.4	-	8.4	-
	B	8.5	8.5	-	8.4	-	8.4	-
	C	8.5	-	8.5	-	8.5	-	8.5
	D	8.5	-	8.5	-	8.5	-	8.5

- = Not determined

Table A7.4.3.4-9: pH (cont'd)

APPENDIX 5 contd

WATER QUALITY
(B) pH OF TEST SOLUTIONS

Mean measured concn of [14C] permethrin equivalents (ng l^{-1})	Replicate	Day (date)						
		0 (21.4.95)	4 (25.4.95)	7 (28.4.95)	10 (1.5.95)	14 (5.5.95)	17 (8.5.95)	21 (12.5.95)
39	A	8.5	8.5	-	8.4	-	8.5	-
	B	8.5	8.5	-	8.4	-	8.5	-
	C	8.5	-	8.5	-	8.4	-	8.5
	D	8.5	-	8.5	-	8.5	-	8.5
84	A	8.5	8.5	-	8.4	-	8.5	-
	B	8.5	8.5	-	8.4	-	8.5	-
	C	8.5	-	8.5	-	8.4	-	8.5
	D	8.5	-	8.5	-	*	-	8.5
190	A	8.5	8.5	-	8.4	-	8.4	-
	B	8.5	8.5	-	8.4	-	8.4	-
	C	8.5	-	8.5	-	8.5	-	8.5
	D	8.5	-	8.5	-	8.5	-	8.5
340	A	8.5	8.5	-	8.4	-	8.5	-
	B	8.5	8.5	-	8.4	-	8.4	-
	C	8.5	-	8.5	-	8.5	-	8.5
	D	8.5	-	8.5	-	8.5	-	8.5

- = Not determined
* = Not determined due to operator error

Table A7.4.3.4-10: Analytical data

CONCENTRATIONS OF [^{14}C] PERMETHRIN EQUIVALENTS IN THE TEST VESSELS DETERMINED BY LIQUID SCINTILLATION COUNTING (ng l^{-1})

Sponsor:	ZENECA Agrochemicals
Test substance:	[^{14}C] Permethrin
Test organism:	*Daphnia magna*
Test water:	Dilution water (see Appendix 1)

Nominal concn of [^{14}C] permethrin (ng l^{-1})	Day (date)	0 (21.4.95)	1 (22.4.95)	2 (23.4.95)	4 (25.4.95)	7 (28.4.95)	11 (2.5.95)	14 (5.5.95)	18 (9.5.95)	21 (12.5.95)
Dilution water control	A	<1.7	<1.7	<1.7	-	-	-	<1.7	-	-
	B	<1.7	<1.7	<1.7	<1.7	-	-	<1.7	<1.7	-
	C	<1.7	<1.7	<1.7	-	<1.7	-	<1.7	-	-
	D	<1.7	<1.7	<1.7	-	-	<1.7	<1.7	-	<1.7
Solvent control	A	<1.7	<1.7	<1.7	<1.7	-	-	<1.7	<1.7	-
	B	<1.7	<1.7	<1.7	<1.7	-	-	-	<1.7	-
	C	<1.7	<1.7	<1.7	<1.7	<1.7	-	-	<1.7	<1.7
	D	<1.7	<1.7	<1.7	<1.7	-	<1.7	-	<1.7	-
40	A	18	17	16	-	-	18	20	-	22
	B	18	20	20	18	-	20	-	20	22
	C	18	18	17	-	18	18	-	-	22
	D	18	20	18	-	-	18	-	-	20
80	A	35	37	40	-	38	-	37	35	-
	B	37	43	38	37	42	-	-	38	-
	C	37	42	40	-	45	-	-	38	38
	D	38	42	40	-	42	37	-	38	-

- = not determined

Nominal concn of [^{14}C] permethrin (ng l^{-1})	Day (date)	0 (21.4.95)	1 (22.4.95)	2 (23.4.95)	4 (25.4.95)	7 (28.4.95)	11 (2.5.95)	14 (5.5.95)	18 (9.5.95)	21 (12.5.95)
160	A	73	73	75	-	-	98	87	-	87
	B	73	82	77	83	-	92	85	110	88
	C	73	87	73	-	120	95	85	-	83
	D	67	77	77	-	-	85	83	-	80
320	A	180	190	180	200	-	-	190	170	-
	B	190	190	200	200	-	-	-	190	-
	C	180	210	180	210	200	-	-	170	180
	D	170	180	180	200	-	190	-	170	-
640	A	320	320	370	-	330	-	300	-	-
	B	360	330	400	330	380	-	-	350	-
	C	340	350	390	-	330	-	-	-	330
	D	330	320	370	-	310	300	-	-	-

- = not determined

Table A7.4.3.4-10: Analytical data (cont'd)

SUMMARY OF ANALYTICAL RESULTS BY LIQUID SCINTILLATION COUNTING (LSC)

Sponsor:	ZENECA Agrochemicals
Test substance:	[^{14}C] Permethrin
Test organism:	*Daphnia magna*
Test water:	Dilution water (see Appendix 1)

Nominal concn of [^{14}C] permethrin (ng l^{-1})	Replicate	Mean measured concn of [^{14}C] permethrin equivalents (ng l^{-1})	Standard deviation (ng l^{-1})	Range (ng l^{-1})	No of samples	Percentage of nominal
Dilution water control	A	<1.7	-	-	4	-
	B	<1.7	-	-	6	-
	C	<1.7	-	-	5	-
	D	<1.7	-	-	6	-
	All data	<1.7	-	-	21	-
Solvent control	A	<1.7	-	-	6	-
	B	<1.7	-	-	5	-
	C	<1.7	-	-	7	-
	D	<1.7	-	-	6	-
	All data	<1.7	-	-	24	-
40	A	19	2	16 - 22	6	48
	B	20	1	18 - 22	7	50
	C	19	2	17 - 22	6	48
	D	19	1	18 - 20	5	48
	All data	19	2	16 - 22	24	48
80	A	37	2	35 - 40	6	46
	B	39	3	37 - 43	6	49
	C	40	3	37 - 45	6	50
	D	40	2	37 - 42	6	50
	All data	39	3	35 - 45	24	49

160	A	82	10	73 - 98	6	51
	B	86	11	73 - 110	8	54
	C	88	16	73 - 120	7	55
	D	78	6	67 - 85	6	49
	All data	84	12	67 - 120	27	53
320	A	190	10	170 - 200	6	59
	B	190	5	190 - 200	5	59
	C	190	16	170 - 210	7	59
	D	180	12	170 - 200	6	56
	All data	190	12	170 - 210	24	59
640	A	330	26	300 - 370	5	52
	B	360	28	330 - 400	6	56
	C	350	25	330 - 390	5	55
	D	330	27	300 - 370	5	52
	All data	340	28	300 - 400	21	53

Table A7.4.3.4-10: Analytical data (cont'd)

ANALYSIS OF PARENT [^{14}C] PERMETHRIN BY THIN LAYER CHROMATOGRAPHY (TLC) IN THE TEST SOLUTIONS

Sponsor:	ZENECA Agrochemicals
Test substance:	[^{14}C] Permethrin
Test organism:	*Daphnia magna*
Test water:	Dilution water (see Appendix 1)

Day (date)	Nominal concn of [^{14}C] permethrin (ng l^{-1})	Mean measured concn of [^{14}C] permethrin equivalents by LSC (Appendix 2) (ng l^{-1})	Percentage of total activity extracted into hexane (Appendix 3)	Percentage of [^{14}C] permethrin determined in sample by TLC (Appendix 3)	Percentage as [^{14}C] permethrin in original sample
-1 (20.4.95)	Dilution water control	<1.7	-	-	-
	Solvent control	<1.7	-	-	-
	40	19	100	88.3	88
	80	39	89.1	90.3	80
	160	84	92.7	91.2	85
	320	190	90.2	89.1	80
	640	340	98.0	94.8	93
6 (27.4.95)	Dilution water control	<1.7	-	-	-
	Solvent control	<1.7	-	-	-
	40	19	100	90.6	91
	80	39	100	94.7	95
	160	84	85.7	95.0	81
	320	190	89.3	94.0	84
	640	340	98.1	95.8	94

13 (4.5.95)	Dilution water control	<1.7	-	-	-
	Solvent control	<1.7	-	-	-
	40	19	100	93.9	94
	80	39	91.9	94.0	86
	160	84	96.2	92.5	89
	320	190	95.5	96.1	92
	640	340	98.0	92.8	91
20 (11.5.95)	Dilution water control	<1.7	-	-	-
	Solvent control	<1.7	-	-	-
	40	19	100	87.8	88
	80	39	100	94.8	95
	160	84	94.5	92.5	87
	320	190	95.0	91.6	87
	640	340	97.0	93.8	91

- = No data

Table A7.4.3.4-11: Effect data

PERMETHRIN: Chronic toxicity to *Daphnia magna*

MORTALITY AND OBSERVED EFFECTS

Sponsor:	ZENECA Agrochemicals
Test substance:	[^{14}C] Permethrin
Test organism:	*Daphnia magna*
Test water:	Dilution water (see Appendix 1)

Date	Day	Cumulative number dead						
		Mean measured concn of [^{14}C] permethrin equivalents (ng l^{-1})						
		Dilution water control	Solvent control	19	39	84	190	340
21.4.95	0	0	0	0	0	0	0	0
22.4.95	1	0	0	0	0	0	0	0
23.4.95	2	0	0	0	0	0	0	0[a]
24.4.95	3	0	0	0	0	0	0	1[ab]
25.4.95	4	0	0	0	0	0	0	2[ab]
26.4.95	5	0	0	0	0	0	0[b]	2[ab]
27.4.95	6	0	0	0	0	0	0[b]	2[ab]
28.4.95	7	0	0	0	0	0	0[b]	3[ab]
29.4.95	8	0	0	0	0	0	0[b]	3[ab]
30.4.95	9	0	0	0	0	0	0[b]	3[ab]
1.5.95	10	0	0	0	0	0	0[b]	3[ab]
2.5.95	11	0	0	0	0	0	0[b]	3[ab]
3.5.95	12	0	0	0	0	0	0[b]	4[ab]
4.5.95	13	0	0	0	0	0	0[b]	4[ab]
5.5.95	14	0	0	0	0	0	0[b]	4[ab]
6.5.95	15	0	0	0	0	0	0[b]	4[ab]
7.5.95	16	0	0	0	0	0	0[b]	4[ab]
8.5.95	17	0	0	0	0	0	0[b]	4[ab]
9.5.95	18	0	0	0	0	0	0[b]	4[b]
10.5.95	19	0	0	1	0	0	0	4[b]
11.5.95	20	0	0	1	0	0	0	4[b]
12.5.95	21	0	0	1	0	0	0	4[b]

40 *Daphnia* were tested per concentration. Mortality data on which this table is based are given in Appendix 4
Observed effects: [a] = pale compared to the control [b] = reduced in size compared to the control

Table A7.4.3.4-11: Effect data (cont'd)

PERMETHRIN: Chronic toxicity to *Daphnia magna*

REPRODUCTION DATA

Sponsor:	ZENECA Agrochemicals
Test substance:	[14C] Permethrin
Test organism:	*Daphnia magna*
Test water:	Dilution water (see Appendix 1)

Mean measured concn of [14C] permethrin equivalents (ng l^{-1})	Replicate	Numbers of offspring (F_1) removed on each occasion (numbers of surviving $P_0^{\$}$ animals, if <10, in parenthesis)						Total F_1	F_1 per P_0	mean F_1 per P_0		Standard deviation	
		Day 10	Day 12	Day 14	Day 17	Day 19	Day 21						
Dilution water control	A	132	199	0	158	199	0	688	69	70	68#	2.5	3.8#
	B	164	140	67	150	83	70	674	67				
	C	174	171	15	151	138	38	687	69				
	D	178	172	37	174	145	27	733	73				
Solvent control	A	170	191	0	131	197	0	689	69	66		4.0	
	B	192	163	0	112	180	0	647	65				
	C	150	174	0	105	167	0	596	60				
	D	185	197	0	128	174	0	684	68				
19	A	188	223	0	118	189	0	718	72	70 (NSD)		4.8	
	B	174	241	0	135	188	0	738	74				
	C	177	174	0	111	169	0	631	63				
	D	151	192	0	116	171	0 (9)	630	70				

$ = Number of P_0 surviving on previous counting occasions (see 5.3) # = Value for pooled control
* = Significant decrease (P=0.05, one sided) from pooled control) NSD = No significant decrease (P=0.05, one sided) from pooled control

Table A7.4.3.4-11: Effect data (cont'd)

PERMETHRIN: Chronic toxicity to *Daphnia magna*

REPRODUCTION DATA

Mean measured concn of [^{14}C] permethrin equivalents (ng l^{-1})	Replicate	Numbers of offspring (F_1) removed on each occasion (numbers of surviving $P_0^{\$}$ animals, if <10, in parenthesis)						Total F_1	F_1 per P_0	Mean F_1 per P_0	Standard deviation
		Day 10	Day 12	Day 14	Day 17	Day 19	Day 21				
39	A	184	182	0	149	198	0	713	71	71 (NSD)	1.7
	B	197	199	0	136	199	0	731	73		
	C	164	186	0	140	204	0	694	69		
	D	185	182	19	162	139	30	717	72		
84	A	139	204	14	116	156	3	632	63	60 (*)	2.5
	B	150	173	0	123	156	0	602	60		
	C	105	178	12	106	172	0	573	57		
	D	169	183	0	112	143	0	607	61		
190	A	91	109	0	120	176	2	498	50	52 (*)	1.7
	B	70	115	17	159	151	13	525	53		
	C	73	105	44	133	108	38	501	50		
	D	60	132	41	158	98	41	530	53		
340	A	0	0	0	25(8)	2(8)	67(8)	94	12	16 (*)	4.6
	B	0	0	0	58	13	109	180	18		
	C	0	0	0	64(9)	0(9)	49(9)	113	13		
	D	0	0	16(9)	70(9)	36(9)	72(9)	194	22		

$ = Number of P_0 surviving on previous counting occasions (see 5.3)
* = Significant decrease (P=0.05, one sided) from pooled control NSD = No significant decrease (P=0.05, one sided) from pooled control

Table A7.4.3.4-11: Effect data (cont'd)

PERMETHRIN: Chronic toxicity to *Daphnia magna*

LENGTH MEASUREMENTS

Sponsor:	ZENECA Agrochemicals
Test substance:	[^{14}C] Permethrin
Test organism:	*Daphnia magna*
Test water:	Dilution water (see Appendix 1)

Replicate	Number	Length in scale divisions (day 21)						
		Mean measured concn of [^{14}C] permethrin equivalents (ng l^{-1})						
		Dilution water control	Solvent control	19	39	84	190	340
A	1	47	47	50	47	47	46	42
	2	51	47	47	50	50	46	40
	3	47	50	47	46	50	47	41
	4	48	46	48	50	48	48	39
	5	46	49	47	48	46	44	41
	6	49	50	47	51	50	46	42
	7	46	48	49	47	48	45	41
	8	47	46	48	47	50	47	39
	9	51	46	50	50	47	47	M
	10	50	49	51	46	45	46	M
B	1	50	46	47	47	45	49	43
	2	48	51	51	47	49	44	44
	3	50	50	48	49	46	46	44
	4	47	47	46	47	49	44	41
	5	50	47	48	47	50	47	40
	6	50	47	49	47	48	45	40
	7	46	50	49	50	44	47	40
	8	51	46	51	46	47	45	43
	9	49	51	45	50	48	46	42
	10	49	47	51	51	48	46	41

M = mortality

Table A7.4.3.4-11: Effect data (cont'd)

PERMETHRIN: Chronic toxicity to *Daphnia magna*

LENGTH MEASUREMENTS

Replicate	Number	Length in scale divisions (day 21)						
		Mean measured concn of [^{14}C] permethrin equivalents (ng l^{-1})						
		Dilution water control	Solvent control	19	39	84	190	340
C	1	49	47	46	47	45	46	40
	2	47	49	48	50	47	45	40
	3	47	48	48	46	45	45	40
	4	46	46	50	50	46	43	40
	5	51	48	50	50	45	49	44
	6	50	49	46	50	45	46	42
	7	48	47	48	51	45	47	40
	8	50	50	45	48	44	45	39
	9	46	49	46	48	48	47	40
	10	50	50	48	47	45	44	M
D	1	50	45	46	50	47	49	45
	2	51	50	50	46	46	47	41
	3	47	49	47	48	46	46	43
	4	51	48	50	50	45	44	45
	5	46	48	49	51	51	47	44
	6	50	49	47	48	45	46	42
	7	50	50	46	48	44	47	42
	8	51	50	46	48	45	46	41
	9	49	49	50	49	46	46	40
	10	47	48	M	47	46	49	M
Mean of 4 replicates		49	48	48 (NSD)	48 (NSD)	47 (*)	46 (*)	41 (*)
		48#						
Standard deviation		1.79	1.61	1.78	1.66	1.97	1.47	1.71
		1.71#						
Mean		Length in mm (1 mm = 12.24 scale divisions)						
		4.00	3.92	3.92	3.92	3.84	3.76	3.35
		3.92#						

\# = Value for pooled control data
* = Significant decrease (P=0.05, one sided) from pooled control
NSD = No significant decrease (P=0.05, one sided) from pooled control
M = Mortality

Table A7.4.3.4-11: Effect data (cont'd)

PERMETHRIN: Chronic toxicity to *Daphnia magna*

DRY WEIGHT MEASUREMENTS

Sponsor:	ZENECA Agrochemicals
Test substance:	[^{14}C] Permethrin
Test organism:	*Daphnia magna*
Test water:	Dilution water (see Appendix 1)

Replicate	Number	Dry weight (µg)						
		Mean measured concn of [^{14}C] permethrin equivalents (ng l^{-1})						
		Dilution water control	Solvent control	19	39	84	190	340
A	1	733	770	1001	787	715	703	758
	2	845	500	762	574	741	780	848
	3	654	733	868	726	977	699	511
	4	730	802	581	472	604	758	839
	5	750	699	627	865	1015	726	912
	6	613	630	583	601	798	717	600
	7	671	866	1029	471	533	759	666
	8	755	550	1007	661	511	709	1045
	9	575	929	873	588	598	709	M
	10	623	537	738	620	767	726	M
B	1	522	781	850	687	647	746	1057
	2	747	681	615	756	802	953	984
	3	630	603	865	654	638	951	906
	4	523	932	475	706	870	467	787
	5	706	707	779	680	614	789	1438
	6	706	715	683	720	674	698	1156
	7	614	874	828	694	756	746	913
	8	816	657	765	552	534	778	1085
	9	783	736	797	582	514	847	792
	10	543	918	620	873	734	716	830

M = Mortality

Table A7.4.3.4-11: Effect data (cont'd)

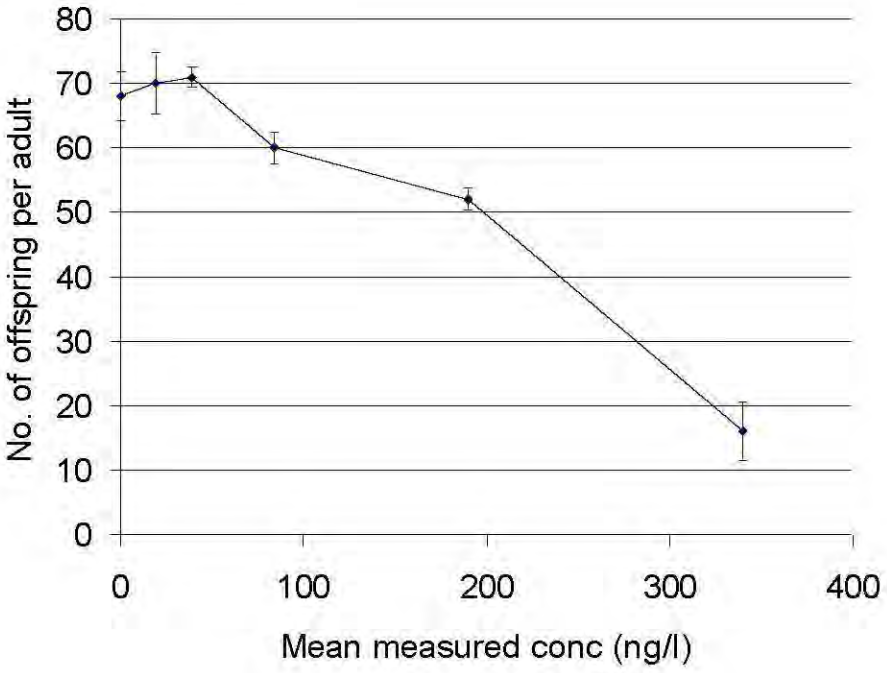
PERMETHRIN: Chronic toxicity to *Daphnia magna*

DRY WEIGHT MEASUREMENTS

Replicate	Number	Dry weight (µg)						
		Mean measured concn of [^{14}C] permethrin equivalents (ng l^{-1})						
		Dilution water control	Solvent control	19	39	84	190	340
C	1	577	2043†	776	770	790	944	828
	2	536	1394†	696	619	563	692	876
	3	674	-1162†	886	704	619	702	898
	4	854	2015†	728	580	348	843	911
	5	637	-999†	605	850	613	846	1049
	6	650	736	703	658	784	739	798
	7	733	905	709	846	702	747	590
	8	562	831	604	850	534	552	866
	9	677	810	695	698	709	691	1161
	10	958	520	875	534	772	796	M
D	1	833	588	435	795	745	623	596
	2	896	866	797	877	463	683	598
	3	600	379	995	745	656	739	888
	4	639	702	837	639	749	555	545
	5	570	630	489	742	740	744	714
	6	816	794	473	721	725	1130	1432
	7	575	873	725	991	708	783	1347
	8	650	622	657	878	557	765	1042
	9	908	690	746	1143	493	711	1040
	10	718	832	M	795	643	1531	M
Mean of 4 replicates		690	726	738 (NSD)	718 (NSD)	674 (NSD)	770 (NSD)	897 (NSD)
		707#						
Standard deviation		113.4	136.6	150.5	137.5	134.4	167.6	230.8
		125.2#						

\# = Value for pooled control data
NSD = No significant (P=0.05, one sided) decrease from pooled control
M = Mortality
† = Anomalous data (see 5.5)

Figure 1: Effect data



Section A7.4.3.5.1 (1)
Annex Point IIIA, XIII.3.4

Effects on any other specific, non-target organisms believed to be at risk:

Effects on sediment dwelling organism

Key Study

Official use only

		1 REFERENCE
1.1	**Reference**	Conrad, A.U., Fleming, R.J., Crane, M.; 1999; Laboratory and field response of *Chironomus riparius* to a pyrethroid insecticide. Water Research, 33, 7, 1603-1610; Not GLP; Published
1.2	**Data protection**	No
1.2.1	**Data owner**	No data protection claimed
1.2.2	**Companies with letter of access**	No data protection claimed
1.2.3	**Criteria for data protection**	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No
2.2	**GLP**	No
2.3	**Deviations**	No: Protocol was not to any guidelines
		3 MATERIALS AND METHODS
3.1	**Test material**	Permethrin formulation 'Picket'
3.1.1	**Lot/Batch number**	No data
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**	Low water solubility
3.1.6	**Method of analysis**	GC-ECD
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	
3.3	**Reference substance**	
3.4	**Testing procedure**	The objective of the study was to investigate the relationship between the results of laboratory toxicity tests with the response of a field population exposed to the same pesticide.
3.4.1	**Test organisms**	*Species/strain*: *Chironomus riparius* *Source of initial stock*: Not reported

Section A7.4.3.5.1 (1)
Annex Point IIIA, XIII.3.4

Effects on any other specific, non-target organisms believed to be at risk:

Effects on sediment dwelling organism

Culturing techniques: Egg ropes from in-house stock were hatched in groundwater held at 20+/- 2°C, 16:8 hour light:dark regime. Transferred to water vessels and fed a suspension of tetramin fish food.

Age/weight: Within 8 days of hatching.

Kind of food: Tetramin fish food

Amount of food: No data

Pre-treatment: None

3.4.2 Test system

LABORATORY WATER ONLY TEST

Renewal of test solution: No

Volume/type of vessels: glass beakers, aerated

Amount of water/vessel: 100 ml

Concentration of test solutions: 0, 0.625, 1.25, 2.5, 3.2, 5.0, 6.3, 10, 12.5, 31.3, 62.5 µg l^{-1}.

Number of replicates/concentration: 1

Number of test animals/vessel: 15

Controls: Yes

Test duration: 96 hours

Sampling: Survival was monitored after 24, 48, 72, 96 hours.

Statistical analysis: LC50 was determined using Debtox.

LABORATORY SEDIMENT TEST

Renewal of test solution: No

Volume/type of vessels: 300 ml glass beakers, aerated

Source of sediment: Uncontaminated experimental pond, Medmenham, Kent. 500 µm sieved.

Dosing of sediment: All sediments were spiked with permethrin on a dry weight basis. This was achieved by mixing wet sediment in an industrial food mixer and adding permethrin in acetone solution, dropwise as it mixed, the mixtures being left to mix for an additional hour. The sediments were distributed into triplicate vessels (50 ml aliquots in 300 ml beakers). 200 ml of uncontaminated water was added to each vessel and allowed to stand.

Amount of sediment/vessel: 50 ml

Amount of water/vessel: 200 ml

Concentration of test solutions: 0, 0.43, 4.3, 22, 43, 220, 430, 4300 µg g^{-1} dry weight.

Number of replicates/concentration: 3

Number of test animals/vessel: 15

Controls: Yes

Section A7.4.3.5.1 (1)
Annex Point IIIA, XIII.3.4

Effects on any other specific, non-target organisms believed to be at risk:

Effects on sediment dwelling organism

Test duration: 10 days

Sampling: After 10 days sediments were sieved and the number of surviving larvae determined.

Statistical analysis: LC50 was determined using Spearman-Karber.

FIELD TOXICITY

Four unreplicated doses were randomised among five ponds, one of which was a control. The ponds measured 5x5m at the surface, sloping to 4x4 m at the bottom lined with butyl rubber. The ponds contained a 5-10 cm layer of uncontaminated sediment (source: C S Lewis Nature Reserve, Oxford) and 60 cm of uncontaminated water (Source: River Thames). Plants and invertebrates were present through natural colonisation, although a dense growth of pondweed was removed by raking 27 days before dosing. The ponds were dosed (4 July 1995) to achieve nominal concentrations of 1, 10, 50 and 100 $\mu g\ l^{-1}$ by sub-surface injection.

Sediment bioassays:

Sediment samples were removed from the ponds on days 2, 4, 6, 8, 10, 15, 17, 24, 31, 45, 52 after dosing. Ekman grab samples were taken from random positions in the ponds, the sediments sieved to 500 um to remove indigenous organisms, and the toxicity tested as described above.

Airlift samples:

Airlift samples were taken twice before dosing to establish the number of larval chironomids present in the benthos. One sample was taken 17 days after dosing to monitor larval chironomid numbers. The mean number was transformed and regressed against log permethrin concentration to determine any significant trends in abundance after application.

Emergence traps:

Three boxes with fine mesh sides and polystyrene floats were placed at random positions on each pond to trap emerged midges. The traps were emptied and replaced on days -5, -3, -1, 2, 4, 6, 8, 10, 15, 17, 24, 31, 45, 52. A log transformation of the emergence data was used to linearise the data before performing a linear regression of emergence against concentration for each time point. A dose response was inferred if the slope of the regression line differed significantly from zero.

4 RESULTS

4.1 Limit Test

Not performed

4.2 Results test substance

The objective of the study was to investigate the relationship between the results of laboratory toxicity tests with the response of a field population exposed to the same pesticide.

LABORATORY WATER TOXICITY TEST

Section A7.4.3.5.1 (1)
Annex Point IIIA, XIII.3.4

Effects on any other specific, non-target organisms believed to be at risk:

Effects on sediment dwelling organism

LC50 values were;

24 hour	34.4 µg l^{-1}
48 hour	9.27 µg l^{-1}
72 hour	4.62 µg l^{-1}
96 hour	2.89 µg l^{-1}

LABORATORY SEDIMENT TOXICITY TEST

The 10 day LC50 for spiked sediment was 2.11 µg g^{-1}. Control survival was >90%.

FIELD TOXICITY

No difference in weed density could be observed at the end of the study.

Knockdown of aquatic invertebrates, particularly hemipterans, was apparent after dosing in the three highest concentrations.

Sediment bioassays:

On day 2 after dosing, dead chironomid larvae were observed in the grab samples taken from the ponds dosed at 50 and 100 µg l^{-1}. The bioassays on the field grab samples did not show any toxicity, with survival >80% in all samples, regardless of dosing or sampling time.

Airlift Samples:

Before application, there was high variability between samples taken both within and between ponds, although there was no significant tend in abundance between ponds. A significant dose response was observed after treatment, although there was still high variability within samples from the same pond.

Emergence traps:

Before treatment, emergence was variable. After treatment, at 50 and 100 µg l^{-1} no emergence was observed until days 24 and 31 respectively. At 10 µg l^{-1} insects were collected on all days, although numbers were significantly reduced up to day 15 samples. At 1.0 µg l^{-1}, there was no significant difference to the controls.

Regression data are presented for each time point.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In the laboratory, chironomids were exposed to permethrin at a range of concentrations both in purely aqueous systems and in water/sediment systems. Exposure was for 96 hours (water only) and 10 days (sediment).

For assessment of impact on ponds, four unreplicated doses were randomised among five ponds, one of which was a control. The ponds measured 5x5m at the surface, sloping to 4x4 m at the bottom lined with butyl rubber. The ponds contained a 5-10 cm layer of

Section A7.4.3.5.1 (1)
Annex Point IIIA, XIII.3.4

Effects on any other specific, non-target organisms believed to be at risk:

Effects on sediment dwelling organism

uncontaminated sediment (source: C S Lewis Nature Reserve, Oxford) and 60 cm of uncontaminated water (Source: River Thames).

5.2 Results and discussion

The authors suggest a water only test was more suitable for the prediction of toxic effects to sediment organisms.

No chronic or sub-lethal effects due to sediment bound permethrin were observed, indicating the toxicity is knockdown lethality, and permethrin is then not bioavailable once bound to sediment.

Permethrin caused significant decline in larval density and emergence of adult midges when ponds were dosed at 10µg l^{-1} and above. There were no adverse effects observed when dosed at 1 µg l^{-1}. Field populations recovered rapidly, suggesting that chronic and sub-lethal effects on larval density and emergence did not occur. The capacity of an aquatic ecosystem to recover after chemical perturbation is affected by many factors, including the persistence and bioavailability of the toxicant. In this study, organisms emerged from the highest dosed ponds within 4 weeks of dosing.

5.2.1 EC_0

Laboratory water toxicity test

LC0 values were;

24 hour	2.64 µg l^{-1}
48 hour	1.32 µg l^{-1}
72 hour	0.88 µg l^{-1}
96 hour	0.66 µg l^{-1}

Laboratory sediment toxicity test: Not reported

Field toxicity: 1 µg l^{-1}. No toxicity to pond weed was observed at any concentration.

5.2.2 LC_{50}

Laboratory water toxicity test

LC50 values were;

24 hour	34.4 µg l^{-1}
48 hour	9.27 µg l^{-1}
72 hour	4.62 µg l^{-1}
96 hour	2.89 µg l^{-1}

Laboratory sediment toxicity test

The 10 day LC50 for spiked sediment was 2.11 µg g^{-1}. Control survival was >90%.

Field toxicity: Effects were observed in all ponds treated at 10 µg l^{-1} or greater

5.3 Conclusion

The test presents a complex experimental design to determine the

Section A7.4.3.5.1 (1) **Annex Point IIIA, XIII.3.4**	**Effects on any other specific, non-target organisms believed to be at risk:** **Effects on sediment dwelling organism**
	impact of a single point contamination of a pond system, and to try to predict those effects with laboratory based testing methodologies. Although non-standard, the experimental design is well described, and the organism response could be seen to be predictive of real-life examples.
5.3.1 Reliability	2
5.3.2 Deficiencies	The report does not provide any analytical data to support dosing concentrations.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27/04/05
Materials and Methods	Applicant's version is acceptable
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	1-2
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Section A7.4.3.5.1(2)
Annex Point IIIA, XIII.3.4

Effects on any other specific, non-target organisms believed to be at risk:

Effects on sediment dwelling organisms

Key Study

Official use only

		1 REFERENCE
1.1	**Reference**	Fleming, R.J., Holmes, D. and Nixon, S.J.; 1998 ; Toxicity of permethrin to *Chironomus riparius* in artificial and natural sediments. Environmental Toxicity and Chemistry, Vol 17, N°7 pp 1332-1337; Not GLP; Published
1.2	**Data protection**	No
1.2.1	**Data owner**	No data protection claimed
1.2.2	**Companies with letter of access**	No data protection claimed
1.2.3	**Criteria for data protection**	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No
2.2	**GLP**	No
2.3	**Deviations**	No: Protocol was not to any guidelines
		3 MATERIALS AND METHODS
3.1	**Test material**	Permethrin
3.1.1	**Lot/Batch number**	No data
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**	Low water solubility
3.1.6	**Method of analysis**	GC-ECD
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Acute toxicity test: Spiking was achieved by rolling wet sediment with permethrin in an acetone carrier for 90 min with additional overlying water. Each treatment was left to stand for 24 h to allow suspended material to settle, after which the overlying water was decanted off and the sediment distributed into test vessels. Chronic toxicity test: An alternative method of spiking was used to that described above to avoid any loss of test substance in excess water decanted off after spiking. This was achieved by placing 300 ml of wet sediment in an industrial food mixer (Crypto Peerless KNM6) and adding test substance in an acetone carrier drop wise onto the sediment as it mixed. The sediment was left to mix for an additional hour.
3.3	**Reference substance**	

Section A7.4.3.5.1(2) Annex Point IIIA, XIII.3.4		**Effects on any other specific, non-target organisms believed to be at risk:** **Effects on sediment dwelling organisms**
3.4	**Testing procedure**	The aim of the study was to compare acute and chronic effect of permethrin in artificial and natural sediment using larvae of midge *Chironomus riparius*.
3.4.1	**Dilution water**	Uncontaminated borehole groundwater
3.4.2	**sediment**	Natural sediment was collected from an experimental freshwater pond at WRc, Medmenham, UK, using an Ekman grab and was sieved to 500 µm.. Artificial sediments constructed using acid-washed sand, kaolin clay, 1% calcium carbonate and either finely ground *sphagnum* moss peat or α-cellulose. Two additional artificial sediments were constructed with peat and α-cellulose to match the natural sediment in terms of organic carbon content (1.23%) and particle size distribution (38% .100 mm as sand, 60%, 100 mm as clay). Both artificial sediments were based on OECD earthworm soil rcipe using sand, clay and calcium carbonate as a buffer
3.4.3	**Test organisms**	Species/strain: Chironomus riparius Source of initial stock: Not specified Culturing techniques: Egg ropes from in-house continuous culture were hatched in groundwater held at 20+/- 2°C, 16:8 hour light:dark regime. For chronic tests larvae wer inoculated into test system s within 48 h of hatching. For acute tests, first-instar larvae were transferred from water-only cultures to 5-L glass aquariums with a 1-cm layer of acit-washed sand. These culture vessels were continuously aerated. Age/weight: Within 10 days posthatch Kind of food: Tetramin fish food every 48 h. Amount of food: No data Pre-treatment: None
3.4.4	**Test system**	Acute toxicity Renewal of test solution: No Volume/type of vessels: glass beakers, aerated Amount of water/vessel: 100 ml Concentration of test solutions: All sediments (14 artificial sediments **constructed using either peat or α-cellulose as organic source**) were spiked with permethrin at :0, 400, 800, 1600, and 3200ng/g (dry wt. sediment) Number of replicates/concentration: 3 Number of test animals/vessel: 15 Controls: Yes Test duration: 96 hours Sampling: Survival was monitored after 24, 48, 72, 96 hours. Statistical analysis: LC50 was determined using Debtox.

Section A7.4.3.5.1(2) **Annex Point IIIA, XIII.3.4**	**Effects on any other specific, non-target organisms believed to be at risk:** **Effects on sediment dwelling organisms**
	Chronic toxicity Renewal of test solution: No Volume/type of vessels: glass beakers, aerated Amount of water/vessel: 100 ml Concentration of test solutions: All sediments (14 artificial sediments constructed using either peat or α-cellulose as organic source) were spiked with permethrin at :0, 400, 800 and 1600 ng/g (dry wt. sediment) Number of replicates/concentration: 3 Number of test animals/vessel: 20 Controls: Yes Test duration: 15 days test was terminated 5 d after the last emergence of was seen in control vessels Sampling: After 10 days sediments were sieved and the number of surviving larvae determined. Statistical analysis: LC_{50} was determined using Spearman-Karber.
3.4.5 Test conditions	The vessels were continuously aerated. Temperature 20 ±0.5°C, illumination of 16 hours light : 8 hours darkness (see table A7.4.3.5.1-4).
3.4.6 Test parameter	Acute toxicity test: mortality. Chronic toxicity test and artificial versus natural sediment test: adult emergence.
	4 RESULTS
4.1 Results test substance	
4.1.1 Initial concentrations of test substance	Nominal concentrations Acute toxicity test: 0, 400, 800, 1600 and 3200 ng/g (dry weight sediment) Chronic toxicity test: 0, 200, 400, 800 and 1600 ng/g (dry weight sediment) Artificial versus natural sediment test: 0, 200, 400, 800 and 1600 ng/g (dry weight sediment)
4.1.2 Actual concentrations of test substance	Acute toxicity test: permethrin concentration measured in the highest treatment of each sediment ranged from 150 to 250% depending on organic matter type, organic carbon content and clay content as shown in table A7.4.3.5.1-5 Chronic toxicity test and artificial versus natural sediment test: no measured concentration due to a poor recovery during sample extraction.
4.1.3 Effect data	Organism survival was higher than 80% for all control sediment in acute and chronic tests. Acute toxicity test As sediment concentrations were higher than nominals mortality was higher than expected. For those sediments with lower levels of carbon and clay , complete mortality of larvae was observed in the overlying water of the two highest treatments. Discussion is provided for the sediment types which is

Section A7.4.3.5.1(2) Annex Point IIIA, XIII.3.4

Effects on any other specific, non-target organisms believed to be at risk:

Effects on sediment dwelling organisms

not reproduced here.

Chronic toxicity test
Due to poor recovery of permethrin concentrations in bulk sediment samples nominal concentrations are reported.As seen with the acute tessts, larval mortality was seen immediately in overlying wter of the two highest permethrin treatments for those sedimetns with lower organic carbon and clay contents.
In general, increases in clay and organic content led to increased survuval, as was also observed in the acute tests.
Comparison between the acute and chronic test results is hindered by the lack of bulk sediment chemistry data for the latter. For sediment types containing 50% clay and 2.0% peat, which were used in both tests, comparison of effects based on nominal concentrations suggest that the chronic test was more sensitive. In the chronic test mean percentage number of emerged adults at nominal concentration of 200 ng/g wsa reduced to 27 % compared to 100 % emergence in control vessels. In the acute test, no reduction in larval survival was seen at a nominal concentration of 400 ng/g.

Artificial versus natural sediment test: Logistic regression analysis showed that the nominal permethrin concentration strongly affected the total number of adults emerging, although this response was also influenced by sediment type. In the natural sediment, a significant reduction in mean emergence of 63% at a nominal permethrin concentration of 800 ng/g compared to the controls. In the peat sediment, a significant reduction in emergence of 62% was observed at 200 ng/g. In the α-cellulose sediment, no emergence was seen at the lowest permethrin concentration of 200 ng/g compared with 100% emergence in the controls. Therefore, the toxicity of permethrin in the three sediment test systems was α-cellulose> peat > natural sediment. Mean number of adults emerged (%) can be seen in Figure 2 for the three sediment types at the nominal permethrin concentrations tested.

4.2 NOEC

A NOEC can be derived from the test performed with natural sediment. A significant reduction in mean emergence of 63% at a nominal permethrin concentration of 800 ng/g was measured compared to the controls.
From the figure 1, an effect on emergence of about 20% could be seen at the dose of 200 ng/g for the natural sediment system. This value could be regarded as a LOEC. According to the TGD (2003), part II, p 106, table 15 a NOEC can be calculated as LOEC/2 if the LOEC is showing an effect between 10 and 20%. Therefore, a NOEC of 100 ng/g can be derived from this publication.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

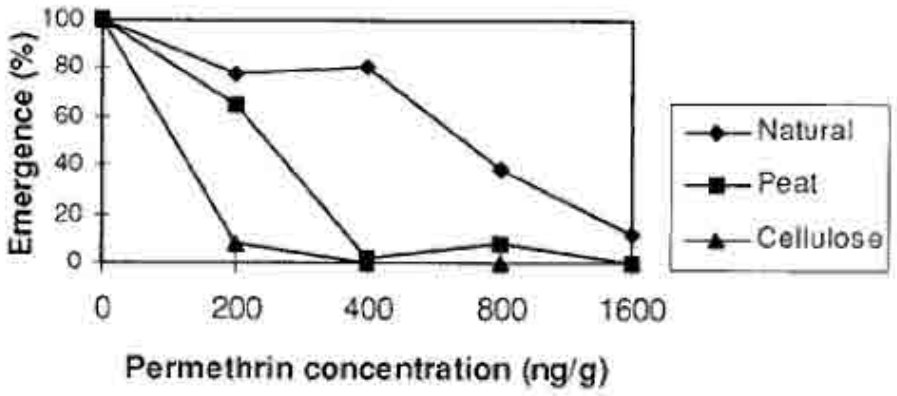
In the laboratory, chironomids were exposed to permethrin at a range of concentrations in water/sediment systems. The sediments were spiked with permethrin at 0, 400, 800, 1600 and 3200 ng/g (dry weight sediment) and 2, 200, 400, 800 and 1600 ng/g (dry weight sediment) for acute and chronic toxicity test, respectively.

Section A7.4.3.5.1(2) Annex Point IIIA, XIII.3.4		**Effects on any other specific, non-target organisms believed to be at risk:** **Effects on sediment dwelling organisms**
		The acute toxicity study was performed over a period of 10 days for the test. The chronic toxicity tests were terminated 5 days after the last adult emergence.
5.2	**Results and discussion**	In both tests the nature of the sediment affected the level of toxicity. Generally increases in clay content and organic content both led to increased survival In the acute toxicity test, complete mortality of larvae was observed in the overlaying water of the two highest treatments (1600 and 3200 ng/g dry weight sediment) on test initiation for sediment types with lower levels of carbon and clay. In the chronic test, larval mortality was seen immediately in the overlying water of the two highest permethrin treatments for those sediments with lower organic carbon and clay contents as seen in the acute test. Comparison between the acute and chronic test results is hindered by the lack of bulk sediment chemistry data for the latter. For sediment types containing 50% clay and 2.0% peat, which were used in both tests, comparison of effects based on nominal concentrations suggest that the chronic test was more sensitive. In the chronic test, mean percentage number of emerged adults at a nominal concentration of 200 ng/g was reduced to 27% compared to 100% emergence in control vessels. In the acute test, no reduction in larval survival was seen at a nominal concentration of 400 ng/g.
5.2.1	**NOEC Acute**	400 ng/g (dry wt. sediment)
5.2.2	**NOEC Chronic**	100 ng/g based upon adult emergence
5.3	**Conclusion**	
5.3.1	**Reliability**	2
5.3.2	**Deficiencies**	Not specifically designed to determine NOEC values

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14/1/09
Materials and Methods	The NOEC of 100 ng/g was derived from the LOEC of 200 ng/g derived from the test performed with natural sediment (20% reduction in emergence of was seen at 200 ng/g), following the recommendations outlined in the TGD (2003), part II, p 106, table 15, which states that a NOEC can be calculated as LOEC/2 if the LOEC is showing an effect between 10 and 20%. This was considered acceptable.
Results and discussion	Adopt applicant's version

Conclusion	Adopt applicant's version
Reliability	2
Acceptability	acceptable
Remarks	Comparison between the acute and chronic test results is hindered by the lack of bulk sediment chemistry data for the latter. For sediment types containing 50% clay and 2.0% peat, which were used in both tests, comparison of effects based on nominal concentrations suggested that the chronic test was more sensitive.
	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	

Fig 1 Mean number of adult emerged (%) in natural an artificial sediment at differing permethrin concentration



Section A7.4.3.5.2 Annex Point IIIA, XIII.3.4	**Effects on any other specific, non-target organisms believed to be at risk:** **Aquatic plant toxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The Technical Guidance on Data Requirements for Active Substances states that this is required when the preliminary risk assessment indicates additional testing may be required. Based upon the water solubility and aquatic dissipation of permethrin, an assessment of the behaviour of permethrin would indicate there will not be continuous aquatic exposure, therefore exposure will be minimal. Existing information on permethrin indicates it is of low toxicity to aquatic algae (**ErC50** > 1.13 mg a.s./L. Furthermore, Conrad et al[1] reported that the pondweed Elodea canadensis rapidly recolonized all of the experimental ponds tested with 0, 1, 10, 50 and 100 µg permethrin per liter. No differences in weed density could be observed between the ponds at the end of the study. This indicates that at concentrations again higher than the limit of solubility (100 µg l^{-1}) there was no effect observed on pondweed. This information on low aquatic phytotoxicity is broadly in line with the terrestrial phytotoxicity of permethrin, which has been used as a broad spectrum insecticide on different crop types since discovery with no impact on plants when used at recommended levels. These levels are significantly higher than those expected in releases to the environment through use as a wood preservative. Therefore a justification for non-submission is suggested on the grounds of limited exposure and secondary observations of phytotoxicity. 1) Conrad, A.U., Fleming, R.J., Crane, M.; 1999; Laboratory and field response of *Chironomus riparius* to a pyrethroid insecticide. Water Research, 33, 7, 1603-1610; Not GLP; Published	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27/04/05
Evaluation of applicant's justification	Applicant's justification is robust.
Conclusion	Adopt applicant' s justification for non-submission of data
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.5.1.1 **Inhibition to microbial activity (terrestrial)**
Annex Point IIA7.4

Key study

Official use only

		1 REFERENCE
1.1	**Reference**	Johnen, B.G, Slinger, J.M, Bridgman, P.A.; 1977; P557: Effect on carbon and nitrogen turnover by soil microorganisms. ICI internal report AR2659/B; Not GLP; Unpublished
1.2	**Data protection**	Yes
1.2.1	**Data owner**	Syngenta
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 - The testing protocol far exceeded the guidelines laid out in OECD 216 and 217 in that 2 soils were tested at 2 concentrations, and multiple substrates were added to the soils to allow for multiple soil functions to be assessed.
2.2	**GLP**	No - GLP was not compulsory at the time the study was performed
2.3	**Deviations**	No – Guidelines were not followed
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2 – PP557 (ICI sourced material)
3.1.1	**Lot/Batch number**	Not reported
3.1.2	**Specification**	As given in section 2
3.1.3	**Purity**	Not reported
3.1.4	**Composition of Product**	Formulated as a 10% emulsifiable concentrate
3.1.5	**Further relevant properties**	Very low water solubility
3.1.6	**Method of analysis**	No analysis
3.2	**Reference substance**	No
3.3	**Testing procedure**	Permethrin was applied at two rates, 0.5 and 10 kg ai/ha, equivalent to 0.80 and 16 mg/kg (soil 1) and 0.70 and 14 mg/kg (soil 2). Controls were run concurrently for comparison. Carbon turnover soils were treated (4 replicates of each) with either soil organic matter, glucose, plant material, sucrose, urea, starch, pectin, cellulose, tripalmitin, phenol or vanillin. Soils were then incubated for up to 40 days and evolved carbon dioxide measured

Section A7.5.1.1 **Inhibition to microbial activity (terrestrial)**
Annex Point IIA7.4

		from each replicate, and compared to the controls. Nitrogen turnover soils were treated (4/5 replicates of each) with ammonium sulphate and plant material (wheat straw and Lucerne meal). Soils were incubated for up to 18 days and the rate of ammonification assessed. Ammonia, nitrite (where applicable) and nitrate were extracted with K2SO4 solution and quantities determined colourimetrically, and compared to control values.
3.3.1	**Soil sample**	see table A7.5.1.1-1
3.3.2	**Test system**	see table A7.5.1.1-2
3.3.3	**Application of TS**	see table A7.5.1.1-2
3.3.4	**Test conditions**	see table A7.5.1.1-2
3.3.5	**Test parameter**	Inhibition of microbial carbon transformation
3.3.6	**Analytical parameter**	Carbon turnover - evolved carbon dioxide measured from each replicate, and compared to the controls. Nitrogen turnover - ammonia, nitrite (where applicable) and nitrate were extracted with K2SO4 solution and quantities determined colourimetrically, and compared to control values
3.3.7	**Duration of the test**	see table A7.5.1.1-2
3.3.8	**Sampling**	Varied per test
3.3.9	**Monitoring of TS concentration**	No
3.3.10	**Controls**	Controls without test substance
3.3.11	**Statistics**	Data were analysed statistically by two-way analysis of variance.
		4 RESULTS
4.1	**Range finding test**	Not performed
4.2	**Results test substance**	
4.2.1	**Initial concentrations of test substance**	Permethrin was applied at two rates, 0.5 and 10 kg ai/ha, equivalent to 0.80 and 16 mg/kg (soil 1) and 0.70 and 14 mg/kg (soil 2). Controls were run concurrently for comparison.
4.2.2	**Actual concentrations of test substance**	Not measured
4.2.3	**Growth curves**	Not measured
4.2.4	**Cell concentration data**	Not measured
4.2.5	**Concentration/ response curve**	See Figures 1-9

Section A7.5.1.1 **Inhibition to microbial activity (terrestrial)**
Annex Point IIA7.4

4.2.6	**Effect data**	See Tables A7.5.1.1-3 to 15
4.2.7	**Other observed effects**	None
4.3	**Results of controls**	See Figures 1-9, Tables A7.5.1.1-3 to 15
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	**Soils**: Two soils were used, a coarse sand and a coarse sandy loam. Full soil characterisation data (pH, OM, MHC, P, K, Mg, CEC) are given in the report. **Test material**: The test material was PP557, 25:75 *cis*:*trans*-permethrin **Soil preparation and treatment**: Freshly sampled soil was processed and prepared for testing, and permethrin applied at two rates, 0.5 and 10 kg ai/ha, equivalent to 0.80 and 16 mg/kg (soil 1) and 0.70 and 14 mg/kg (soil 2). **Carbon turnover** soils were treated (4 replicates of each) with either soil organic matter, glucose, plant material, sucrose, urea, starch, pectin, cellulose, tripalmitin, phenol or vanillin. Soils were then incubated for up to 40 days and evolved carbon dioxide measured from each replicate, and compared to the controls. **Nitrogen turnover** soils were treated (4/5 replicates of each) with ammonium sulphate and plant material (wheat straw and Lucerne meal). Soils were incubated for up to 18 days and the rate of ammonification assessed. Ammonia, nitrite (where applicable) and nitrate were extracted with K2SO4 solution and quantities determined colourimetrically, and compared to control values.
5.2	**Results and discussion**	Results indicate that the permethrin treatments did not adversely effect ammonification and nitrification in two soils amended with lucerne meal wheat straw or ammonium sulphate. Slight stimulatory effects (generally <10%) were only transient. Permethrin treatment also had no effect on the organic matter turnover or the decomposition of glucose, sucrose, urea, starch, phenol, pectin, cellulose, vanillin, tripalmitin.
5.2.1	**NOEC**	> 16 mg kg^{-1} Converted to artificial soil **> 9.9 mg/kg dwt** > 14 mg kg^{-1} Converted to artificial soil **: > 31.7 mg/kg dwt** Calculations are given in annex I
5.2.2	**EC_{10}**	Not determined
5.2.3	**EC_{50}**	Not determined
5.3	**Conclusion**	Validity criteria can be considered as fulfilled

Section A7.5.1.1 **Inhibition to microbial activity (terrestrial)**
Annex Point IIA7.4

5.3.1	**Reliability**	1
5.3.2	**Deficiencies**	No – the experimental design is well described, and multiple functions of soil impact have been assessed and 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	28/04/05
Materials and Methods	Applicant's version is comprehensive and acceptable
Results and discussion	Adopt applicant's version
Conclusion	Adopt applicant's version
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_5_1_1-1: Soil samples

Criteria	**Details**	
Nature	soil sample	soil sample
Sampling site:	Lower sand field, Lower Farm, Bury St. Edmunds	Pear Tree Meadow, Jealotts Hill Farm, Bracknell
Geographical reference on the sampling site	UK OS 892728	UK OS 875738
Data on the history of the site	Conventional crop rotation until 1975	Vegetation, no treatments with fertilizers
Use pattern	Agricultural soil	Open field
Depth of sampling [cm]	20 cm	20 cm
Sand / Silt / Clay content [% dry weight]	92:4:4	59:14:27
pH	7.8	6.0
Organic matter content [% dry weight]	1.5	5.5
Nitrogen content [% dry weight]	-	-
Cation exchange capacity [meq/100g]	5.0	13.0
Available P (mg/100 g soil)	5.5	5
Available K (mg/100 g soil)	13	18
Available Mg (mg/100 g soil)	3.5	14
Initial microbial biomass	Not reported	Not reported
Reference of methods	Not reported	Not reported
Collection / storage of samples	Prior to use samples were stored in a moist cold room.	Prior to use samples were stored in a moist cold room.
Preparation of inoculum for exposure	Not reported	Not reported
Pretreatment	No pretreatment	No pretreatment

Table A7_5_1_1-2: Test conditions

Table 2: Experimental details of carbon and nitrogen turnover tests.

Test	Substrate	Amount applied mg C/100 g soil	Method of application in		Incubation temperature °C	Incubation time (days)		No. of replicates per treatment		
			LSF soil	PT soil		LSF soil	PT soil			
Carbon Turnover	Soil organic matter	none - already present in soil			21°-27°	13	14	4		
	[U-^{14}C] glucose	80	aqueous solution		21°-27°	6	12	4		
	[U-^{14}C] plant material	225	dried, ground (<1 mm) wheat straw		21°-27°	40	39	4		
	[U-^{14}C] phenol	50	aqueous solution		25°±1°	34	40	4		
	[U-^{14}C] urea	50	aqueous solution		25°±1°	34	12	4		
	[U-^{14}C] starch	25	aqueous solution		25°±1°	34	12	4		
	[U-^{14}C] sucrose	50	-	aqueous solution	25°±1°	-	12	4		
	Tripalmitin	50	powder	aqueous solution	25°±1°	34	40	4		
	Vanillin	50	powder	-	25°±1°	34	-	4		
	Pectin	50	powder	-	25°±1°	34	-	4		
	Cellulose	50	powder	-	25°±1°	34	-	4		
Test	Substrate	Amount applied µg N/g soil	Method of application in		Incubation temperature °C	Incubation time in (weeks)		No. of replicates per treatment		
			LSF soil	PT soil		LSF soil	PT soil	LSF	PT	
Nitrogen Turnover	$(NH_4)_2SO_4$	100	aqueous solution		25°±1°	5	6.5	5	4	
	Plant material	100	-	dried, ground (<1 mm) wheat straw	25°±1°	-	18	-	5	
		160	dried, powder-fine ground lucerne		25°±1°	8	-	5	-	

Table A7_5_1_1-3 - 15: Results

Table 3: Effect of PP557 on soil organic matter turnover in 100 g LSF soil (mg C/100 g soil).

Treatment and Rate	mg C evolved as CO_2 per sampling interval (days after treatment)			
	0-1	1-2	2-6	6-13
Untreated Control	0.94	0.73	2.78	3.97
PP557 Low Rate	0.69	0.64	2.68	4.19
PP557 High Rate	1.01	0.47	2.85	3.94
Standard error (single plot)	0.15	0.18	0.33	0.27
Difference between treatment means	Sig	N/S	N/S	N/S
LSD for P=5%/1%	0.26/ 0.36			
Probability level		20.5%	80.5%	42.6%

Treatment and Rate	Cumulative values (days after treatment)			
	1	2	6	13
Untreated Control	0.94	1.67	4.44	8.41
PP557 Low Rate	0.69	1.33	4.01	8.20
PP557 High Rate	1.01	1.49	4.34	8.27
Standard error (single plot)	0.15	0.29	0.40	0.50
Difference between treatment means	Sig	N/S	N/S	N/S
LSD for P=5%/1%	0.26/ 0.36			
Probability level		37.5%	38.5%	86.3%

37460145

Table 4: Effect of PP557 on soil organic matter turnover in 100 g PT soil (mg C/100 g soil).

Treatment and Rate	mg C evolved as CO_2 per sampling interval (days after treatment)				
	0-1	1-2	2-3	3-6	6-14
Untreated Control	2.3	2.2	2.4	7.2	14.3
PP557 Low Rate	2.7	2.1	2.2	6.9	14.4
PP557 High Rate	2.4	2.2	2.8	6.5	14.8
Standard error (single plot)	0.3	0.1	0.6	0.5	0.3
Difference between treatment means	N/S	N/S	N/S	N/S	N/S
Probability level	15.1%	58.3%	37.1%	18.0%	15.7
Treatment and Rate	**Cumulative values (days after treatment)**				
	1	2	3	6	14
Untreated Control	2.3	4.5	6.8	14.0	28.3
PP557 Low Rate	2.7	4.8	7.0	13.9	28.3
PP557 High Rate	2.4	4.7	7.4	13.8	28.6
Standard error (single plot)	0.3	0.3	0.7	0.8	0.9
Difference between treatment means	N/S	N/S	N/S	N/S	N/S
Probability level	15.1%	52.6%	52.5%	96.8%	83.5%

37460146

Table 5: Effect of PP557 on microbial activity in 100 g LSF soil amended with ^{14}C-labelled glucose (2000 mg/kg). (mg ^{14}C/100 g soil).

Treatment and Rate	mg ^{14}C evolved as $^{14}CO_2$ per sampling interval (hours after treatment)			
	0-24	24-48	48-72	72-144
Untreated Control	10.2	17.2	5.97	8.29
PP557 Low Rate	10.6	18.6	5.03	8.13
PP557 High Rate	9.6	19.1	5.51	8.07
Standard error (single plot)	0.4	1.0	1.07	0.43
Difference between treatment means	SIG	N/S	N/S	N/S
LSD for P=5%/1%	0.7/ 1.0			
Probability level		8.9%	49.2%	77.5%
Treatment and Rate	Cumulative values (hours after treatment)			
	24	48	72	144
Untreated Control	10.2	27.4	33.4	41.7
PP557 Low Rate	10.6	29.2	34.2	42.3
PP557 High Rate	9.6	28.7	34.2	42.3
Standard error (single plot)	0.4	1.2	0.5	0.7
Difference between treatment means	SIG	N/S	N/S	N/S
LSD for P=5%/1%	0.7/ 1.0			
Probability level		19.9%	6.0%	38.5%

37460149

Table 6: Effect of PP557 on microbial activity in 100 g PT soil amended with ^{14}C-labelled glucose (2000 mg/kg). (mg ^{14}C/100 g soil).

Treatment and Rate	mg ^{14}C evolved as $^{14}CO_2$ per sampling interval (hours after treatment)					
	0-16	16-24	24-48	48-120	120-168	168-288
Untreated Control	18.4	6.06	4.43	5.27	1.91	3.77
PP557 Low Rate	18.3	6.29	4.73	5.32	1.90	3.70
PP557 High Rate	17.1	6.74	4.77	5.71	2.07	3.94
Standard error (single plot)	1.3	0.36	0.46	0.29	0.11	0.14
Difference between treatment means	N/S	N/S	N/S	N/S	N/S	N/S
Probability level	35.2%	6.8%	54.8%	10.9%	9.3%	10.3%

Treatment and Rate	Cumulative values (hours after treatment)					
	16	24	48	120	168	288
Untreated Control	18.4	24.5	28.9	34.2	36.1	39.9
PP557 Low Rate	18.3	24.6	29.3	34.6	36.5	40.2
PP557 High Rate	17.1	23.9	28.6	34.3	36.4	40.4
Standard error (single plot)	1.3	1.2	0.9	0.6	0.6	0.3
Difference between treatment means	N/S	N/S	N/S	N/S	N/S	N/S
Probability level	35.2%	67.1%	54.9%	62.6%	55.4%	41.0%

37460150

Table 7: Decomposition of 0.5 g ^{14}C-labelled plant material treated with PP557 in 100 g LSF soil. (mg ^{14}C/100 g soil).

Treatment and Rate	mg ^{14}C evolved as $^{14}CO_2$ per sampling interval (days after treatment)							
	0-2	2-4	4-7	7-11	11-15	15-22	22-29	29-40
Untreated Control	13.9	20.0	18.7	15.8	12.3	16.6	8.3	8.2
PP557 10% EC:								
Low Rate	14.8	19.5	18.9	15.1	12.2	17.6	8.6	8.8
High Rate	13.7	19.7	18.9	15.9	12.7	16.7	8.3	8.9
PP557 25% EC:								
Low Rate	14.7	19.8	18.2	15.2	12.2	16.4	8.2	8.8
High Rate	14.6	19.6	18.4	15.1	12.2	16.5	8.4	8.7
Standard error (single plot)	0.9	1.2	0.4	0.5	0.4	0.7	0.3	0.5
Difference between treatment means	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
Probability level	42.7%	98.3%	10.2%	5.4%	35.2%	12.9%	34.1%	42.5%

Treatment and Rate	Cumulative values (days after treatment)							
	2	4	7	11	15	22	29	40
Untreated Control	13.9	33.9	52.6	68.5	80.7	97.3	105.6	113.9
PP557 10% EC:								
Low Rate	14.8	34.3	53.3	68.4	80.5	98.2	106.8	115.6
High Rate	13.7	33.5	52.4	68.3	81.0	97.7	104.2	115.1
PP557 25% EC:								
Low Rate	14.7	34.4	52.7	67.9	80.1	96.5	104.7	113.5
High Rate	14.6	34.1	52.6	67.7	79.9	96.4	104.8	113.4
Standard error (single plot)	0.9	2.0	2.2	2.4	2.6	2.5	2.5	2.7
Difference between treatment means	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
Probability level	42.7%	96.4%	98.2%	98.8%	97.0%	81.5%	71.6%	68.4%

37460154

Table 8: Decomposition of 0.5 g ^{14}C-labelled plant material treated with PP557 in 100 g PT soil (mg ^{14}C/100 g soil).

Treatment and Rate	mg ^{14}C evolved as $^{14}CO_2$ per sampling interval (days after treatment)							
	0-1	1-3	3-5	5-8	8-13	13-21	21-28	28-39
Untreated Control	8.8	13.6	14.2	15.5	14.5	10.4	5.7	6.6
PP557 10% EC:								
Low Rate	8.5	13.9	14.3	15.8	14.8	11.0	6.1	6.8
High Rate	8.3	13.4	14.1	15.9	14.5	10.5	5.5	6.0
PP557 25% EC:								
Low Rate	7.4	14.4	14.7	16.2	15.1	10.7	6.1	6.5
High Rate	8.1	13.8	14.1	15.7	14.3	9.4	5.6	5.2
Standard error (single plot)	0.9	0.9	0.5	0.5	0.4	0.6	0.4	0.5
Difference between treatment means	N/S.	N/S	N/S	N/S	N/S	SIG	N/S	N/S
LSD for P=5%/1%						0.9/ 1.2		
Probability level	26.9%	60.4%	60.5%	40.6%	21.4%		15.9%	15.3%

Treatment and Rate	Cumulative values (days after treatment)							
	1	3	5	8	13	21	28	39
Untreated Control	8.8	22.4	36.6	52.1	66.6	77.0	82.7	89.3
PP557 10% EC:								
Low Rate	8.5	22.5	36.7	52.5	67.4	78.4	84.5	91.3
High Rate	8.3	21.7	35.9	51.8	66.4	76.9	82.4	88.4
PP557 25% EC:								
Low Rate	7.4	21.8	36.4	52.6	67.7	78.4	84.5	91.0
High Rate	8.1	21.9	36.0	51.7	66.2	75.6	81.2	87.4
Standard error (single plot)	0.9	1.2	1.4	1.5	1.6	1.9	2.1	2.2
Difference between treatment means	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
Probability level	26.9%	85.5%	87.6%	88.0%	62.3%	25.7%	16.7%	9.7%

37460155

Table 9A: Effect of PP557 on turnover of organic substances in 100 g LSF soil (mg C/100 g soil).

Substrate (amount applisd) Treatment and Rate	mg C evolved as CO_2 per sampling interval (days after treacment)						Cumulative values (days after treatment)					
	0-2	2-5	5-9	9-16	16-23	23-34	2	5	9	16	23	34
Pectin (50 mg C)												
Untreated Control	18.0	8.5	3.90	3.02	2.11	3.62	18.0	26.5	30.4	33.4	35.5	39.1
PP557 Low Rate	20.1	5.3	4.88	3.34	2.49	3.85	20.1	25.4	30.3	33.6	36.1	40.0
PP557 High Rate	20.6	5.9	6.46	3.38	2.09	1.63	20.6	26.5	32.9	36.3	38.4	40.0
Standard error (single plot)	1.6	2.9	0.74	0.51	0.22	0.90	1.6	2.3	2.9	3.1	3.1	2.8
Difference between treatment means	N/S	N/S	S.SIG	N/S	N/S	SIG	N/S	N/S	N/S	N/S	N/S	N/S
LSD for P=5%/1%			1.18/ 1.70			1.44/ 2.07						
Probability level	10.3%	31.0%		38.7%	5.7%		10.3%	75.3%	38.8%	36.9%	41.9%	[redacted]
	0-5		5-9	9-16	16-23	23-34	5		9	16	23	[redacted]
Cellulose (50 mg C)												
Untreated Control	7.2		11.7	9.2	6.64	6.81	7.2		19.0	28.2	34.3	41.6
PP557 Low Rate	7.3		10.7	11.7	5.53	4.11	7.3		18.0	29.8	35.4	39.5
PP557 High Rate	7.6		12.0	12.2	5.32	3.58	7.6		19.6	31.8	37.2	40.7
Standard error (single plot)	0.5		2.5	3.3	1.19	1.22	0.5		2.3	3.3	3.2	4.2
Difference between treatment means	N/S		N/S	N/S	N/S	S.SIG	N/S		N/S	N/S	N/S	N/S
LSD for P=5%/1%						1.95/ 2.80						
Probability level	55.3%		76.1%	43.2%	30.7%		55.3%		63.6%	34.3%	58.3%	77.6%
Tripalmitin (50 mg C)												
Untreated Control	5.13		7.72	6.52	5.12	8.18	5.1		12.9	19.4	24.5	32.7
PP557 Low Rate	4.47		7.98	6.90	5.71	9.25	4.5		12.5	19.3	25.1	34.3
PP557 High Rate	5.74		7.93	6.95	4.93	6.48	5.7		13.7	20.6	25.6	32.0
Standard error (single plot)	0.48		0.84	0.56	0.73	0.66	0.5		0.7	1.2	1.7	2.1
Difference between treatment means	SIG		N/S	N/S	N/S	S.SIG	SIG		N/S	N/S	N/S	N/S
LSD for P=5%/1%	0.77/ 1.11					1.06/ 1.53	0.8/ 1.1					[redacted]
Probability level			90.2%	52.5%	33.0%				10.5%	29.3%	67.9%	32.6%
Vanillin (50 mg C)												
Untreated Control	23.5		4.15	1.41	0.51	0.76	23.5		27.6	29.0	29.5	30.3
PP557 Low Rate	23.9		2.89	1.39	1.14	1.95	23.9		26.8	28.2	29.3	31.3
PP557 High Rate	23.6		2.43	1.65	0.95	1.31	23.6		26.0	27.7	28.6	30.0
Standard error (single plot)	1.5		0.68	0.45	0.44	0.64	1.5		1.9	2.0	2.1	2.4
Difference between treatment means	N/S		SIG	N/S	N/S	N/S	N/S		N/S	N/S	N/S	N/S
LSD for P=5%/1%			1.09/ 1.57									
Probability level	90.1%			67.1%	16.6%	7.4%	90.1%		54.0%	65.7%	82.8%	71.6%

37460158

-23-

Table 9b: Effect of PP557 on turnover of ^{14}C-labelled organic substances in 100 g LSF soil (mg ^{14}C/100 g soil).

Substrate (amount applied) Treatment and Rate	mg ^{14}C evolved as $^{14}CO_2$ per sampling interval (days after treatment)								Cumulative values (days after treatment)							
	0-1	1-2	2-4	4-7	7-9	9-16	16-23	23-34	1	2	4	7	9	16	23	34
^{14}C-Urea (50 mg C)																
Untreated Control	3.76	3.52	7.14	7.35	2.70	5.11	1.65	0.85	3.8	7.3	14.4	21.8	24.5	29.6	31.2	32.1
PP557 Low Rate	3.77	3.54	7.13	7.24	2.90	5.17	1.69	0.87	3.8	7.3	14.4	21.7	24.6	29.8	31.4	32.3
PP557 High Rate	3.66	3.52	7.31	7.18	2.68	4.22	2.08	1.06	3.7	7.2	14.8	22.0	24.7	28.9	31.0	32.0
Standard error (single plot)	0.14	0.16	0.40	0.18	0.10	0.73	0.36	0.11	0.1	0.2	0.4	0.5	0.5	0.8	0.5	0.4
Difference between treatment means	N/S	N/S	N/S	N/S	SIG	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
LSD for P=5%/1%					0.16/ 0.23											
Probability level	50.6%	96.6%	77.7%	46.5%		23.3%	29.7%	7.3%	50.6%	73.6%	43.0%	73.3%	37.4%	39.9%	46.6%	59.3%
	0-2	2-5	5-9	9-16	16-23	23-34			2	5	9	16	23	36		
^{14}C-Starch (25 mg C)																
Untreated Control	7.70	2.13	1.08	1.49	1.17	1.52			7.7	9.8	10.9	12.4	13.6	15.1		
PP557 Low Rate	8.00	2.00	1.08	1.33	1.18	1.39			8.0	10.0	11.1	12.4	13.6	15.2		
PP557 High Rate	8.37	2.18	1.07	1.48	1.15	1.48			8.4	10.5	11.7	13.2	14.4	15.8		
Standard error (single plot)	0.65	0.20	0.10	0.27	0.17	0.22			0.7	0.8	0.8	0.9	1.0	1.1		
Difference between treatment means	N/S	N/S	N/S	N/S	N/S	N/S			N/S	N/S	N/S	N/S	N/S	N/S		
Probability level	38.7%	44.9%	34.2%	64.4%	97.5%	76.7%			38.7%	46.2%	39.9%	37.6%	44.7%	57.3%		
^{14}C-Phenol (50 mg C)																
Untreated Control	0.04	0.60	15.1	6.48	1.24	1.24			0.04	0.64	15.8	22.2	23.5	24.7		
PP557 Low Rate	0.06	0.70	14.4	6.98	1.23	0.96			0.06	0.76	15.1	22.1	23.3	24.3		
PP557 High Rate	0.06	1.99	17.0	7.24	1.32	1.06			0.06	2.05	19.0	26.3	27.6	28.6		
Standard error (single plot)	0.02	0.96	1.2	0.61	0.08	0.27			0.02	0.96	1.9	2.0	2.0	2.0		
Difference between treatment means	N/S	N/S	SIG	N/S	N/S	N/S			N/S	N/S	N/S	SIG	SIG	SIG		
LSD for P=5%/1%			1.9/ 2.8									3.2/ 4.7	3.3/ 4.8	3.3/ 4.8		
Probability level	15.3%	14.2%		25.4%	25.3%	43.0%			15.3%	14.0%	5.0%					

37460159

-24-

Table 10: Effect of PP557 on turnover of ^{14}C-labelled and unlabelled organic substances in 100 g PT soil. (mg ^{14}C or mg C/100 g soil).

Substrate (amount applied) Treatment and Rate	mg C evolved as CO_2 per sampling interval (days after treatment)					Cumulative values (days after treatment)				
	0-1	1-2	2-5	5-8	8-12	1	2	5	8	12
^{14}C-Urea (50 mg C)										
Untreated Control	22.2	14.9	9.2	0.9	0.25	22.2	37.1	46.3	47.2	47.4
PP557 Low Rate	21.4	15.1	9.5	0.9	0.35	21.4	36.5	45.9	46.8	47.2
PP557 High Rate	21.3	15.5	9.5	0.9	0.27	21.3	36.8	46.3	47.1	47.4
Standard error (single plot)	0.8	0.4	0.5	0.	0.09	0.8	1.0	0.8	0.7	0.6
Difference between treatment means	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
Probability level	14.1%	11.6%	74.6%	99.1%	37.1%	34.1%	77.7%	32.9%	76.1%	33.7%
^{14}C-Sucrose (50 mg C)										
Untreated Control	14.0	2.27	3.30	1.34	1.77	14.0	16.3	19.6	21.4	23.2
PP557 Low Rate	14.4	2.26	3.33	1.34	1.68	14.4	16.7	20.0	21.9	23.6
PP557 High Rate	14.6	2.04	3.04	2.08	2.14	14.6	16.7	19.7	21.8	23.9
Standard error (single plot)	0.8	0.17	0.20	0.14	0.17	0.8	0.9	0.9	0.9	0.9
Difference between treatment means	N/S	N/S	N/S	N/S	SIG	N/S	N/S	N/S	N/S	N/S
LSD for P=5%/1%					0.27/ 0.40					
Probability level	61.3%	17.3%	14.2%	7.6%		61.3%	78.1%	79.2%	80.7%	59.9%
^{14}C-Starch (25 mg C)										
Untreated Control	4.46	1.40	1.72	0.96	0.90	4.5	5.9	7.6	8.6	9.5
PP557 Low Rate	4.92	1.28	1.70	0.97	0.92	4.9	6.2	7.9	8.9	9.8
PP557 High Rate	5.40	1.47	1.73	0.94	0.95	5.4	6.9	8.6	9.6	10.5
Standard error (single plot)	0.52	0.13	0.15	0.12	0.06	0.5	0.5	0.6	0.6	0.7
Difference between treatment means	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
Probability level	8.4%	17.0%	96.0%	93.8%	42.3%	8.4%	6.6%	9.2%	12.6%	11.6%

Cont/d.....

Cont/d.....

Table 10: Effect of PP557 on turnover of ^{14}C-labelled and unlabelled organic substances in 100 g PT soil. (mg ^{14}C or mg C/100 g soil).

Substrate (amount applied) Treatment and Rate	mg C evolved as CO_2 sampling interval (days after treatment)							Cumulative values (days after treatment)						
	0-5	5-9	9-13	13-16	16-21	21-29	29-40	5	9	13	16	21	29	40
^{14}C-Phenol (50 mg C)														
Untreated Control	9.16	7.72	0.97	0.43	0.52	0.88	0.55	9.2	16.9	17.9	18.3	18.8	19.7	20.2
PP557 Low Rate	8.35	8.72	1.11	0.45	0.52	0.83	0.38	8.4	17.1	18.2	18.6	19.2	20.0	20.4
PP557 High Rate	7.65	9.65	0.99	0.42	0.51	0.82	0.58	7.7	16.3	17.3	17.7	18.2	19.0	19.6
Standard error (single plot)	0.76	0.54	0.10	0.02	0.02	0.05	0.06	0.8	0.8	0.8	0.8	0.8	0.8	0.9
Difference between treatment means	N/S	SIG	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
LSD for P=5%/1%		0.87/ 1.25												
Probability level	5.7%		14.3%	13.5%	71.9%	15.4%	63.4%	5.7%	38.3%	30.7%	29.9%	30.0%	31.1%	35.6%
	0-9		9-13	13-16	16-21	21-29	29-40	9		13	16	21	29	40
Tripalmitin (50 mg C)														
Untreated Control	2.95		3.58	1.91	2.85	2.40	3.50	3.0		6.5	8.4	11.3	13.7	17.2
PP557 Low Rate	4.86		3.82	2.56	2.82	2.59	2.89	4.9		8.7	11.2	14.1	16.7	19.5
PP557 High Rate	5.30		4.19	2.86	2.97	1.43	1.61	5.3		9.5	12.4	15.3	16.8	18.4
Standard error (single plot)	1.48		0.70	0.35	0.45	0.73	1.29	1.5		2.0	2.0	2.3	2.8	3.7
Difference between treatment means	N/S		N/S	SIG	N/S	N/S	N/S	N/S		N/S	N/S	N/S	N/S	N/S
LSD for P=5%/1%				0.57/ 0.84										
Probability level	12.0%		48.8%		89.2%	12.4%	17.2%	12.0%		15.1%	6.7%	9.0%	28.2%	71.9%

37460161

Table 11: Effect of PP557 on ammonification and nitrification of nitrogen derived from lucerne (360 ppm N) and wheat straw (100 ppm) in PT and LSF soil respectively. (µg N/g soil).

Treatment and Rate	NH_4^+-N (days after treatment)					
	0	7	14	21	32	56
Lower Sand Field Soil:						
Untreated Control	0	51.2	4.6	1.8	0	0
PP557 Low Rate	0	55.6	5.3	1.5	0	0
PP557 High Rate	0	48.6	6.0	2.5	0	0
Standard error (single plot)	-	3.9	1.5	2.2	-	-
Difference between treatment means	-	SIG	N/S	N/S	-	-
LSD for P=5%/1%	-	5.3/ 7.5			-	-
Probability level			37.1%	75.6%	-	-

Treatment and Rate	NO_3^--N (days after treatment)					
	0	7	14	21	32	56
Untreated Control	26.1	16.1	76.6	92.4	133.2	152.8
PP557 Low Rate	25.5	13.5	81.6	83.7	122.4	155.3
PP557 High Rate	25.7	16.5	80.8	101.3	178.4	170.6
Standard error (single plot)	5.0	2.3	4.1	4.0	7.1	11.7
Difference between treatment means	N/S	N/S	N/S	H.SIG	H.SIG	N/S
LSD for P=5%/1%				5.6/ 7.9	9.8/ 13.8	
Probability level	98.4%	11.9%	20.1%			6.8%

Treatment and Rate	NO_3^--N (weeks after treatment)				
	0	3	13	15	18
Pear Tree Soil:					
Untreated Control	38.9	31.1	95.3	109.1	109.8
PP557 Low Rate	36.6	29.6	95.2	104.1	104.0
PP557 High Rate	33.0	32.5	100.3	117.4	116.1
Standard error (single plot)	3.5	5.8	3.2	1.3	4.0
Difference between treatment means	N/S	N/S	N/S	H.SIG	H.SIG
LSD for P=5%/1%				2.2/ 3.1	5.5/ 7.8
Probability level	10.4%	74.8%	7.9%		

37460165

Table 12: Effect of PP557 on nitrification of 100 ppm $(NH_4)_2SO_4$ nitrogen in LSF soil. (µg N/g soil).

Treatment and Rate	NO_3^--N (days after treatment)					
	0	3	7	14	21	35
Untreated Control	26.1	57.6	123.9	138.1	139.7	140.3
PP557 Low Rate	20.5	67.9	123.7	138.3	144.2	135.2
PP557 High Rate	22.6	62.7	122.8	135.8	136.0	135.7
Standard error (single plot)	5.2	2.3	2.8	4.0	4.1	3.1
Difference between treatment means	N/S	H.SIG	N/S	N/S	SIG	SIG
LSD for P=5%/1%		3.2/ 4.4			5.7/ 7.9	4.3/ 6.0
Probability level	25.7%		81.0%	55.8%		

Treatment and Rate	NH_4^+-N (days after treatment)				
	0	3	7	14	21
Untreated Control	88.1	53.2	0.3	0	0
PP557 Low Rate	87.1	53.9	0.0	0	0
PP557 High Rate	87.8	49.8	0.5	0	0
Standard error (single plot)	3.2	3.6	-	-	-
Difference between treatment means	N/S	N/S	-	-	-
Probability level	88.7%	20.7%	-	-	-

37460170

Table 13: Effect of PP557 on nitrification of 100 ppm $(NH_4)_2SO_4$ nitrogen in PT soil. (μg N/g soil).

Treatment and Rate	NO_3^--N (days after treatment)					
	3	7	14	21	28	45
Untreated Control	50.7	61.8	82.8	96.0	109	137
PP557 Low Rate	40.6	59.0	82.8	96.5	112	137
PP557 High Rate	51.4	60.8	76.2	90.7	102	136
Standard error (single plot)	6.6	4.0	1.9	6.4	6	3
Difference between treatment means	N/S	N/S	H.SIG	N/S	N/S	N/S
LSD for P=5%/1%			3.1/ 4.4			
Probability level	7.9%	62.0%		40.3%	12.3%	79.1%

Treatment and Rate	NH_4^+-N (days after treatment)					
	3	7	14	21	28	45
Untreated Control	59.1	53.0	37.5	25.7	15.0	3.2
PP557 Low Rate	65.0	58.0	39.9	26.0	13.8	3.1
PP557 High Rate	63.6	52.4	40.7	30.6	15.9	2.7
Standard error (single plot)	2.7	1.6	1.0	1.3	0.8	0.5
Difference between treatment means	SIG	H.SIG	H.SIG	H.SIG	SIG	N/S
LSD for P=5%/1%	4.3/ 6.2	2.5/ 3.6	1.6/ 2.3	2.1/ 3.0	1.3/ 1.9	
Probability level						46.1%

37460171

Figures 1-9

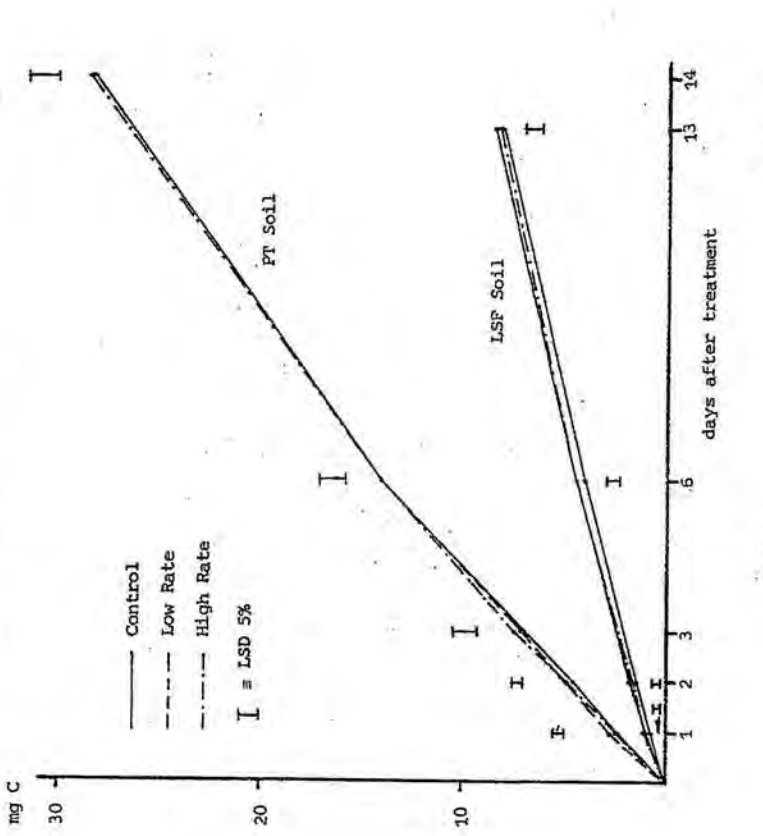


Figure 1: Effect of PP557 on soil organic matter turnover in PT and LSF soil (mg C evolved as CO_2/100 g soil)

37460144

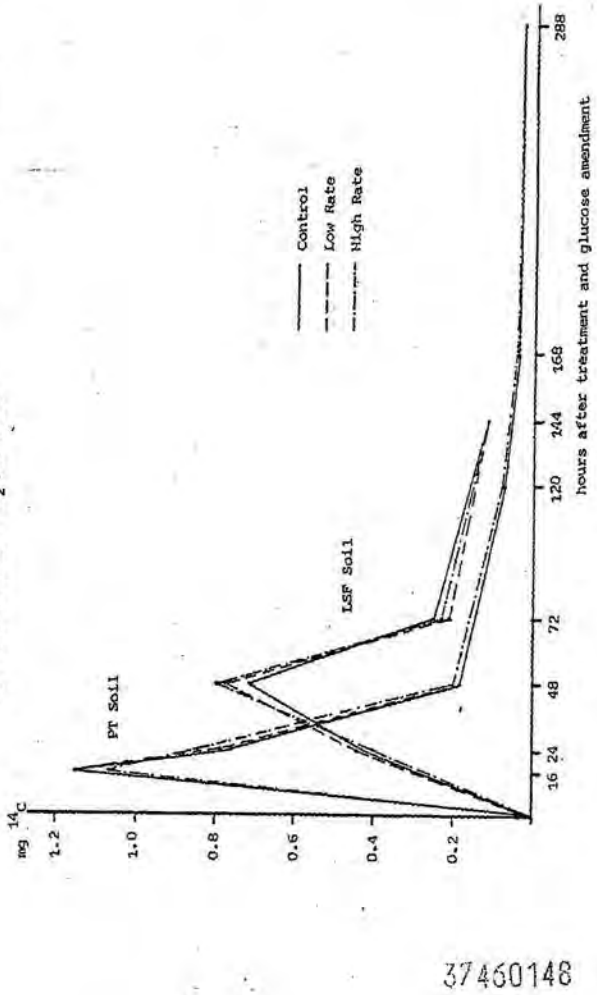


Figure 2: Effect of PP557 on microbial activity in 100 g LSF and PT soil amended with ^{14}C-labelled glucose (2000 mg/kg)
($mg\ ^{14}C$ evolved as $^{14}CO_2$ per hour)

37460148

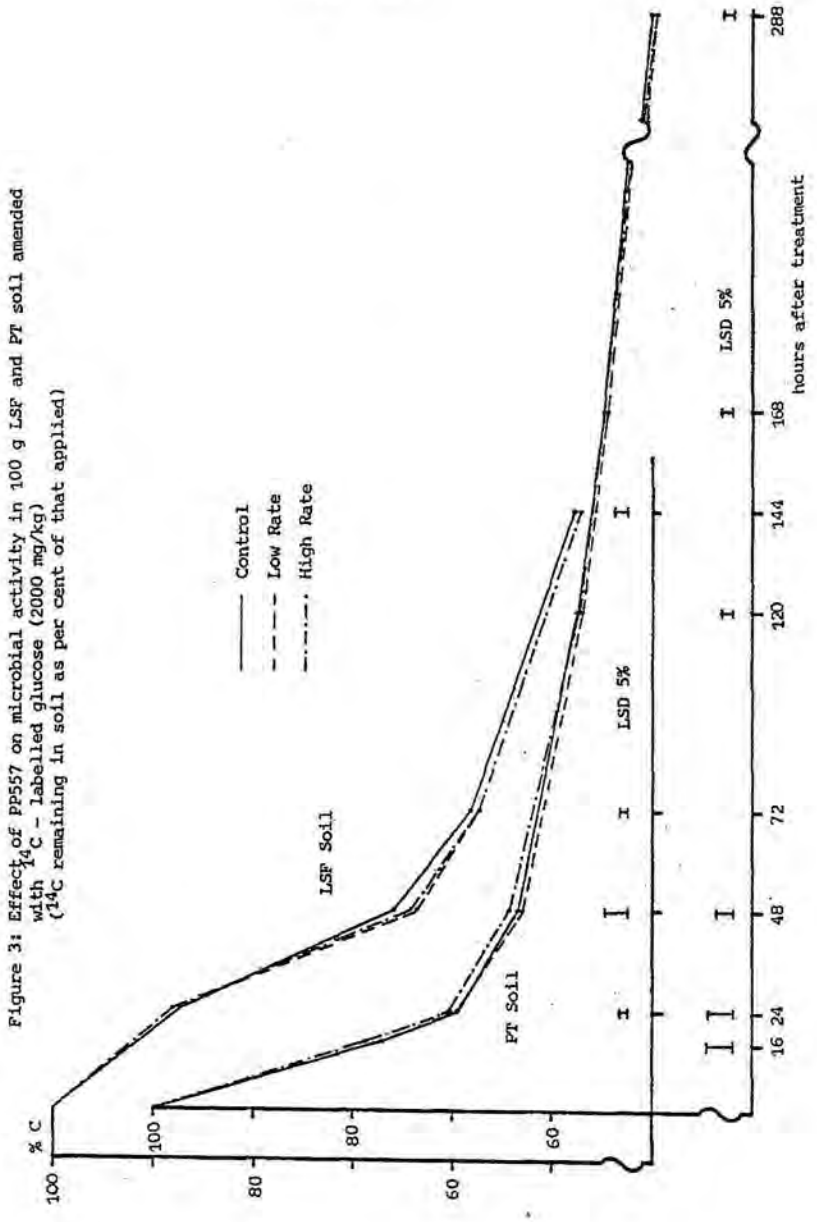


Figure 3: Effect of PP557 on microbial activity in 100 g LSF and PT soil amended with ^{14}C - labelled glucose (2000 mg/kg)
(^{14}C remaining in soil as per cent of that applied)

37460151