6,7 (2)	Ishmael, J. & Litchfield, M.H.	1988	Chronic Toxicity and Carcinogenic Evaluation of Permethrin in Rats and Mice. Fundamental and Applied Toxicology. Vol. 11. pp308-322	No	N/A
6,7 (1)	[redacted]	1980	21z: Potential Toxicity and Oncogenicity in Dietary Administration to Rats for a Period of 104 weeks. [redacted]. Report No. 80/WRL003/283 (Unpublished)	Yes	Sumitomo Chemical
6,8,1 (1)	[redacted]	1974	Foetal Toxicity of 21z73 (NRDC 143) in the Rat. [redacted] Report No. BPAT 74/10 (Unpublished)	Yes	Sumitomo Chemical
6,8,1 (2)	[redacted]	1979	21z: Effects of Oral Administration upon Pregnancy in the Rabbit. [redacted] Report No. HEFG 80-4.	Yes	Sumitomo Chemical
6,8,2	[redacted]	1979	A Multigeneration Reproduction Study of 21z73 (Permethrin) in the Rat. [redacted] No. BPAT 79/3.	Yes	Sumitomo Chemical
6,9	[redacted]	1997	Motor activity measurements in male and female mice postnatally exposed to Permethrin by inhalation; [redacted] unpublished Report No. 26418; 03.07.1997.	Yes	Sumitomo Chemical
6,13	[redacted]	1978	Permethrin Oral Administration to Dogs for 6 Months. [redacted] Report No. HEFG 78-14	Yes	Sumitomo Chemical

Competent Authority Report

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

Permethrin (PT 8)

Document III (A7)

Evaluation Report

March 2011

RMS: Ireland

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Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

Key Study

			Official use only
		1 REFERENCE	
1.1	**Reference**	Alvarez, M. & Dziedzic, J.E.; 1977; Hydrolysis of FMC 33297. FMC Corporation. Report No. CGP-77-12; Not GLP; Unpublished	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**	No	
2.2	**GLP**	No: GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Yes. The methodology followed is substantially different from more recent guidelines. Major differences are: 1. Testing was conducted in 20:80 acetonitrile buffer and 20:80 acetonitrile water to increase solubility and minimise loss to the test vessel walls. 2. Testing was conducted at 25 and 45°C	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	^{14}C-Permethrin (40:60 *cis:trans*) was created *in situ* by the addition of appropriate amounts of: *cis* permethrin radiolabelled with ^{14}C in the acid and alcohol moiety, and *trans* permethrin radiolabelled with ^{14}C in the acid and alcohol moiety. Determination of rate of hydrolysis was undertaken using a non-radiochemical detection technique, HPLC-UV. The radiolabel was utilised for analysis of potential breakdown products, but the report does not include these data, therefore no attempt has been made to interpret data other than those provided by HPLC-UV analysis.	
3.1.1	**Lot/Batch number**	Not available	
3.1.2	**Specification**	Not available	
3.1.3	**Purity**	Data not available	
3.1.4	**Further relevant properties**	Alcohol-^{14}C *cis* isomer had a Specific Activity of 57.01 mCi mmol^{-1} Alcohol-^{14}C *trans* isomer had a Specific Activity of 57.01 mCi mmol^{-1} Acid-^{14}C *cis* isomer had a Specific Activity of 54.8 mCi mmol^{-1}	

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

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Key Study

	Acid-^{14}C *trans* isomer had a Specific Activity of 54.8 mCi mmol^{-1}	
3.2 Reference substance	No	
3.2.1 Initial concentration of reference substance	Not applicable	
3.3 Test solution	Test substance was 40:60 *cis:trans* permethrin, prepared by the addition of appropriate quantities of individual isomers (3.1.4) to suitable volumes of prepared buffer solutions. Buffer solutions (tabular form; see table A7_1_1_1_1-1) Replications and experimental conditions (tabular form; see table A7_1_1_1_1-2)	X
3.4 Testing procedure	The report includes information on the assessment of hydrolysis using two separate systems, one designed for HPLC analysis of the test solutions, the other for TLC analysis. The TLC system required significant sample extraction to allow enough radioactivity to quantify, but provides no recovery information and results are only reported for one pH value. The purpose of the TLC was for preliminary identification of potential metabolites, and substantial information and data are not reported. For these reasons, the data are excluded as incomplete and of less reliability than the data generated *via* the system designed for HPLC analysis.	
3.4.1 Test system	Limited information on the test system is available. Flasks were 125 ml pyrex Erlenmeyers with ground glass-stoppers ed flat-bottomed boiling flasks. The flasks were placed in a constant temperature water-bath for the duration of the tesing period. The authors provide no indication that the buffer solutions were sterile, and do not discuss sterilisation method. No information on temperature control system or light exclusion is provided.	
3.4.2 Temperature	No test temperatures recorded during the test are reported	
3.4.3 pH	The report states the observed pH of the hydrolysis solutions were 5.7, 7.6 and 9.6, and the observed pH of the distilled water was 7.7. No other pH values recorded during the test are reported.	
3.4.4 Duration of the test	20 days at 25°C, 11 days at 45°C	
3.4.5 Number of replicates	One per pH and temperature	
3.4.6 Sampling	Samples were taken according to the following schedule; At 25°C: Days 1, 4, 6, 8, 11, 13, 16, 20 At 45°C: Days 0, 1, 4, 5, 6, 7, 8, 11	
3.4.7 Analytical	Solutions were analysed directly using HPLC under the following	

Section A7.1.1.1.1 **Hydrolysis as a function of pH and identification of breakdown products**

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Key Study

methods	chromatographic conditions; Column: μBondapak C18, 40 × 4.0 mm id Mobile phase: 100% water to 40:60 water:acetonitrile over 12 minutes, then to 28:72 water:acetonitrile over 12 minutes Flow rate: 1.0 ml min^{-1} Temperature: Ambient Detector: UV @ λ = 234 nm
3.5 Preliminary test	No
	4 RESULTS
4.1 Concentration and hydrolysis values	No numerical data are presented on the hydrolysis of permethrin. Data are presented graphically where relevant (Figures 1 and 2), and half-lives provided by the author are presented in Table A7_1_1_1_1-4(1) pH 5.7 (buffer) and 7.6 (buffer). At these pHs, no hydrolysis was observed at either temperature. pH 7.7 (distilled water). No hydrolysis was observed at 25°C. At 45°C slight loss was observed indicating half-lifes as given in Table A7_1_1_1_1-4(1) pH 9.7 (buffer). At both temperatures, hydrolysis was observed, with half-lifes as given in Table A7_1_1_1_1-4(1)
4.2 Hydrolysis rate constant (k_h)	Where possible, rate constants were calculated by the author and are presented in Table A7_1_1_1_1-4(2):
4.3 Dissipation time	See 4.1
4.4 Concentration – time data	See 4.1
4.5 Specification of the transformation products	The authors identify one peak in a chromatogram as phenoxybenzyl alcohol, although no co-chromatographic evidence are given to support this. Similarly in the discussion section of the report the authors discuss the production of DCVA, although no data are presented to support this.
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	Limited information were provided by the authors to assess the conduct of the test, however the report provides enough detail to indicate that the basic study design would be suitable to provide data which could be indicative of hydrolysis. Light exclusion and the use of distilled water, coupled with quantitative recovery at lower pH values would suggest photolysis, biodegradation and adsorption were minimised in the study design, intimating that any losses observed were due to hydrolysis.
5.2 Results and	At the higher temperature (45°C) transformation is rapid, giving half-

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

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Key Study

discussion		lives as shown in Table A7_1_1_1_1-4(1). At slightly elevated temperatures (25°C) permethrin appears to undergo some hydrolysis at high pH. While the results and study design would not be suitable for use in risk assessment if hydrolysis had been observed at environmentally relevant temperatures, it is proposed that, coupled with other data, they carry sufficient weight to support the conclusion that hydrolysis will not be a relevant loss mechanism in environmental risk assessment.
5.2.1	**k_H**	Not calculable
5.2.2	**DT_{50}**	Not calculable
5.2.3	**r^2**	Not calculable
5.3	**Conclusion**	Limited information on the protocol were provided, however, the data presented are sufficient to support several conclusions regarding the hydrolysis of permethrin 1. The main conclusion of the study is that permethrin is not subject to abiotic hydrolysis at acidic to neutral pH. Under basic conditions, hydrolysis is observed. 2. Environmentally relevant temperatures and pHs are unlikely to be sufficient to cause significant hydrolysis of permethrin based on these findings.
5.3.1	**Reliability**	2
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	*25 April 2005*	
Materials and Methods	The applicants version is acceptable with the following revision: **Comments:** (Section 3.3) Acetonitrile was used as a co-solvent owing to the hydrophobic nature of permethrin. The amount of acetonitrile used (~20%) as the co-solvent was greater than 1% of the test solution.	
Results and discussion	Adopt applicant's version.	
Conclusion	Adopt applicant's version.	
Reliability	2	
Acceptability	acceptable / ~~not acceptable~~	
Remarks	**Comments:** In line with current guidelines (SETAC 1995 and OECD 111) the	

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

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Key Study

	following deviations were observed: (Section 2.3) High levels of acetonitrile (~20%) were required in the test system owing to the hydrophobic nature of permethrin. The amount of solvent used to dissolve permethrin was greater than 1% of the test solution. However, it is considered unlikely that the solvent used should interfere with the hydrolysis process and the scientific validity of the study is not deemed to be compromised. (Section 3.4.4) The duration of the hydrolysis test was not 30 days, as preferred in the current test guidelines. The duration of the test under temperatures of 25 °C and 45 °C were 20 days and 11 days, respectively. However, this is considered a minor deviation owing to the relative stability of permethrin to abiotic hydrolysis under environmentally significant pH levels.
	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_1_1_1_1-1: Type and composition of buffer solutions (specify kind of water if necessary)

pH	Type of buffer (final molarity)	Composition
5	0.007 M phthalate	50 mL of 0.1 M potassium hydrogen phthalate and 22.6 ml of 0.1 M sodium hydroxide
7	0.006 M phosphate	50 mL of 0.1 M potassium dihydrogen phosphate and 29.1 mL of 0.1M sodium hydroxide
9	0.002 M borax	50 mL of 0.025 M borax and 4.6 mL of 0.1M hydrochloric acid
-	Distilled water	-

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	Distilled

Preparation of test medium	The buffer solutions were prepared as given in Table A7_1_1_1_1-1. Test media were prepared by adding 100 ml of the appropriate buffer solutions to 200 ml actonitrile and diluting to 1 litre with distilled water.
Test concentrations (mg a.i./L)	Test solutions were prepared by adding 84 g of distilled water, 23 ml acetonitrile and 12 ml of the appropriate buffer to a 125 ml Erlenmeyer flask. To this mixture was added 1 ml of an acetonitrile solution containing 3.6 µg of *trans* and 2.4 µg of *cis* isomer, to give test concentration of 50 µg l^{-1} permethrin (40:60 *cis:trans* ratio)
Temperature (°C)	25 and 45°C
Controls	None
Identity and concentration of co-solvent	Acetonitrile at 20%
Replicates	No information

Table A7_1_1_1_1-3: Description of test system

Glassware	Testing was conducted in 125 ml Erlenmeyer flasks with glound glass stoppers.
Other equipment	Temperature was maintained in constant temperature water baths.
Method of sterilization	No information.

Table A7_1_1_1_1-4(1):Hydrolysis of test compound expressed as average half-life at pH 5.7, 7.6, 9.6 (all buffered) and pH 7.7 (unbuffered) at 25°C and 45°C.

Permethrin isomer	Temp	Average t½ (days)			
		pH 5.7 buffered	pH 7.6 buffered	pH 9.6 buffered	pH 7.7 unbuffered
trans	25°C	>200	>200	42	>200
	45°C	>200	>200	5	130
cis	25°C	>200	>200	35	>200
	45°C	>200	>200	9	200

Table A7_1_1_1_1-4(2):Rate constants of test compound at pH 5.7, 7.6, 9.6 (all buffered) and pH 7.7 (unbuffered) at 25°C and 45°C.

Permethrin isomer	Temp	Rate constant ($\times 10^{-4}$ day^{-1})			
		pH 5.7 buffered	pH 7.6 buffered	pH 9.6 buffered	pH 7.7 unbuffered
trans	25°C	<35	<35	160	<35
	45°C	<35	<35	1200	55
cis	25°C	<35	<35	200	<35
	45°C	<35	<35	750	35

Figure 1: LnConcentration (100%/% remaining) vs. Time plot; pH 9, 25°C

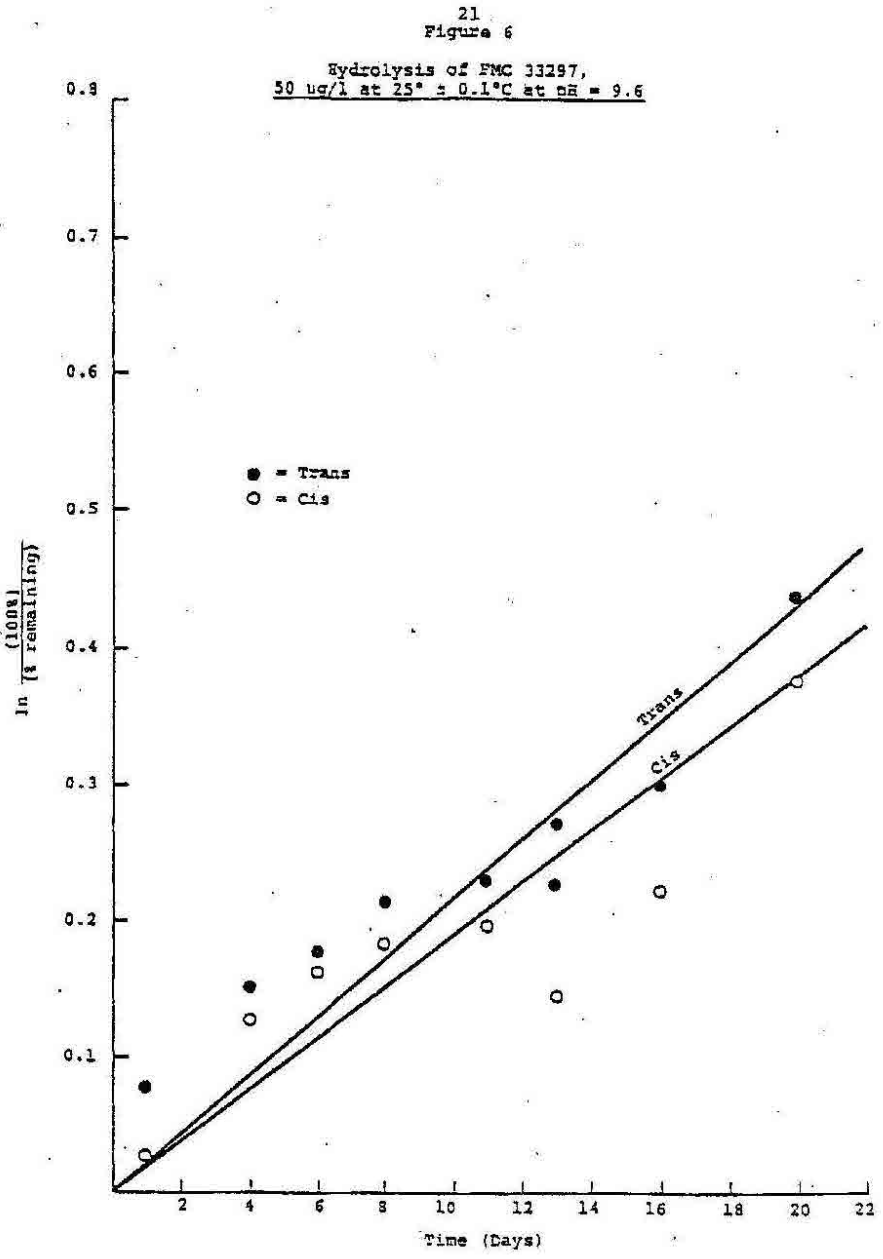
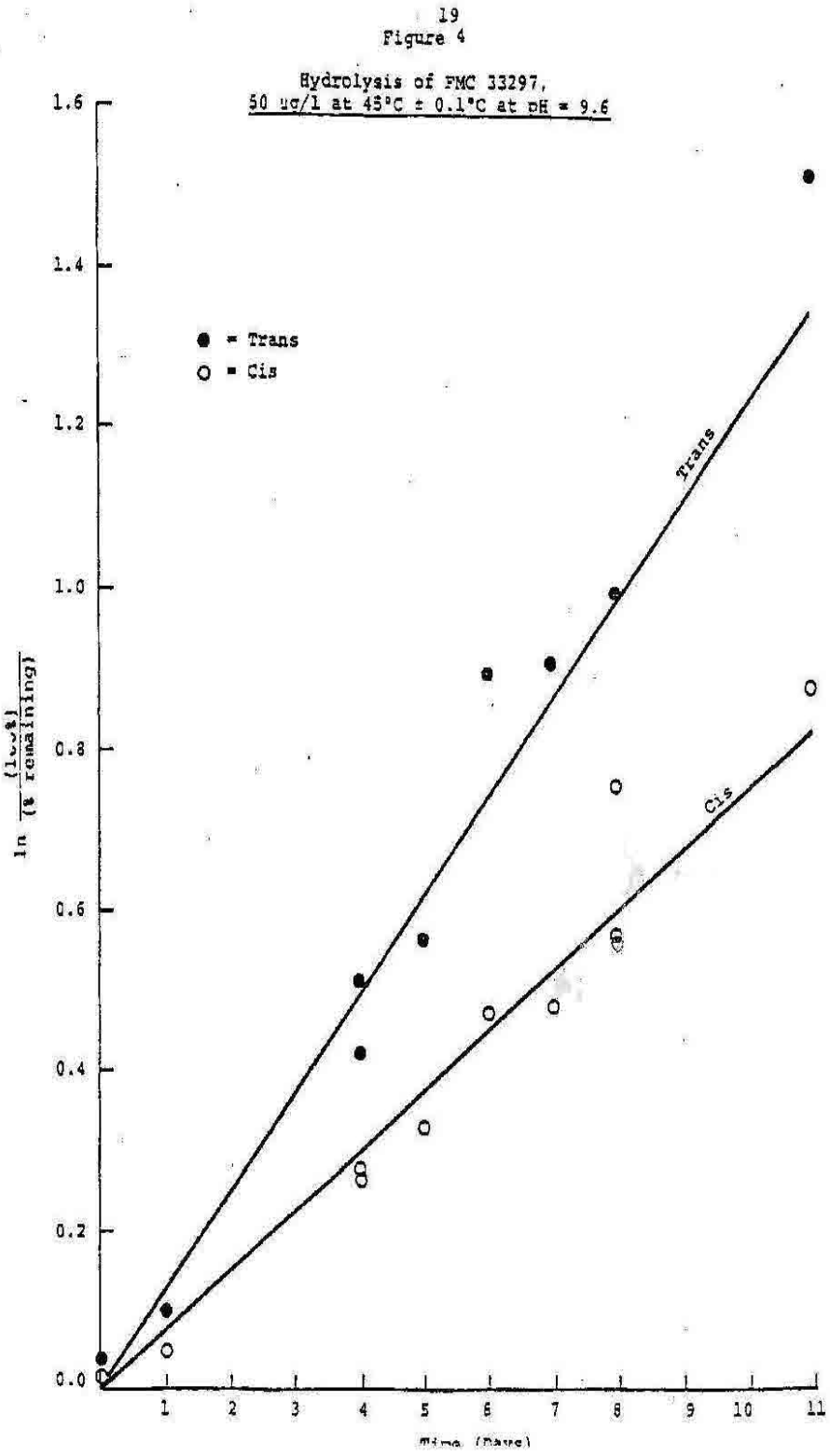


Figure 2: LnConcentration (100%/% remaining) vs. Time plot; pH 9, 45°C



Section A7.1.1.1.1 (2)
Annex Point IIA7.6.2.1

Hydrolysis as a function of pH and identification of breakdown products

Key Study

Official use only

		1 REFERENCE
1.1	**Reference**	Allsup, T.L. & Russell, K. H.; 1976; Hydrolysis of FMC 33297 Insecticide. FMC Corporation. Report No. W-0103. Not 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: EPA 6-25-75
2.2	**GLP**	No: GLP was not compulsory at the time the study was performed
2.3	**Deviations**	Yes. The guidelines followed are substantially different from more recent guidelines. Major differences are: 1. No preliminary testing at 50°C was undertaken – instead testing was performed at 3 temperatures, 25, 35, 45°C 2. Buffer solutions were pH 3, 6, 9, not pH 4, 7, 9 as recommended in current guidelines 3. Not enough datapoints were onbtained to enable rate constants to be determined
		3 MATERIALS AND METHODS
3.1	**Test material**	FMC33297 (*cis:trans* permethrin) radiolabelled with 14C in the alcohol moiety, and FMC33297 (*cis:trans* permethrin) radiolabelled with 14C in the acid moiety.
3.1.1	**Lot/Batch number**	Not available
3.1.2	**Specification**	As given in section 2
3.1.3	**Purity**	Data not available
3.1.4	**Further relevant properties**	Alcohol-14C had a radiochemical purity of 98% Acid-14C had a radiochemical purity of 100%
3.2	**Reference substance**	No
3.2.1	**Initial concentration of reference substance**	Not applicable

Section A7.1.1.1.1 (2)
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Hydrolysis as a function of pH and identification of breakdown products

Key Study

3.3 Test solution

Test substance:

Alc-14C: FMC33297 alcohol-14C was 25:75 *cis:trans* ratio. To this was added non-radiolabelled *cis* isomer, sufficient to produce a *cis:trans* ratio of 40:60 total permethrin.

Ac-14C: FMC33297 acid-14C was 25:75 *cis:trans* ratio. To this was added non-radiolabelled *trans* isomer, sufficient to produce a *cis:trans* ratio of 40:60 total permethrin. X

These values were confirmed by GC analysis (details not provided). X

Buffer solutions (tabular form; see table A7_1_1_1_1-1)

Replications and experimental conditions (tabular form; see table A7_1_1_1_1-2)

3.4 Testing procedure

3.4.1 Test system

Limited information on the test system is available. Flasks were 250 ml glass-stoppered flat-bottomed boiling flasks. The authors state the buffer solutions were sterile, but do not discuss sterilisation method. No information on temperature control system is provided. The report also states samples were maintained in the dark, but no detail as to light exclusion techniques are given.

3.4.2 Temperature

No test temperatures recorded during the test are reported

3.4.3 pH

No pH values recorded during the test are reported

3.4.4 Duration of the test

28 days at 25°C, 42 days at 35°C, 7 days at 45°C

3.4.5 Number of replicates

Three per pH and temperature

3.4.6 Sampling

Samples were taken according to the following schedule;

Temp	Label	pH	Day No.					
25	alc,ac	3,6	0	7	14	21	28	
	alc,ac	9	0	7	14	21	28	42
35	alc,ac	3,6	0	7	14	21	28	
	alc,ac	9	0	7	14	21	28	42
45	alc,ac	3,6	0	7	14	21	28	
	alc,ac	9	0 3	7	14	21	28	

Samples were analysed directly on sampling

3.4.7 Analytical methods

20 ml aliquots were placed in a 60 ml separator funnel, and approx. 0.5 g NaCl added. Samples were extracted with 15 ml chloroform, which was then dry filtered through anhydrous sodium sulphate. The sodium sulphate was rinsed with further aliquots of chloroform which were then combined. The volume was reduced to approximately 300 µl for TLC analysis. Analysis of the samples was by 2 dimensional TLC, benzene:carbontetrachloride (1:1) and formic acid saturated benzene:diethyl ether (10:3). The radioactive distribution was quantified by scraping radioactive areas from the plate, followed by direct liquid scintillation counting.

3.5 Preliminary test

No

4 RESULTS

4.1 Concentration and hydrolysis values

In tabular form (see table A7_1_1_1_1-4). Replicate data not reported, reported results had been adjusted for recovery of radioactivity from the TLC plate (normalised). The presented data have been readjusted and TLC recovery data reported, which has resulted in slight numerical difference, but these are not considered to

Section A7.1.1.1.1 (2) **Hydrolysis as a function of pH and identification of breakdown products**

Annex Point IIA7.6.2.1

Key Study

		affect the conclusions of this summary. Extraction recovery from solution was considered to be quantitative.
4.2	**Hydrolysis rate constant (k_h)**	Not calculable
4.3	**Dissipation time**	Not calculable
4.4	**Concentration – time data**	There was no convincing evidence of hydrolysis at any pH at 25°C, therefore these data are not presented graphically. At 45°C there was either no hydrolysis (pH 3, 6) or it was too rapid (pH 9) and not enough timepoints are available, therefore these data are not presented graphically. At 35°C pH 3, 6 no hydrolysis was observed, therefore these data are not presented. At pH 9 at 35°C hydrolysis was observed, these data are presented in Figures 1 and 2 (alcohol and acid radiolabelled)
4.5	**Specification of the transformation products**	Transformation products were tentatively identified by co-chromatography against non-radiolabelled samples of expected metabolites. Metabolite production was most marked in the pH 9 samples at 45°C, and these will be discussed. Similar profiles were observed at 35°C, but to a lesser extent. The sample labelled in the acid moiety gave one principle (>50% of applied radioactivity) breakdown product, identified as *cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCVA). The sample labelled in the alcohol moiety gave a single metabolite (>50% of applied radioactivity), identified as 3-phenoxybenzyl alcohol (PBA). A tentative degradation route is presented in Figure 3.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Limited information were provided by the authors to assess the conduct of the test, however the report states the study was conducted using a protocol suggested by the EPA guidelines for registering pesticides (6-25-75).
5.2	**Results and discussion**	At higher temperatures (35, 45°C) transformation to two metabolites, PBA and DCVA (See Table A7_1_1_1_1-6, Fig. 3) is rapid, with 64% and 43% transformation in 7 days at the higher temperature. At slightly elevated temperatures (35°C) permethrin appears to undergo some hydrolysis, forming the same degradation products. However, after a time period, an equilibrium between *cis:trans* isomers and breakdown products appears to be achieved and further hydrolysis does not occur. Because of this, 50% hydrolysis is not achieved, and a DT50 cannot be calculated because the loss is not first- or pseudo-first oder kinetics.
5.2.1	**k_H**	Not calculable
5.2.2	**DT_{50}**	Not calculable
5.2.3	**r^2**	Not calculable
5.3	**Conclusion**	Limited information on the protocol were provided, however, the data presented are sufficient to support several conclusions regarding the hydrolysis of permethrin 1. The main conclusion of the study is that permethrin is not subject to abiotic hydrolysis at acidic to neutral pH. Under basic conditions, hydrolysis is observed, although the data are inconclusive. 2. Environmentally relevant temperatures and pHs are unlikely

Section A7.1.1.1.1 (2) **Hydrolysis as a function of pH and identification of breakdown products**

Annex Point IIA7.6.2.1

Key Study

to be sufficient to cause significant hydrolysis of permethrin based on these findings.

3. When hydrolysis does occur, it does not appear to follow first- or pseudo-first order kinetics, therefore it is not possible to calculate or estimate DT50 or rate constants.

5.3.1	**Reliability**	2
5.3.2	**Deficiencies**	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	6 EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*4 April 2005*
Materials and Methods	*The applicants version is acceptable; however, the relevant section discrepancies were observed:* ***Comments:*** *(Section 3.3) Permethrin was radiolabelled in the acid moiety with a cis:trans permethrin ratio of 48:52.* *(Section 3.3) Methanol was used as a co-solvent owing to the hydrophobic nature of permethrin. However, the concentration of methanol used as the co-solvent is not reported.*
Results and discussion	*Adopt applicants version.*
Conclusion	*Adopt applicants version.*
Reliability	*2*
Acceptability	acceptable / ~~not acceptable~~
Remarks	*The study by Allsup and Russell (1976) provides sufficient data to support conclusions regarding the hydrolysis of permethrin. However, the limited information on the test protocol and limited sampling points, means that the RMS considers that this study only partially fulfils data requirements of this data point. It is considered that the study by Alvarez* et al *(1977) provides additional information pertinent for a more complete assessment of the hydrolytic behaviour of permethrin, and, hence, should be included as a key study with the Allsup and Russell (1976) study to cover the hydrolysis data point.*
	7 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_1_1_1_1-1: Type and composition of buffer solutions (specify kind of water if necessary)

pH	Type of buffer (final molarity)	Composition
3	0.0087 M phthalate	50 mL of 0.1 M potassium acid phthalate and 22.3 ml of 0.1 M hydrochloric acid made up to 578 mL with water
6	0.011 M phosphate	50 mL of 0.1 M potassium dihydrogen phosphate and 5.6 mL of 0.1M sodium hydroxide made up to 445 mL with water
9	0.0028 M borax	50 mL of 0.025 M borax and 4.6 mL of 0.1M hydrochloric acid made up to 437 mL with water

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	*Distilled, sterilised*
Preparation of test medium	*For each prepared substance (alc-14C and ac-14C), nine sterile, glass-stoppered flasks containing 200 ml of sterile buffered solution (3 at each pH) were prepared.*
Test concentrations (mg a.i./L)	*To the glass-stoppered flasks was added the test substances to the following concentrations:* *Alc-14C: 4 µg l^{-1}.* *Ac-14C: 5 µg l^{-1}.*
Temperature (°C)	25, 35 and 45°C
Controls	None
Identity and concentration of co-solvent	*Methanol, glass distilled, concentration not specified*
Replicates	*Triplicate flasks for each pH and temperature*

Table A7_1_1_1_1-3: Description of test system

Glassware	*250 ml glass stoppered flat bottom boiling flasks*
Other equipment	*No information available*
Method of sterilization	*No information available*

Table A7_1_1_1_1-4(1): Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 3 pH 6 and pH 9 at 25°C for both alcohol and acid ^{14}C labelled permethrin.

Temp	Label	pH	Compound	0	3	7	14	21	28	42
25	alc	3	Cis	27.8		23.3	23	20.9	30.5	
			Trans	73.1		67	73	65.9	82.2	
			Unknown	2.06		2.79	4	2.23	1.73	
			Recovery	103		93	100	89	114	
		6	Cis	27.3		26	21.5	31.3	20.2	
			Trans	71.7		72	64.5	74.7	63.6	
			Unknown	2.02		2			2.15	
			Recovery	101		100	86	106	86	
		9	Cis	27.4		23.4	21.3	24	26.2	20
			Trans	70.6		60.3	56.1	56.4	64.9	56.9
			Unknown			6.3	6.8	13.6	18	14.1
			Recovery	98		90	84.2	94	109	91
25	ac	3	Cis	32.9		39.6	37	42.8	41.9	
			Trans	40.2		48.4	40	52.3	51.2	
			Unknown							
			Recovery	73		88	77	95	93	
		6	Cis	44		41.9	45.4	45.5	40.9	
			Trans	56		49.1	62.6	52.5	51.1	
			Unknown					0.99		
			Recovery	100		91	108	99	92	
		9	Cis	43.1		38.5	46.4	45.4	33.1	44.1
			Trans	54.9		51.7	56.2	55.6	38	48.5
			Unknown				4.86	7.02	4.94	6.93
			Recovery	98		90.2	107	108	76	99.5

Table A7_1_1_1_1-4(2): Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 3 pH 6 and pH 9 at 35°C for both alcohol and acid ^{14}C labelled permethrin.

Temp	Label	pH	Compound	0	3	7	14	21	28	42
35	alc	3	Cis	21.8		30.2	22.3	21.1	23	
			Trans	63.5		79	68.8	63.2	68.6	
			Unknown	1.74		2.8	1.86	1.29	1.88	
			Recovery	87		112	93	85.6	93.5	
		6	Cis	22.6		22.9	21.4	25	30.5	
			Trans	69.6		63.4	67.6	69.1	73	
			Unknown	1.88		1.76		1.44	1.58	
			Recovery	94		88	89	95.5	105	
		9	Cis	21.8			15.1	15.6	15.2	16.5
			Trans	63.5			39.5	36.5	33.5	34.4
			Unknown	1.74			29.4	36.5	38.3	46.1
			Recovery	87			84	88.6	87	97
35	ac	3	Cis	38.7		42.3	41.4	40.5	44.1	
			Trans	47.3		52.7	49.7	49.5	53.9	
			Unknown				0.92			
			Recovery	86		95	92	90	98	
		6	Cis	34.7		45.7	37.8	35.7	39.6	
			Trans	42.4		59.3	45.4	46.6	48.7	
			Unknown				0.84	1.68	2.28	
			Recovery	77		105	84	84	90.5	
		9	Cis	38.3		31.5	32.7	25.5	28.5	32.3
			Trans	49.8		32.4	29.2	22.5	23.8	26.6
			Unknown	0.89		19.1	23.7	26.3	32.3	36.1
			Recovery	89		83	85.6	74.3	84.6	95

Table A7_1_1_1_1-4(3):Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 3 pH 6 and pH 9 at 45°C for both alcohol and acid ^{14}C labelled permethrin.

				Day number					
Temp	Label	pH	Compound	0	3	7	14	21	28
45	alc	3	Cis	21.1		20.7	23.4	19.9	19.8
			Trans	64.2		65.1	63.9	63.1	67.5
			Unknown	2.2		2.2	2.7		2.7
			Recovery	87.6		88	90	83	90
		6	Cis	21.4		21.6	21.5	18.5	18.5
			Trans	65.9		66.6	62.8	58.5	57
			Unknown	1.87		2.25	1.72		1.54
			Recovery	89.1		90.5	86	77	77
		9	Cis	20.9	10.8	6.96			
			Trans	62.6	23.5	10.4			
			Unknown	3.31	42.7	69.6			
			Recovery	86.8	77	87			
45	ac	3	Cis	43.1		38.3	39.8	44.6	42.6
			Trans	54.9		46.8	55.3	54.5	56.4
			Unknown				1.94		
			Recovery	98		85	97	99	99
		6	Cis	41.3		35.6	39.4	40.7	37.8
			Trans	54.7		45.4	54.7	56.3	51.4
			Unknown				1.92		1.82
			Recovery	96		81	96	97	91
		9	Cis	37.8	23.4	17			
			Trans	45.4	23.8	10.5			
			Unknown	0.84	38.3	54.3			
			Recovery	84	85.4	81.8			

Table A7_1_1_1_1-4(4): TLC Recovery TLC plate recovery data

Temp	Label	pH	0	3	7	14	21	28	42
25	alc	3	103		93	100	89	115	
		6	101		100	86	106	86	
		9	98		90	85	94	109	91
	ac	3	73		88	77	95	93	
		6	100		91	108	99	92	
		9	98		94	108	108	76	99
35	alc	3	87		112	93	86	94	
		6	94		88	89	96	105	
		9	87			84	89	87	97
	ac	3	86		95	92	90	98	
		6	77		105	84	84	91	
		9	89		83	86	75	85	95
45	alc	3	88		88	90	83	90	
		6	89		90	86	77	77	
		9	87	77	87				
	ac	3	98		85	97	99	99	
		6	96		81	96	97	91	
		9	84	85	81				

Table A7_1_1_1_1-6: Specification and amount of transformation products

CAS-Number	*CAS and/or IUPAC* Chemical Name*(s)*	Amount [%] of parent compound measured at		
		pH 3	**pH 6**	**pH 9**
13826-35-2	3-phenoxybenzenemethanol, 3-phenoxybenzyl alcohol (PBA)	25°C – nd 35°C - nd 45°C - nd	25°C – nd 35°C - nd 45°C - nd	25°C – 13.8[a] 35°C – 44.7[b] 45°C – 64.0[c]
-	*cis/trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCVA)	25°C – nd 35°C - nd 45°C - nd	25°C – nd 35°C - nd 45°C - nd	25°C – 5.9[a] 35°C – 33.7 45°C – 43.0[c]

a)After 42 days, b)After 42 days, c)After 7 days

Figure 1: Log_{10}Concentration vs. Time plot; pH 9, 35°C (alcohol ^{14}C-radiolabelled)

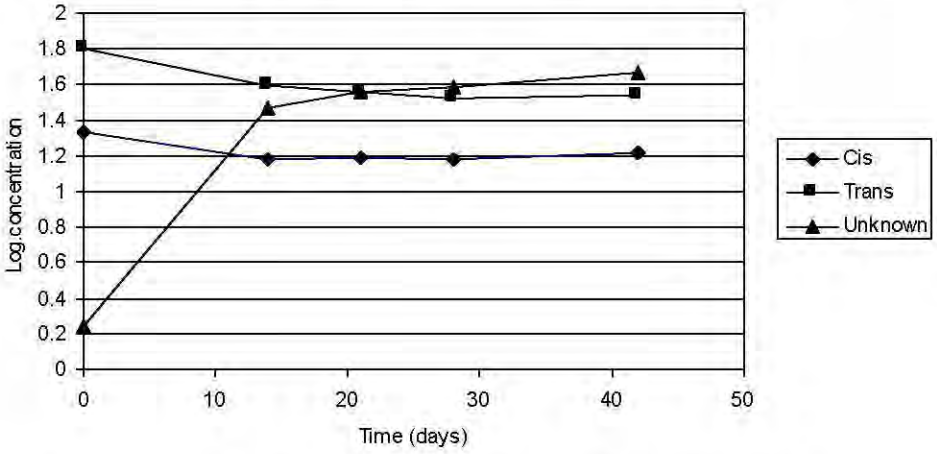


Figure 2: Log_{10}Concentration vs. Time plot; pH 9, 35°C (acid ^{14}C-radiolabelled)

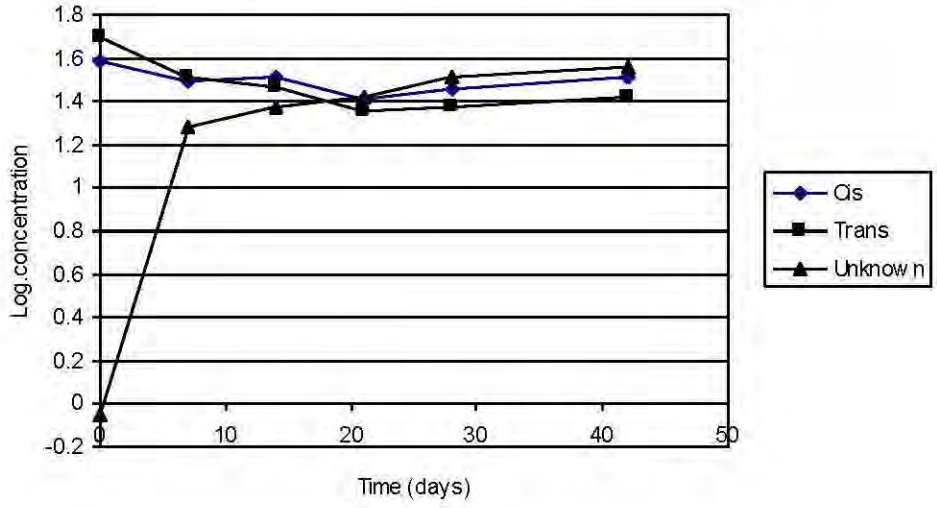
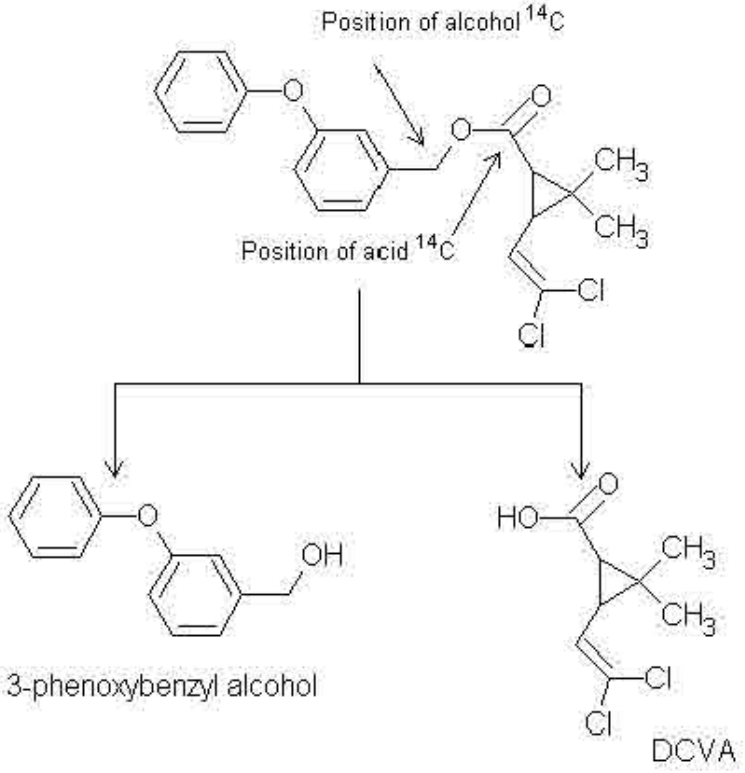


Figure 3: Suggested degradation pathway


Position of alcohol ^{14}C
Position of acid ^{14}C
3-phenoxybenzyl alcohol
DCVA

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Key Study

			Official use only
		1 REFERENCE	
1.1	**Reference**	Amos, R. and Donelan, R. B; 1987; Permethrin: Photolysis in sterile water at pH5. Report No. RJ0577B, 15 June 1987; Not GLP; Unpublished Buerkle, L.W, 2007; Calculation of the Environmental Photolysis Half Lives for permethrin, Report number MEF-07/414, 2007-09-21	
1.2	**Data protection**	Yes	
1.2.1	**Data owner**	Syngenta (Amos, R. and Donelan, R. B; 1987) Bayer CropScience AG (Buerkle, L.W, 2007)	
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: Guidelines not available at time of testing	
2.2	**GLP**	No	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Two separate radiolabelled [^{14}C]permethrin test materials were used in the study, labelled in the cyclopropane position (acid[^{14}C]permethrin) and methylene (alc[^{14}C]permethrin), as shown in Figure 1. The structures of reference materials used for co-chromatography are shown in Figure 2.	
3.1.1	**Lot/Batch number**	Both synthesised in-house at ICI Plant Protection division, Jealott's Hill Research Station. acid[^{14}C]permethrin: Batch 86-J28 alc[^{14}C]permethrin: Batch 86-J29	
3.1.2	**Specification**	acid[^{14}C]permethrin: *cis:trans* ratio 49:51 alc[^{14}C]permethrin: *cis:trans* ratio 49:51	
3.1.3	**Purity**	acid[^{14}C]permethrin: 97.4% alc[^{14}C]permethrin: 97.2%	
3.1.4	**Radiolabelling**	As shown in Figure 1	
3.1.5	**UV/VIS absorption spectra and absorbance value**	As shown in Figure 3	
3.1.6	**Further relevant properties**	acid[^{14}C]permethrin: Specific activity 1.02 GBq mmol^{-1} alc[^{14}C]permethrin: Specific activity 2.32 GBq mmol^{-1}	
3.2	**Reference substances**	None	
3.3	**Test solution**	See table A7_1_1_2-1	
3.4	**Testing procedure**		
3.4.1	**Test system**	See table A7_1_1_2-2, Figure 4.	

Section A7.1.1.1.2 **Phototransformation in water including identity of transformation products**

Annex Point IIA7.6.2.2

Key Study

3.4.2	**Properties of light source**	See table A7_1_1_2-2, Figure 5
3.4.3	**Determination of irradiance**	See table A7_1_1_2-2, Figure 5
3.4.4	**Temperature**	Temperature was maintained at 25°C throughout the exposure period, by a flow of water through the troughs.
3.4.5	**pH**	Not recorded
3.4.6	**Duration of the test**	33 days 3 hours (Florida autumn sunlight equivalents)
3.4.7	**Number of replicates**	2 replicates per time point, per radiolabel
3.4.8	**Sampling**	Nominal irradiation period; 0, 7, 15, 22, 30 (Florida autumn sunlight equivalents) Actual irradiation period; See Results Table 1.
3.4.9	**Analytical methods**	Aqueous sacrificial vessels were allowed to reach room temperature, assayed by LSC and acidified to ~pH2 with cHCl. Aqueous samples and glassware were partitioned with dichloromethane and/or ethyl acetate, and all solutions assayed by LSC. Polyurethane bungs from the inlet and outlet arms were refluxed in acetonitrile (25 ml, 1 hr) and assayed by LSC to determine loss of volatile materials. Silicone tubing joining the vessels were allowed to stand in methanol (4 hours), which was subsequently assayed by LSC. Trapping solutions were assayed by LSC at time of sampling. Extracts were analysed by thin layer chromatography (TLC). TLC was carried out using either pre-coated normal phase $Si_{60}F_{254}$ silica gel plates or pre-coated reverse phase C18 silica gel plates, under the following conditions; Normal phase: Solvent system 1: Hexane:Diethyl ether 20:1 v/v Solvent system 2: Hexane (sat'd w acetonitrile) Solvent system 3: Cyclohexane (sat'd w formic acid):Diethyl ether 3:2 Solvent system 4: Toluene (sat'd w formic acid):Diethyl ether 10:3 Reverse phase: Solvent system 1: Methanol:0.2M ammonium acetate 7:3 Solvent system 2: Ethanol:0.2M ammonium acetate 6:4 Samples were co-chromatographed alongside reference standards of permethrin and metabolites as shown in Figure 2.
3.5	**Transformation products**	No
3.5.1	**Method of analysis for transformation products**	No

4 RESULTS

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Key Study

4.1	**Screening test**	Not performed	
4.2	**Actinometer data**	Not used	
4.3	**Controls**	Control samples stored in the dark yielded quantitative recovery of applied radioactivity, all of which was present as parent material.	
4.4	**Photolysis data**		
4.4.1	**Concentration values**	Recovery data (as % of applied) are shown graphically in Figures 6 and 7.	
4.4.2	**Mass balance**	Total recovery of radiolabelled test substance are shown in Table 2. Individual isomer and total permethrin data are also included.	
4.4.3	**k^c_p**	During the irradiation period, radioactivity precipitated from the photolysis solutions onto the walls of the glass vessels. This does not allow an accurate assessment of the kinetics of photodegradation of permethrin to be made.	
4.4.4	**Kinetic order**	See 4.4.3	
4.4.5	**k^c_p / k^a_p**	See 4.4.3	
4.4.6	**Reaction quantum yield (ϕ^c_E)**	See 4.4.3	
4.4.7	**k_{pE}**	See 4.4.3	
4.4.8	**Half-life ($t_{1/2E}$)**	After 32-33 days irradiation, the authors state 69-77% of the applied radioactivity was identified as permethrin. Assessment of the tabulated data presented in the report, indicate 58-80% of the recovered radioactivity was identified as permethrin. Analysis of these tabulated data indicates the half-life of permethrin is 119 days 8 hours under Florida autumn sunlight. The authors calculate a half-life of 32 days 17 hours under Florida summer sunlight. These values should be treated with caution, because during the irradiation period, radioactivity precipitated from the photolysis solutions onto the walls of the glass vessels, which does not allow an accurate assessment of the kinetics of photodegradation of permethrin to be made. In respect of this quality caveat, estimated environmental half lives for different conditions could be conducted on the basis of comparing the relative solar intensities given in the report of Amos and Donelan and now also given in the OECD Guidance Document on Direct Phototransformation of Chemical in Water (1997) (Buerkle, 2007). The resulting calculated environmental half lives of photodegradation of permethrin in water are shown in the table A7_1_1_1_2-3.	X
4.5	**Specification of the transformation products**	Up to 19 by-products of degradation were observed, the greatest of which being 5.6% of the applied radioactivity.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The test was performed in glass vials with quartz lids, which allowed light from the Xenon Arc lamp to fully irradiated the vials. Temperature was controlled by flowthrough water. Sacrificial samples were removed at time intervals and solvent extracted. The	

Section A7.1.1.1.2 **Phototransformation in water including identity of transformation products**

Annex Point IIA7.6.2.2

Key Study

		extracts were then analysed by TLC (six different systems).
5.2	**Results and discussion**	During the irradiation period, radioactivity precipitated from the photolysis solutions onto the walls of the glass vessels. This does not allow an accurate assessment of the kinetics of photodegradation of permethrin to be made. At the end of the exposure period, adjusted to Florida autumn sunlight, the authors state 69-77% of the applied radioactivity was identified as permethrin. Assessment of the tabulated data presented in the report, indicate 58-80% of the recovered radioactivity was identified as permethrin. It is not possible to determine the cause of these discrepancies. In respect of this quality caveat, estimated half-lives were 119 days 8 hours in Florida autumn sunlight and 32 days 17 hours under Florida summer sunlight. In respect of this quality caveat, estimated environmental half lives for different conditions could be conducted on the basis of comparing the relative solar intensities given in the report of Amos and Donelan and now also given in the OECD Guidance Document on Direct Phototransformation of Chemical in Water (1997) (Buerkle, 2007). The resulting calculated environmental half lives of photodegradation of permethrin in water are 118 days with 12h sunlight per day under outdoor conditions at latitude of 50°N and the fall season. The resulting half lives of photodegradation of permethrin in water indicate that direct photolysis indeed takes place, albeit rather slowly. However, indirect photodegradation via ubiquitous photosensitisers (e.g. dissolved humic substances in surface waters) and photogenerated reactive intermediates (e.g. hydroxyl and peroxy radicals, excited oxygen) typically play a greater role usually resulting in shorter half lives Up to 19 transformation products were observed, the greatest of which accounted for 5.6% of the applied radioactivity.
5.2.1	**k^c_p**	See 4.4.3
5.2.2	**K_{pE}**	See 4.4.3
5.2.3	**ϕ^c_E**	See 4.4.3
5.2.4	**$t_{1/2E}$**	See 4.4.3
5.3	**Conclusion**	The test protocol was designed to minimise loss of test material, allow derivation of DT50, and allow for characterisation of metabolites. Since permethrin has such a long half-life, the sampling points were not widespread enough to cover the measured half life. Also, during the irradiation period, radioactivity precipitated from the photolysis solutions onto the walls of the glass vessels. This does not allow an accurate assessment of the kinetics of photodegradation of permethrin to be made. These factors, coupled with the noted discrepancy between the reported and concluded data, do not allow real confidence in the derived DT50 values.
5.3.1	**Reliability**	2
5.3.2	**Deficiencies**	Yes The study was not completed to recognised GLP procedures; however the testing protocol, although not to any recognised guidelines, incorporated essential elements necessary to enable some confidence in the output. The test system was designed to allow for

Section A7.1.1.1.2 Annex Point IIA7.6.2.2	Phototransformation in water including identity of transformation products

Key Study

mass balance determination, which were high throughout the course of the study, indicating no material was lost and some confidence can be placed in the results. The adsorption onto the glassware was measureable, but would have some effect on the reliability of the derived DT50s, which should therefore be treated with caution.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	4 April 2005 (updated September 2009)
Materials and Methods	The applicant's version is acceptable.
Results and discussion	Adopt applicants version, with the following revision: **Comments:** (Section 4.4.8 and 5.2) Radioactivity precipitating to the walls of the glass vessels hampered radioactive recovery of the test substance, preventing accurate determination of permethrin degradation kinetics. Whilst the adsorption of the radioactivity onto the glassware was measureable the reliability of the derived DT_{50} values is questionable and, therefore, estimated permethrin DT_{50} values of phototransformation should be treated with caution. However, the re-analysis of the tabulated data from this study indicated a DT_{50} value of >119 days under Florida autumn sunlight (corresponding to 118 days for 12h sunlight per day under outdoor conditions at a latitude of 50°N during autumn). Overall, the results indicate that permethrin is relatively resistant to direct photodegradation.
Conclusion	Adopt applicant's version.
Reliability	2
Acceptability	acceptable / ~~not acceptable~~
Remarks	**Comments:** (Section 2.3) In line with current guidelines (SETAC 1995) the following deviation was observed: The test temperature for the photolysis study under sterilised buffer solution at pH 5 was maintained at 25 °C ± 1 °C, instead of 20 °C ± 3 °C. This is considered a minor deviation to current test guidelines.
	6 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_1_1_2-1: Description of test solution and controls

Criteria	Details
Purity of water	Distilled water was used.
Preparation of test chemical solution	pH 5 buffer was prepared by mixing 350 ml of 0.2M sodium acetate trihydrate with 150 ml 0.2M acetic acid. This buffer was diluted to 0.01M with distilled water, and sterilised by autoclave. Radiochemical stock solutions were prepared in acetonitrile, such that the solutions contained 0.75 µg permethrin per 150 µl acetonitrile (1900 and 4381 Bq acid and alcohol labelled respectively).
Test concentrations (mg a.s./L)	150 µl acetonitrile (1900 and 4381 Bq acid and alcohol labelled respectively) were added to 15 ml of pH5 buffer in the test vessels
Temperature (°C)	25 ± 1°C
Preparation of a.s. solution	None
Controls	Dark controls, prepared in exactly the same way as exposure vessels, were included in the experimental design
Identity and concentration of co-solvent	Acetonitrile at 1%

Table A7_1_1_2-2: Description of test system

Criteria	Details
Laboratory equipment	Samples were irradiated with a Hanau NXe 4500 xenon arc lamp (Heraeus Instruments). The photolysis vessels were 20 ml glass vials fitted with air inlet and outlet arms. All vessels were sterilised in an autoclave before use. The vessels were placed in a glass cooling trough, previously coated in light reflective paint to ensure light entered only through quartz vessel lids. Foam bungs were placed in each inlet and outlet to ensure sterility was maintained, and each vessel placed in troughs, 5 per trough. Four of these were joined with silicone tubing leading to a negative pressure pumping system. The exuent air was drawn through a series of traps to remove any volatile ^{14}C by-products. The fifth vessel had a thermocouple inserted for temperature monitoring. See Figure 4.
Test apparatus	Samples were irradiated with a Hanau NXe 4500 xenon arc lamp (Heraeus Instruments).
Properties of artificial light source:	
Nature of light source	Xenon Arc Lamp
Emission wavelength spectrum	See Figure 5
Light intensity	Light intensity was measured using an international Light IL500A Research Radiometer in conjunction with an SEE 038 broad band silicon detector. Narrow and wide band filters wewre used to monitor intensity in the following regions. NBS 297 peak wavelength 297 ± 2 nm } narrow NBS 365 peak wavelength 365 ± 2 nm } band WBS 375 peak transmission 280-420 nm } wide WBS 500 peak transmission 430-700 nm } band Intensity at each vessel position was measured at the start and on sampling. These values were transformed into standard Florida autumn sunshine equivalent days.

Results Table 1: Irradiation period

Radiolabel	Position in arc Lamp (Fig 4)	Intensity (W cm^2)	Hrs irradiation	Equivalent days of Florida autumn sunlight
Cyclopropane1	1	0.032	87.8	7d 3hrs
Cyclopropane2	2	0.036	168.2	15d 8hrs
Cyclopropane3	3	0.040	243.1	24d 15hrs
Cyclopropane4	4	0.041	316.3	32d 21hrs
Cyclopropane5	5	0.042	308.9	32d 21hrs
Cyclopropane6	6	0.040	242.6	24d 14hrs
Cyclopropane7	7	0.038	160.1	15d 0hrs
Cyclopropane8	8	0.034	81.1	7d 0hrs
CyclopropaneSpare1	S1	0.046	285.4	33d 6hrs
CyclopropaneSpare2	S2	0.045	291.1	33d 5hrs
CyclopropaneSpare3	S3	-	-	-
CyclopropaneSpare4	S4	-	-	-
Methylene9	9	0.039	409.9	32d 5hrs
Methylene10	10	0.034	81.1	7d 0hrs
Methylene11	11	0.038	158.9	15d 7hrs
Methylene12	12	0.040	242.6	24d 14hrs
Methylene13	13	0.038	339.6	32d 17hrs
Methylene14	14	0.036	360.0	24d 10hrs
Methylene15	15	0.034	105.8	15d 8hrs
Methylene16	16	0.029	97.2	7d 4hrs
MethyleneSpare5	S5	0.050	264.7	33d 13hrs
MethyleneSpare6	S6	0.044	291.1	33d 3hrs
MethyleneSpare7	S7	-	-	-
MethyleneSpare8	S8	-	-	-

Results Table 2: Recovery data

Radiolabel	Equivalent days of Florida autumn sunlight	Total % recovery	*% cis*-permethrin	*% trans*-permethrin	% Total permethrin
Cyclopropane0	-	95.4	41.6	45.4	87.0
Cyclopropane8	7d 0hrs	98.1	36.4	49.1	85.5
Cyclopropane1	7d 3hrs	115.4	40.3	56.3	96.6
Cyclopropane7	15d 0hrs	110.9	33.4	54.5	87.9
Cyclopropane2	15d 8hrs	108.7	35.7	57.5	93.2
Cyclopropane6	24d 14hrs	112.7	30.6	49.1	79.7
Cyclopropane3	24d 15hrs	109.5	30.9	50.4	81.3
Cyclopropane4	32d 21hrs	93.5	23.7	34.3	58.0
Cyclopropane5	32d 21hrs	112.9	29.7	50.9	80.6
Methylene0	-	96.4	40.8	44.1	84.9
Methylene10	7d 0hrs	102.0	35.9	49.6	85.5
Methylene11	15d 7hrs	96.5	34.0	48.6	82.6
Methylene15	15d 8hrs	99.4	33.5	45.5	79.0
Methylene14	24d 10hrs	100.2	29.9	42.9	72.8
Methylene12	24d 14hrs	96.3	27.5	43.4	70.9
Methylene13	32d 17hrs	96.3	29.9	48.4	78.3
MethyleneSpare6[a]	33d 3hrs	97.9	29.8	45.6	75.4
Methylene9[a]	32d 5hrs	83.7	-	-	-
Methylene16[b]	7d 4hrs	0.6	-	-	-

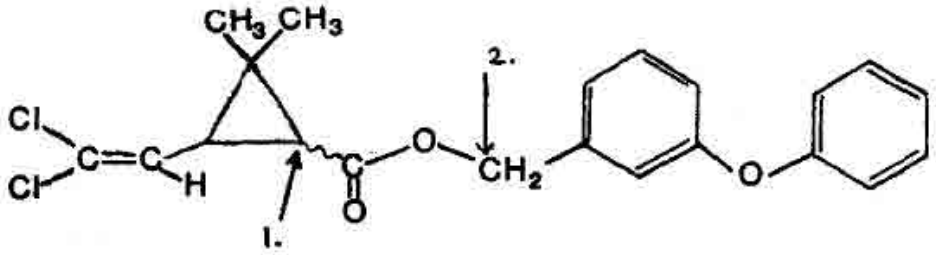
a **Spare sampled because of low recovery from Methylene9.**

b **Not dosed, in error.**

Table A7_1_1_1_2-3 : Environmental half lives of direct photolysis of permethrin in water under environmental conditions range

Latitude	Spring	Summer	Fall	Winter
	[days with 12 hours sunlight per day]			
20°N	43	41	53	64
30°N	45	40	63	84
40°N	48	41	81	128
50°N	54	42	*118*	243

Figure 1: Permethrin structure, showing position of the radioisotope

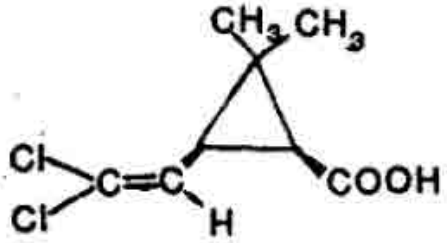


1. ^{14}C-cyclopropane-labelled permethrin, batch no. 86-J28, specific activity 1.02 GBq mmol^{-1}.

2. ^{14}C-methylene-labelled permethrin, batch no. 86-J29, specific activity 2.32 GBq mmol^{-1}.

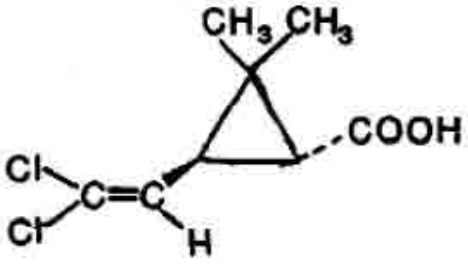
Figure 2: Reference materials

Compound I



(1RS)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid

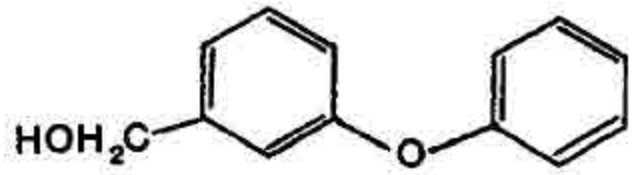
Compound II



(1RS)-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid

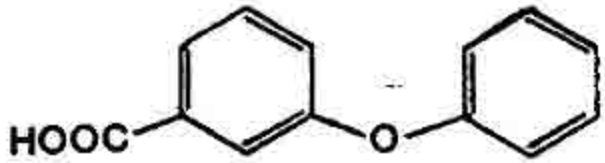
Figure 2: Reference materials (cont'd)

Compound III



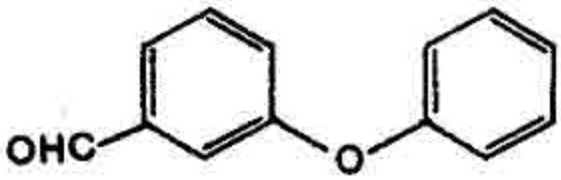
3-phenoxybenzyl alcohol

Compound IV



3-phenoxybenzoic acid

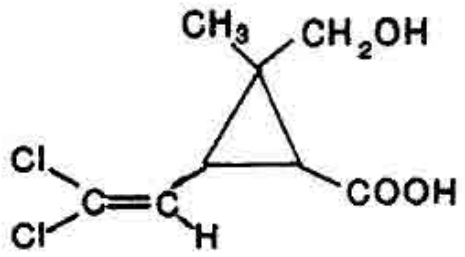
Compound V



3-phenoxybenzaldehyde

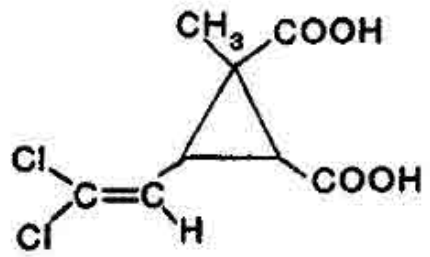
Figure 2: Reference materials (cont'd)

Compound VI



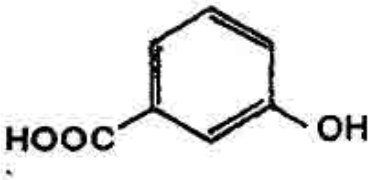
(RS)-3-(2,2-dichlorovinyl)-1-hydroxymethyl-1-methylcyclopropane-2-carboxylic acid

Compound VII



(RS)-3-(2,2-dichlorovinyl)-1-methylcyclopropane-1,2-dicarboxylic acid

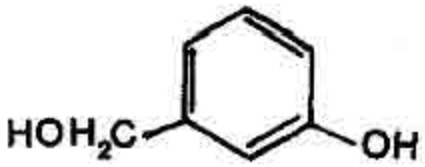
Compound VIII



3-hydroxybenzoic acid

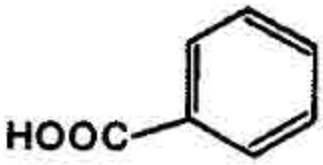
Figure 2: Reference materials (cont'd)

Compound IX



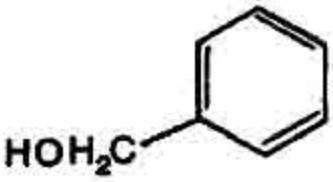
3-hydroxybenzyl alcohol

Compound X



benzoic acid

Compound XI



benzyl alcohol

Figure 3: UV-vis spectrum of permethrin

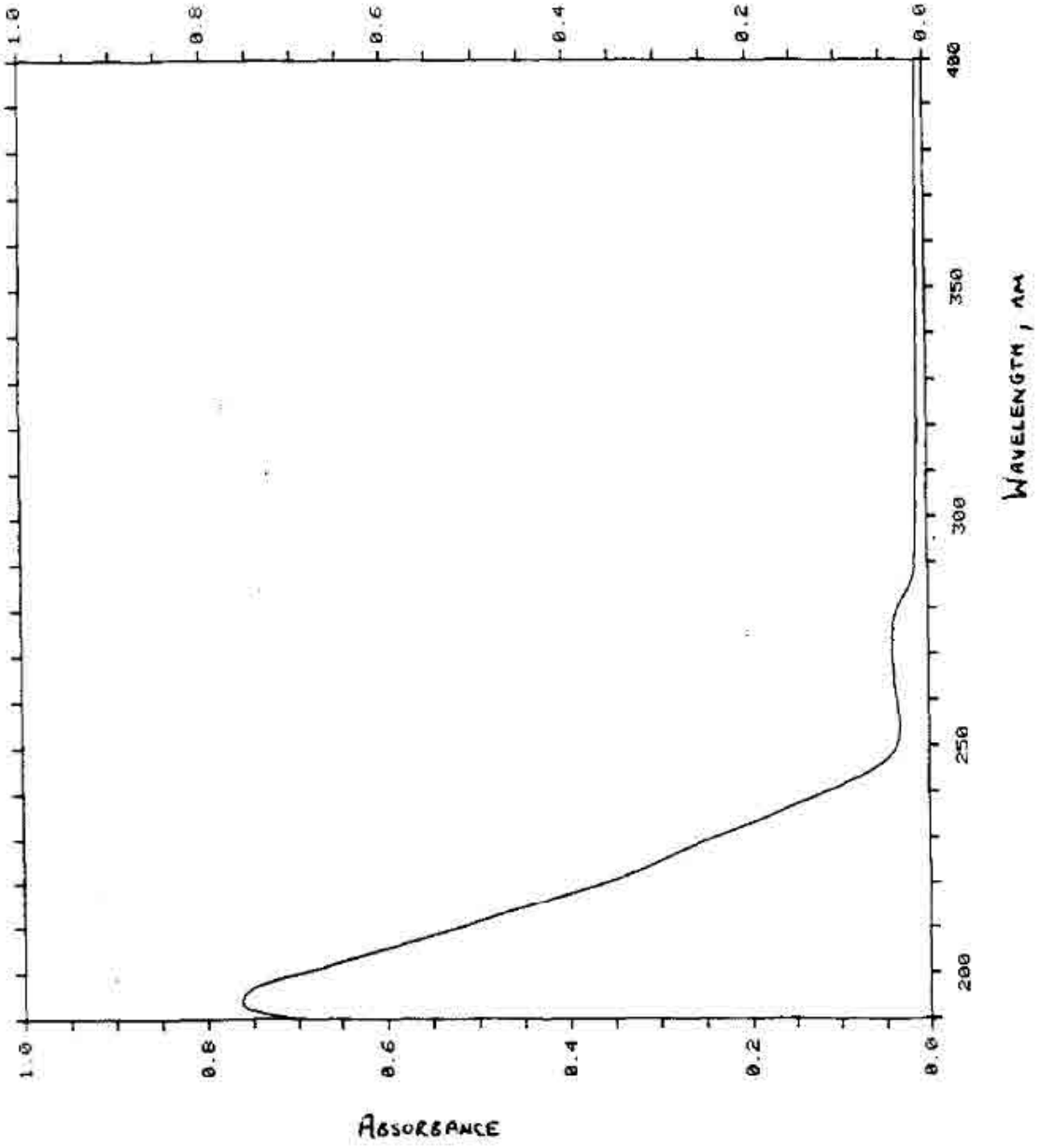


Figure 4: Test system (vessel)

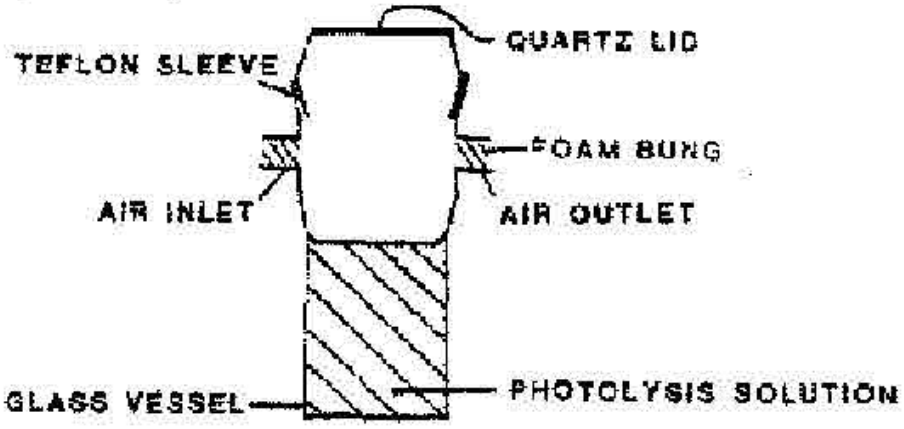


Figure 4: Test system cont'd (vessels in trough)

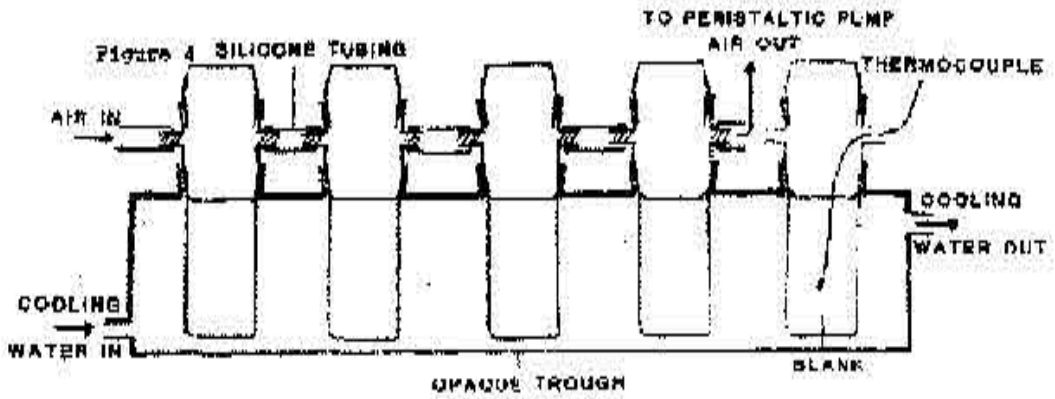


Figure 4: Test system cont'd (troughs in system – overhead view)

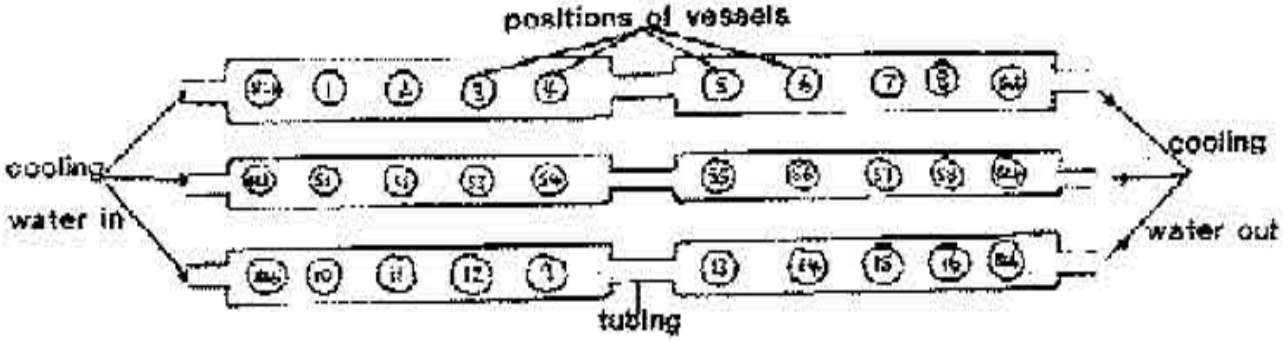


Figure 5: Properties of light source – Comparison of spectral distribution with D65 radiation

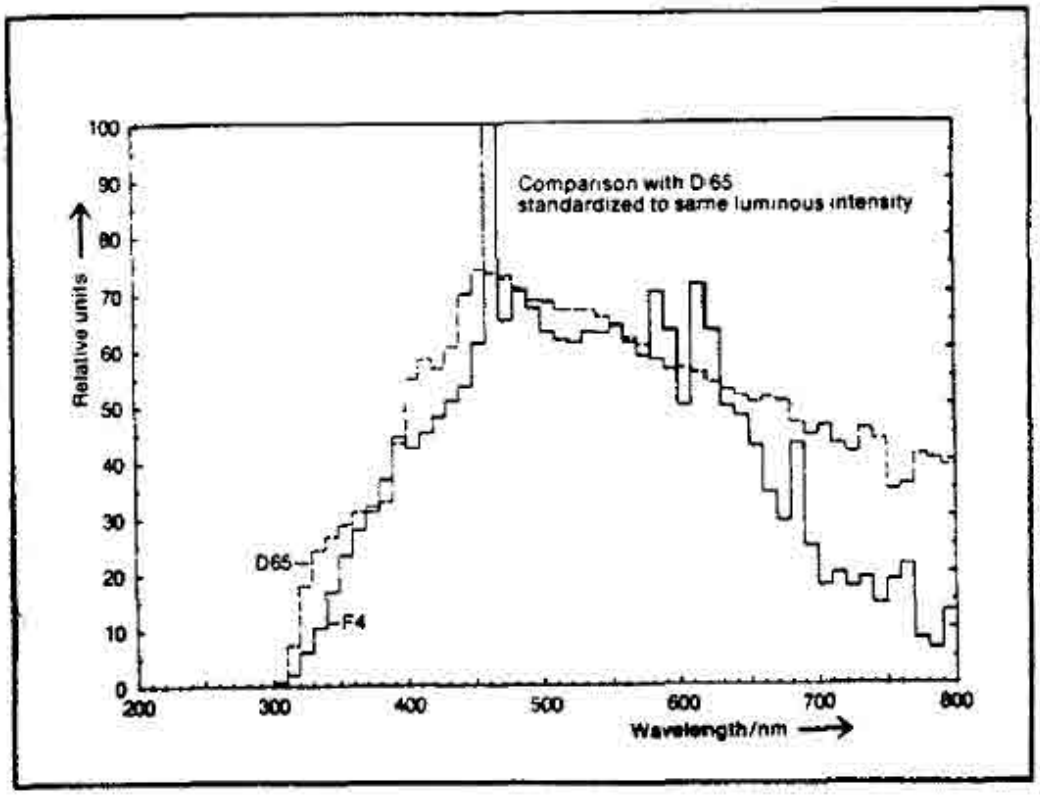


Figure 6: % recovered [^{14}C]permethrin radiolabelled in the cyclopropane (acid[^{14}C]permethrin) position

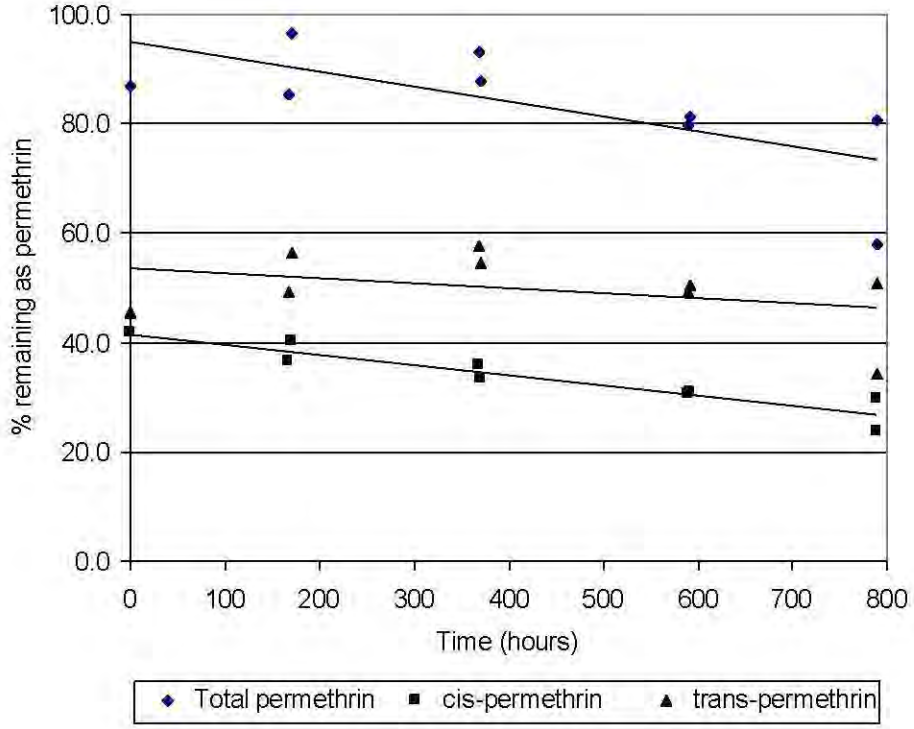
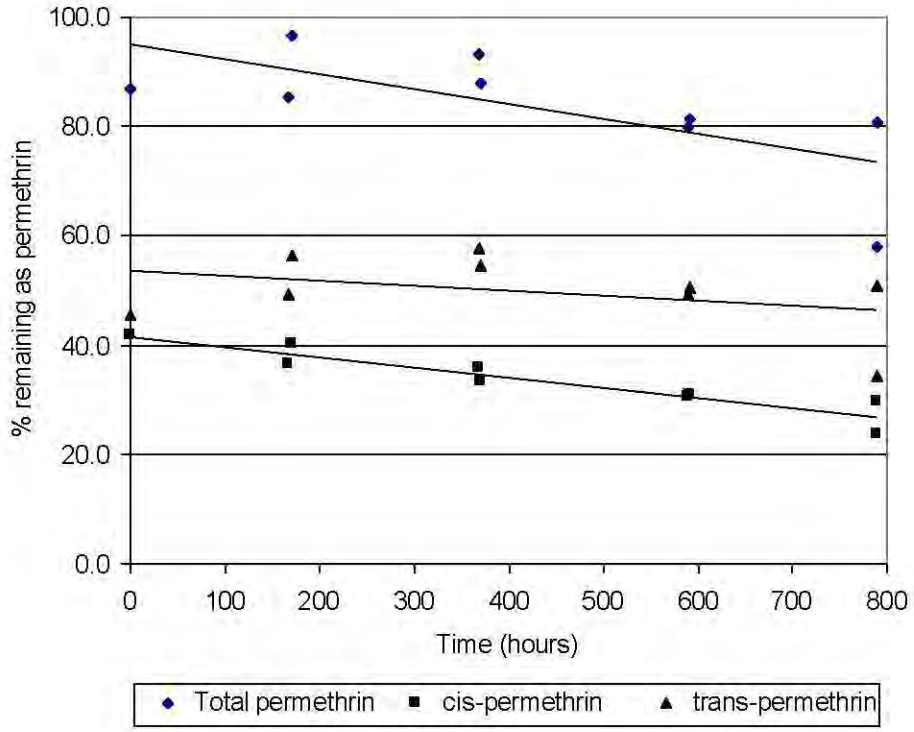


Figure 7: % recovered [^{14}C]permethrin radiolabelled in the methylene (alc[^{14}C]permethrin) position



Section A7.1.1.2.1 Biodegradability (ready/inherent)

Annex Point IIA7.6.1.1 Ready biodegradability

Key Study

			Official use only
		1 REFERENCE	
1.1	**Reference**	Mead, C; 2004; Permethrin: Assessment of Ready Biodegradability; Manometric Respirometry Test; 1430/018;July 2004;GLP; Unpublished	
1.2	**Data protection**	Yes	
1.2.1	**Data owner**	Sumitomo Chemical (UK) Ltd.	
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 – OECD301F	
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**	DGPRTG057	
3.1.2	**Specification**	As given in section 2	
3.1.3	**Purity**	Not reported	
3.1.4	**Further relevant properties**	Very low aqueous solubility (<5 µg l^{-1})	
3.1.5	**Composition of Product**	Not relevant	
3.1.6	**TS inhibitory to microorganisms**	No	
3.1.7	**Specific chemical analysis**	No specific chemical analysis undertaken	
3.2	**Reference substance**	Yes – Aniline	
3.2.1	**Initial concentration of reference substance**	100 mg l^{-1}	
3.3	**Test ing procedure**		
3.3.1	**Inoculum / test species**	see table A7_1_1_2-2	
3.3.2	**Test system**	see table A7_1_1_2-3	
3.3.3	**Test conditions**	see table A7_1_1_2-4	
3.3.4	**Method of preparation of test solution**	50 mg of permethrin was dissolved in 495 ml culture medium and subjected to ultrasonication for approximately 30 minutes prior to addition of 5 ml inoculum	
3.3.5	**Initial TS concentration**	Nominal 100 mg l^{-1}	

Section A7.1.1.2.1 **Biodegradability (ready/inherent)**

Annex Point IIA7.6.1.1 **Ready biodegradability**

Key Study

3.3.6	**Duration of test**	28 days
3.3.7	**Analytical parameter**	BOD
3.3.8	**Sampling**	Oxygen consumption was automatically recorded by the respirometer.
3.3.9	**Intermediates/ degradation products**	No degradation
3.3.10	**Nitrate/nitrite measurement**	No
3.3.11	**Controls**	Culture medium control (x4), Test substance (x4), Aniline control (x3), Toxicity control (x3)
3.3.12	**Statistics**	Statistical analysis of Day 28 BOD values were performed using a Students t-test.
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	**Graph**	No degradation
4.1.2	**Degradation**	No degradation
4.1.3	**Other observations**	None
4.1.4	**Degradation of TS in abiotic control**	Not applicable
4.1.5	**Degradation of reference substance**	Graphs are presented in Figure 1.
4.1.6	**Intermediates/ degradation products**	Not identified
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The test was conducted to OECD301F. Standard solutions of permethrin were prepared and inoculated with effluent from a domestic sewage plant, and the oxygen consumption of the microorganisms measured over 28 days using an automated respirometer which measured the uptake in oxygen in the test flasks. Positive and toxicity controls were included to validate the methodology.
5.2	**Results and discussion**	No degradation of permethrin was observed. Degradation of the test substance and toxicity controls indicated the viability of the sludge organisms. Although the results indicate permethrin is not readily biodegradable under the conditions of the test, it is important to note the sensitivity of the test method, which although performed at a nominal 100 mg l^{-1} would in reality, based upon the solubility, have a bioavailable (soluble) fraction 5-6 orders of magnitude lower. The experimental design may have been more appropriate if

Section A7.1.1.2.1	**Biodegradability (ready/inherent)**
Annex Point IIA7.6.1.1	**Ready biodegradability**

Key Study

		activated sludge had been used as the inoculum to allow more significant adsorption, or if the test had been undertaken at much lower concentrations using ^{14}C labelled material.
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. The test result indicates that permethrin may not be classified as 'readily biodegradable'.
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	4 April 2005
Materials and Methods	The applicant's version is considered acceptable of the materials and methods used in this test.
Results and discussion	Adopt applicant's version.
Conclusion	Adopt applicant's version.
Reliability	1
Acceptability	acceptable / ~~not 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_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Not applicable
Strain	Not applicable
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	Severn Trent Water Plc, Loughborough, UK
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Filtered through coarse filter paper and maintained on aeration in a temperature controlled room at 21 ± 1°C prior to use.
Pretreatment	None
Initial cell concentration	Not reported

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	Manometric respirometer
Number of culture flasks/concentration	2
Aeration device	None
Measuring equipment	CES Multi-channel aerobic respirometer
Test performed in closed vessels due to significant volatility of TS	No

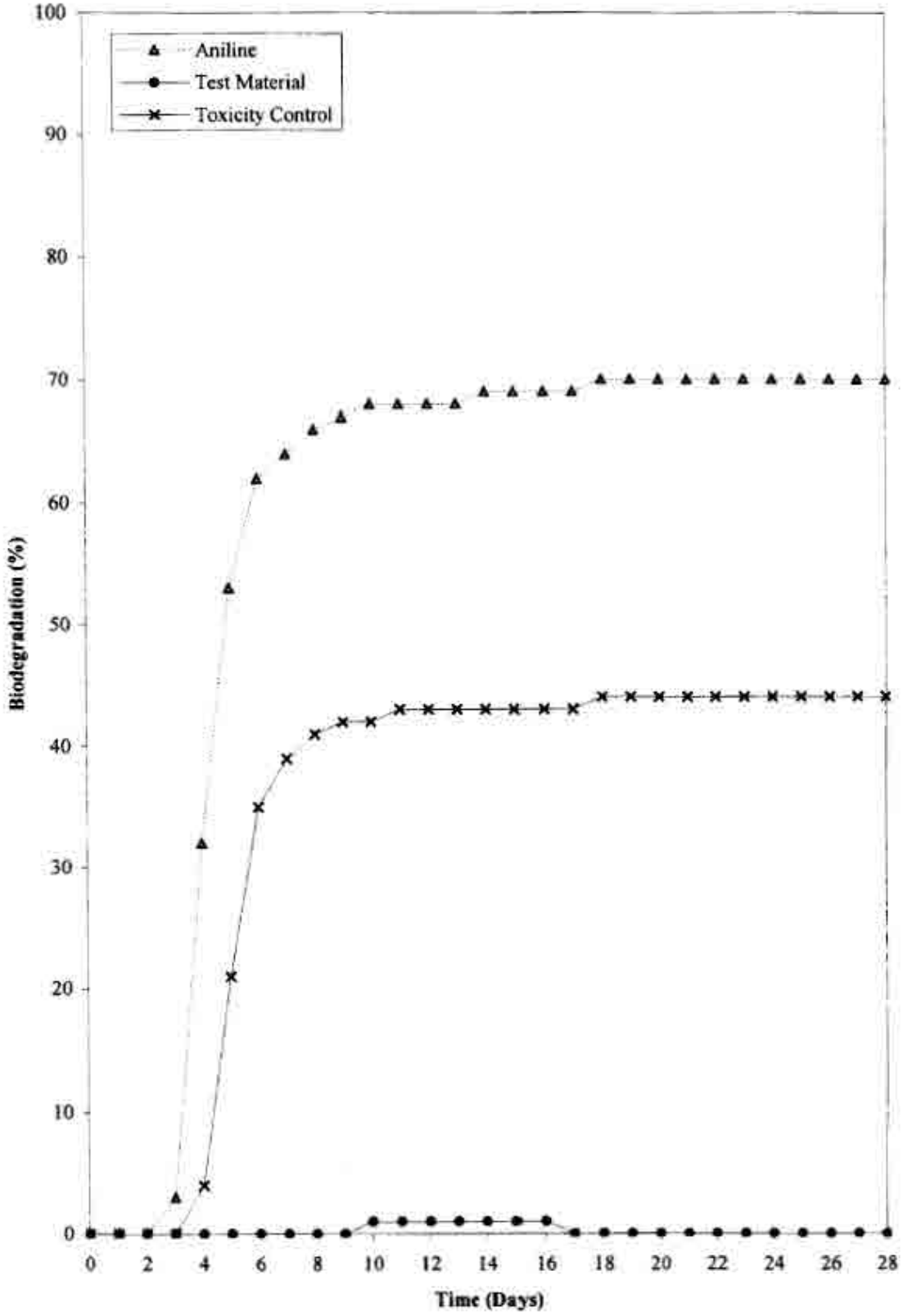
Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	Solution A KH_2PO_4 8.5 g/l K_2HPO_4 21.75 g/l $Na_2HPO_4.2H_20$ 33.40 g/l NH_4Cl 0.50 g/l Solution B $CaCl_2$ 27.5 g/l Solution C $MgSO_4.7H_2O$ 22.5 g/l Solution D $FeCl_3.6H_2O$ 0.25 g/l 10 ml, solution A, 1 ml of other solutions added to 1 L (final volume) of purified water.
Additional substrate	No
Test temperature	21 ± 1°C
pH	7.4 (day 0) 7.8 to 8.5 (day 28)
Aeration of dilution water	No
Suspended solids concentration	Not reported
Other relevant criteria	Stirring of test solution

Table A7_1_1_2-5: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$		X
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		X
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	X	
Percentage of removal of reference substance reaches pass level by day 14	X	

Figure 1: Degradation of aniline, toxicity controls and permethrin



Section A7.1.1.2.2	**Biodegradability (ready/inherent)**	
Annex Point IIA7.6.1.2	**Inherent biodegradability**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [X]	**Other justification** [X]	
Detailed justification:	**Fugacity theory** The equilibrium criterion of fugacity (f) was introduced as a concept in 1901. It can be related to concentration C using the proportionality constant Z, which can be viewed as a kind of solubility or capacity of the phase to absorb the chemical. Chemicals thus tend to migrate into phases in which Z is large, such as lipids of fish for dissolved DDT or aerosol particles for gaseous benzo[a]pyrene. Calculation procedures or recipes are available for estimating Z values using either measured or correlated partition coefficients and other physicochemical properties. Transport and transformation processes can be expressed using D values that are essentially rate constants in fugacity format. Whereas a chemical reaction rate is VCk, where V is volume, C is concentration, and k is a rate constant, the corresponding rate is Df in terms of fugacity. Using f, Z, and D, chemical fate can be quantified multi-compartment systems. The concept of a "unit world" consisting of at least air, water, soil, and sediment compartments is used for assessing chemical fate. The concept is used in the SimpleBox and SimpleTreat models, which are an integral part of the EUSES system. The Equilibrium Criterion (EQC) model was developed and made widely available as a Visual Basic program (Trent University Environmental Modeling Centre) to provide a rational, tiered assessment of chemical fate. It contains level 1, 2, and 3 programs and is applicable to involatile and insoluble substances as well as to conventional organic chemicals. **Model overview** The Level I simulation is of the equilibrium distribution of a fixed quantity of conserved (ie. non-reacting) chemical, in a closed environment at equilibrium, with no degrading reactions, no advective processes, and no intermedia transport processes (eg. no wet deposition, or sedimentation). The medium receiving the emission is unimportant because the chemical is assumed to become instantaneously distributed to an equilibrium condition. **References** Mackay, D.(1991) "Multimedia Environmental Models: The Fugacity Approach", Lewis Publishers, CRC Press, Boca Raton, FL. Mackay D, Paterson, S., Di Guardo, A., Cowan, C.E. (1996) "Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the EQC Model", Environ. Toxicol. Chem. 15 No.9, 1627- 1637. Mackay D, Paterson, S., Kicsi, G., Di Guardo, A., Cowan, C.E. (1996) "Assessing the Fate of New and Existing Chemicals: A Five Stage Process". Environ. Toxicol. Chem.. 15 No.9 , 1618-1626,	

Section A7.1.1.2.2	Biodegradability (ready/inherent)
Annex Point IIA7.6.1.2	Inherent biodegradability

1996.
Mackay D, Paterson, S., Kicsi, G., Cowan, C.E., Di Guardo, A., Kane, D.M. (1996) "Assessment of Chemical Fate in the Environment Using Evaluative, Regional and Local-Scale Models: Illustrative Application to Chlorobenzene and Linear Alkylbenzene Sulfonates" Environ. Toxicol. Chem. 15 No.9, 1638-1648.

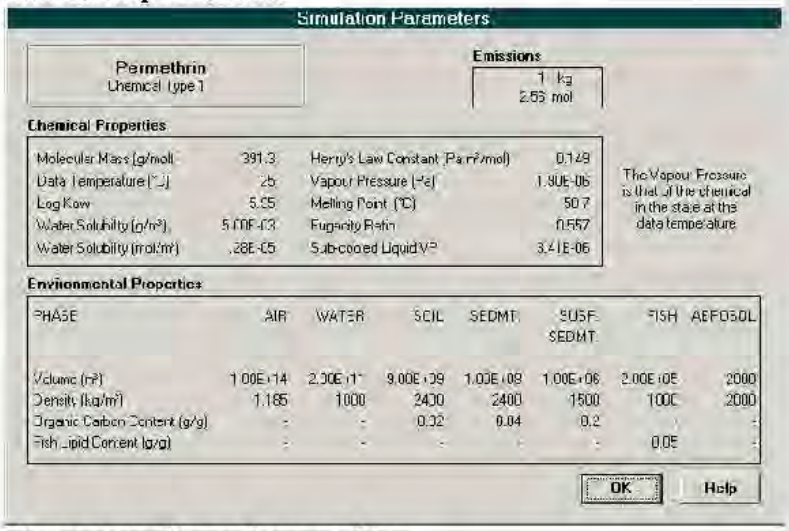
Results

Using known-physical-chemical parameters of permethrin, and a nominal mass input, the Level 1 model yields expected quantities in the following compartments;
Air, Water, Sediment, Suspended sediment, Soil, Fish, Aerosol.

Chemical properties:

Results indicate that 97.5% would partition into the soil compartment, and 2.2% of the remainder would associate with the sediment compartment.
Screendumps of several fields are provided below;

Simulation parameters:



Phase properties and composition:

Section A7.1.1.2.2	**Biodegradability (ready/inherent)**
Annex Point IIA7.6.1.2	**Inherent biodegradability**

Phase Properties and Compositions

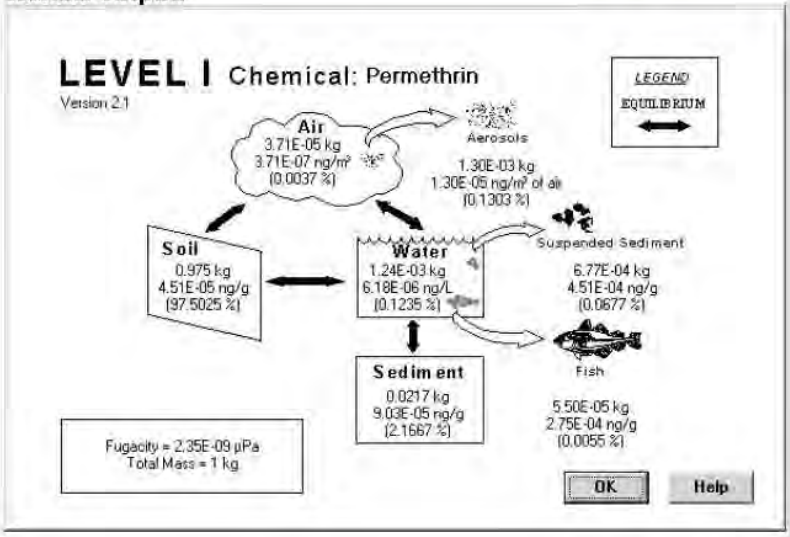
Permethrin
Chemical Type 1

Fugacity Pa 2.35E-15
Total of VZ Products 1.09E+15

PHASE	AIR	WATER	SOIL	SEDMT	SUSP SEDMT	FISH	AEROSOL
Volume: V (m³)	1.00E+14	2.00E+11	9.00E+09	1.00E+08	1.00E+06	2.00E+05	2000
Density (kg/m³)	1.185	1000	2400	2400	1500	1000	2000
Organic Carbon Content (g/g)	-	-	0.02	0.04	0.2	-	-
Fish Lipid Content (g/g)	-	-	-	-	-	0.05	-
Z Value (mol/m³.Pa)	4.03E-04	6.73E+00	1.18E+05	2.36E+05	7.37E+05	3.00E+05	7.10E+08
VZ (mol/Pa)	4.03E+10	1.35E+12	1.06E+15	2.36E+13	7.37E+11	5.99E+10	1.42E+12
Concentration (mol/m³)	9.47E-19	1.58E-14	2.77E-10	5.54E-10	1.73E-09	7.03E-10	1.67E-06
Concentration (g/m³)	3.71E-16	6.18E-12	1.08E-07	2.17E-07	6.77E-07	2.75E-07	6.52E-04
Concentration (µg/g)	3.13E-13	6.18E-12	4.51E-08	9.03E-08	4.51E-07	2.75E-07	3.26E-04
Amount (kg)	3.71E-05	1.24E-03	9.75E-01	2.17E-02	6.77E-04	5.50E-05	1.30E-03
Amount (mol)	9.47E-05	3.16E-03	2.49E+00	5.54E-02	1.73E-03	1.41E-04	3.33E-03
Amount (%)	0.0037	0.1235	97.5025	2.1667	0.0677	0.0055	0.1303

OK Help

Results output:



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 October 2005*
Evaluation of applicant's justification	The RMS considers the applicants justification to be valid for permethrin.
Conclusion	The applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.1.2.1
Section A7.1.1.2.2
Annex Point IIA7.6.1.1
Annex Point IIA7.6.1.2

Biodegradability (ready/inherent)

Key Study

			Official use only
		1 REFERENCE	
1.1	**Reference**	Caplan, J.A., Isbister, J; 1979; ^{14}C-permethrin (Acid and Alcohol label) Activated Sludge Metabolism; Biospherics Incorporated; 9PL-7-SL; 30 April 1979	
1.2	**Data protection**	Yes	
5.3.3	**Data owner**	Syngenta	
5.3.4	**Companies with letter of access**	Bayer Environmental Science	
5.3.5	**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 – Bespoke experimental design to determine the effects of ^{14}C-permethrin on the microbial and protozoan populations and on the operating characteristics (dissolved oxygen, temperature, pH, suspended solids and % settled solids) of an activated sludge system, and to characterise the fate of the test compound in that system.	
2.2	**GLP**	No - GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	Not applicable – test was not performed to any recognised guidelines	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Two separate radiolabelled [^{14}C]permethrin test materials were used in the study, labelled in the cyclopropane position (acid[^{14}C]permethrin) and methylene (alc[^{14}C]permethrin). The reference materials *cis*- and *trans*-DCVA, 3-phenoxybenzaldehyde and 3-phenoxybenzyl alcohol were used for co-chromatography.	
3.1.1	**Lot/Batch number**	Batch information are not reported	
3.1.2	**Specification**	Deviating from specification given in section 2 as follows: *cis*:*trans* ratios nominally 40:60 (0.12mCi:0.18 mCi with specific activities as given in Section 3.1.4)	
3.1.3	**Purity**	Not reported	
3.1.4	**Further relevant properties**	acid[^{14}C]permethrin: Specific activity 54.8 mCi mmol^{-1} alc[^{14}C]permethrin: Specific activity 57.01 mCi mmol^{-1}	
3.1.5	**Composition of Product**	Not applicable	
3.1.6	**TS inhibitory to microorganisms**	Yes/No Purpose of the test was to answer this question	
3.1.7	**Specific chemical analysis**	Yes: Supernatantnt and solids were extracted and the extracts were subsequently analysed by 2-dimensional Thin Layer Chromatography;	

Section A7.1.1.2.1
Section A7.1.1.2.2
Annex Point IIA7.6.1.1
Annex Point IIA7.6.1.2

Biodegradability (ready/inherent)

Key Study

		The filtrates from Cycle 10 were acidified to pH 1 and partitioned with dichloromethane (DCM 2 × 200 ml). The combined DCM fractions were dried through anhydrous sodium sulfate and evaporated to near dryness under a blanket of nitrogen. The Cycle 10 filter pads were extracted first with hexane (10.0 ml) and re-extracted with methanol:water, 9:1 (100 ml) in a Virtis blender. The methanol was evaporated and the aqueous fraction was transferred to a 50 ml volumetric flask and diluted to volume with water. The water was then adjusted to pH 1 with concentrated HCl and partitioned with ethylacetate (2 × 50 ml). The combined ethylacetate fractions were dried through anhydrous sodium sulfate and evaporated to near dryness under a blanket of nitrogen. The solid extracts (hexane and ethylacetate) were combined and adjusted to approximately 38,000 dpm/10 µl, while the filtrate extracts (DCM) were adjusted to 4,000 dpm/100 µl for TLC analysis along with nonlabelled permethrin standards. Plates: Merck Silica plates Solvent 1: Cyclohexane (sat'd with formic acid): ether 3:2 Solvent 2: Hexane:Ether 10:1 Plates were visualised *via* X-ray autoradiography. Radioactive areas were subsequently isolated, scraped and quantified *via* LSC.
3.2	**Reference substance**	No
3.3	**Test ing procedure**	
3.3.1	**Inoculum / test species**	See table A7_1_2_2-1
3.3.2	**Test system**	See table A7_1_2_2-2 The test systems were all-glass aeration chambers (2 control and 4 replicates), designed to contain a maximum of 2,800 ml total volume (operating volume-2,100 ml) with an air purge near the chamber bottom. Exiting air (positive pressure) is passed through a polyurethane volatiles trap and a CO_2 trap (1M NaOH) connected in series. The system was all glass through the volatile traps. Compressed air was purged into the system at 500 ml/min. The daily feed volume is 1,400 ml where 700 ml of settled sludge remained in the system from the previous cycle (2,100 ml total).
3.3.3	**Test conditions**	See table A7_1_2_2-3
3.3.4	**Method of preparation of test solution**	*Cis* and *trans* isomers of each label were combined with nonlabelled permethrin to equal 2100 mg in two 10 ml volumetric flasks (acid and alcohol labels were prepared separately). Each flask was diluted to volume with DMSO (=210 mg/ml). An aliquot (0.1 ml) of solution A from each flask was added to two additional 10 ml volumetric flasks and diluted to volume with DMSO (=2.1 mg/ml). All doses were prepared from these solutions, and (including controls) were diluted to 1.0 ml DMSO for each cycle throughout the

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		study.
3.3.5	**Initial TS concentration**	0.1 to 100 mg/l at increasing levels during the exposure period
3.3.6	**Duration of test**	17 days
3.3.7	**Analytical parameter**	As well as chemical analysis for permethrin (Section 3.1.7), test systems were analysed for physical parameters and microbiological activity; **Physical parameters**: suspended solids, % settled solids, dissolved oxygen, pH and temperature **Microbiological activity**: one ml of the suspension in each flask (during aeration) was removed and diluted to 10 ml with sterile tap water. Serial dilutions.into 9.0 ml of sterile tap water were performed prior to plating. Aliquots (0.1 ml) of the appropriate dilutions were spread-plated and incubated at 35°C for 48 hours on the following media: Nutrient Agar + 1.0 g glucose/1 (Bacteria, Actinomycetes, and Yeasts) Bacto Actinomycetes Isolation Agar (Actinomycetes) Bacto YM Agar (Yeasts) Protozoa were examined by placing one 25 µl drop of sludge suspension from each flask on a glass slide for examination by a phase-contrast microscope (100×). Motile protozoa greater than 50 µm in length were counted.
3.3.8	**Sampling**	**Daily Procedure** Each chamber was charged with 700 ml of activated sludge suspension (suspended solids adjusted g/litre) and 1,400 ml of synthetic sewage solution (adjusted to a BOD of 800). Aeration was begun and continued for 23 hours to acclimate the system. At the end of each 23-hour cycle but prior to discontinuing aeration, 210 ml of the suspension was removed (10% wasting) to measure plate counts, suspended solids, % settled solids, and to obtain solids and supernatant samples for radiocarbon analysis. The sludge solids were collected for combustion using filter pads from the filtration of 10 ml of sludge suspension from cycles 1 - 10. The filtrates and CO_2 traps for each flask were counted directly by addition of 1.0 ml of filtrate and NaOH solution to scintillation cocktail. The polyurethane volatile traps were extracted with acetone (2 × 10 ml) and counted (0.1 ml). All quantitations were performed using a Beckman LS-230 Liquid Scintillation Spectrometer with external standardisation. All vials were counted for five minutes with two carbon isoset channels. Subsequently, aeration was discontinued for 30 minutes (to allow for settling of the solids) and 1,200 ml of the supernatant was withdrawn. During the non-aeration period, the dissolved oxygen, pH and temperature of each flask were measured. Fourteen hundred ml of fresh synthetic sewage stock was added plus ^{14}C-permethrin according to the following schedule: **Experimental design:**

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Cycle	Permethrin Concentration in the System (mg/l)	Wt (mg)	Added Amount of ^{14}C-permethrin: Flasks 1-2 Acid(^{14}C) dpm/ml Sludge Suspension	Added Amount of ^{14}C-permethrin: Flasks 3-4 Alcohol (^{14}C) dpm/ml Sludge Suspension
1	0.1	0.21	29	29
2	0.5	1.05	145	147
3	1.0	2.10	291	294
4	5.0	10.5	1,452	1,472
5	10.0	21.0	2,905	2,945
6	20.0	42.0	5,809	5,889
7	40.0	84.0	11,619	11,778
8	60.0	126.0	17,428	17,667
9	80.0	168.0	23,238	23,557
10	100.0	210.0	29,047	29,446
11-17	100.0	210.0	-	-

Two control vessels were subjected to the same regime, but had no addition of permethrin.

3.3.9 Intermediates/ degradation products

Identified

Samples were quantified for potential metabolites using the same system as for the parent compound, but none were found.

3.3.10 Nitrate/nitrite measurement

No

Controls

Controls without addition of the test substance were included and treated to the same process as the treatment replicates.

3.3.11 Statistics

No statistical analysis was performed

4 RESULTS

4.1 Degradation of test substance

4.1.1 Graph

4.1.2 Degradation

The total radiocarbon recovery in the ^{14}C-permethrin treated flasks was 88.5% (Tables A7_1_2_2-4 to A7_1_2_2-7). Radiocarbon balance calculations are based on the amount of ^{14}C-permethrin added to the cycle, plus the carryover from the previous cycles. The supernatant carryover was one-third of the previous cycle (1,400 ml of 2,100 ml discarded daily after settling and prior to adding fresh synthetic sewage) and the solids carry over was 9/10 of the previous cycle (10% solids wasting prior to settling).

Daily radiocarbon analysis showed that greater than 80% of the

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radioactivity remained in the solids through Cycle 10. The remainder of radioactivity was accounted for primarily in the supernatant. Only small amounts of ^{14}C-volatiles (<1%) and $^{14}CO_2$ (<0.1%) were observed in the treated flasks. The data suggests that the activated sludge system removed greater than 80% of the test chemical or its degradation products from wastewaters. Data are presented graphically in Figure A7_1_2_2-1.

The extraction of radiocarbon from the supernatant in the acid and alcohol labeled ^{14}C-permethrin treated flasks was 80% and 86%, respectively. The extraction of solids was 99% in the ^{14}C-permethrin, acid label and 84% in the ^{14}C-permethrin, alcohol label treated flasks.

Thin layer chromatographic and autoradiographic analysis of the solid extracts yielded *cis*-permethrin (36% of dose in both acid and alcohol labeled flasks) and *trans*-permethrin (53% of dose - acid label; 57% - alcohol label) as the major components recovered (Table A7_1_2_2-8). Characterisation of the filtrate extracts revealed that the major components present were *cis*-permethrin (17 - 17.5%), *trans*-permethrin (24.1 - 27.8%) and polar material at the origin (33 - 43.8%). It should be noted that the filtrate components were all less than l% of the dose (Table A7_1_2_2-8).

4.1.3 Other observations

Physical measurements (pH, temperature, dissolved oxygen, suspended solids), plate counts, and protozoa are presented in Tables A7_1_1_2_2-9 to A7_1_1_2_2-14 and Figures A7_1_1_2_2-2 to A7_1_1_2_2-6.

The temperature remained at 21 ± 2°C for all flasks.

The dissolved oxygen (DO) was 6.6 ppm for all flasks at the beginning of the study and approximately 6.4 ppm by the end of the study.

The pH was 7.7 at the start of the study and gradually decreased to approximately 4.3 for all flasks by the end of the study (Tables A7_1_1_2_2-9 to A7_1_1_2_2-14 and Figure A7_1_1_2_2-2).

No effects due to permethrin were observed on the activated sludge system as measured by plate counts for total bacteria and actinomycetes (Tables A7_1_1_2_2-9 to A7_1_1_2_2-14 and Figures A7_1_1_2_2-3 to A7_1_1_2_2-5). Yeast counts were slightly higher in the treated flasks than in the control flasks.

The suspended solids (Figure A7_1_1_2_2-6) began at 1,400 mg/1 for all flasks and were approximately 2,100 mg/1 for the ^{14}C-permethrin treated flasks by the termination of the experiment. The suspended solids for the control flasks were somewhat lower at the end of the test (1,600 mg/1). Based on the radiochemistry data, the increase in suspended solids for the ^{14}C-permethrin treated flasks was attributed by the authors to the adsorption of test compound to solids.

The % settled solids began at approximately 11.0% for all flasks and reached a peak level of approximately 32-35% (Figure A7_1_1_2_2-7; Tables A7_1_1_2_2-15 to A7_1_1_2_2-20) at Cycle 11 (100 ppm level). By the end of the 17 day period, values had dropped to approximately 15-20%.

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Although protozoa from treated flasks in cycles 12 and 14, (100 ppm addition) appeared stressed and absent respectively (stressed protozoa displayed nondirectional motility i.e., rapid circular movements, the absence and subsequent appearance of protozoa in cycles 15 - 17 was attributed by the authors to a sampling error, based on their subsequent reappearance), protozoa of normal motility were observed in treated flasks from subsequent cycles (cycles 15 - 17, Tables A7_1_1_2_2-15 to A7_1_1_2_2-20).

Based on the physical and biological parameters measured, there appear to be no significant effects of permethrin on this activated sludge system.

4.1.4	**Degradation of TS in abiotic control**	Not performed
4.1.5	**Degradation of reference substance**	Not performed
4.1.6	**Intermediates/ degradation products**	No degradation was observed to occur

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Activated sludge (1.3 g solids/litre), synthetic sewage and ^{14}C-permethrin (Acid and Alcohol label) were aerated in standard reactor vessels for 23 hours per cycle (total 2100 ml). After each cycle, aeration was discontinued (to allow for settling) and 1400 ml of supernatant was removed and replaced with fresh synthetic sewage and an increased amount of ^{14}C-permethrin. The concentration of ^{14}C-permethrin was started at 0.1 ppm and was increased daily for 10 days to 100 ppm. For the next seven days, similar cycles using non-radioactive permethrin were made at a concentration of 100 ppm.
5.2	**Results and discussion**	It is important to note that the dosing rate from the start of the experiment (100 μg l^{-1} on day 0, rising to 100 mg l^{-1}) was considerable higher than the solubility of permethrin in water (<5 μg l^{-1}). This will have had some effect on the outcome of the test, such that the data may not be absolutely quantitative, but nonetheless can be considered as reliable when used with caution. For example, throughout the exposure period, the amount of material measured in the supernatant was consistently higher than the solubility in water (22.7% of 0.5 mg l^{-1} on cycle 2, equivalent to 114 μg l^{-1}, and 2.1% of 100 mg l^{-1} on cycle 10, equivalent to 2.1 mg l^{-1}). No effects due to permethrin were observed on the activated sludge system as measured by pH and plate counts for total bacterial microorganisms. In consideration of the caveat mentioned above, radiocarbon analysis indicated that, except for the first two cycles, greater than 80% of the radioactivity was found in the solids. Less than 1% of the total radioactivity was observed in the volatiles and $^{14}CO_2$ traps. The remainder of the radioactivity was accounted for primarily in the

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supernatant. Characterisation of the extracted radiocarbon from the supernate and solids showed that *cis*-permethrin and *trans*-permethrin were the only major components present. Recovered radioactivity was generally adequate, with maximum recoveries occurring during the middle cycles of the exposure period.

The data indicate that for a newly configured system, adsorption of permethrin is approximately 80%, with the remainder appearing in the supernatant. Once the treatment system matures, the adsorption of applied radioactivity increased, and at the end of the test period only 1-2% of applied radioactivity was associated with the aqueous phase.

Extrapolating these data to behaviour in a STP which would, according to the release model, receive continous exposure to permethrin, it is most appropriate to consider the data from the later stages of the test period, rather than the data from the newly configured system.

The average amount of permethrin in the supernatant phase from Cycle 4 onwards was 3.7% of the applied material.

5.3	**Conclusion**	No validity criteria exist for this bespoke simulation study. The design employed was adequate to describe the impact of permethrin on STP organisms, and provided data on the partitioning behaviour of permethrin.
5.3.1	**Reliability**	2
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	*14 October 2005*
Materials and Methods	The applicants version is acceptable.
Results and discussion	Adopt applicant's version.
Conclusion	Adopt applicant's version.
Reliability	*2*
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_2_2-1: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Not relevant
Strain	Not relevant
Source	Sewage treatment plant treating approximately 85% domestic sewage and 15% industrial waste
Sampling site	Back River (Dundalk, Maryland) STP
Laboratory culture	No
Method of cultivation	Not relevant
Preparation of inoculum for exposure	Prior to initiating the study, the suspended solids was adjusted to 1.3 g/l and a 23 hour acclimation was run.
Pretreatment	Other than the 23 hour acclimation, no pre-treatment was performed.
Initial cell concentration	The suspended solids began at 1,400 mg/1 for all flasks and were approximately 2,100 mg/1 for the ^{14}C-permethrin treated flasks by the termination of the experiment. The suspended solids for the control flasks were somewhat lower at the end of the test (1,600 mg/1)

Table A7_1_2_2-2: Test system

Criteria	Details
Culturing apparatus	All glass Activated Sludge Reaction chamber
Number of culture flasks/concentration	2 control and 4 replicates
Aeration device	Compressed air was purged into the bottom of the chamber at 500 ml/min.
Measuring equipment	Microbiological activity was measured using the following systems; A. Nutrient Agar + 1.0 g glucose/1 B. Bacto Actinomycetes Isolation Agar C. Bacto YM Agar Protozoa were examined microscopically - motile protozoa greater than 50 um in length were counted. Explained in more detail in Section 3.3.2.
Test performed in closed vessels due to significant volatility of TS	Yes – all exuent gases were passed through appropriate traps to retain evolved volatile organic gases and CO_2

Table A7_1_2_2-3: Test conditions

Criteria	Details
Composition of medium	Synthetic sewage stock solution: Glucose..... 13.0 g Nutrient broth..... 13.0 g Beef extract ..... 13.0 g Dipotassium Hydrogen Phosphate ... 13.0 g Ammonium sulphate ..... 2.5 g Diluted to 1 litre with aged tap water, stored at 7°C. Daily feeding solution; 43 ml stock solution diluted to 1.4 l (BOD = 800 at mixed suspended solids of 5000 mg/l)
Additional substrate	No
Test temperature	19.0 to 23.2°C measured daily in each vessel
pH	3.97 to 8.15 measured daily in each vessel
Aeration of dilution water	Yes
Suspended solids concentration	The suspended solids began at 1,400 mg/1 for all flasks and were approximately 2,100 mg/1 for the ^{14}C-permethrin treated flasks by the termination of the experiment. The suspended solids for the control flasks were somewhat lower at the end of the test (1,600 mg/1)
Other relevant citeria	Continuous aeration for 23 hour cycles – settled for one hour for parameter determination.

Table A7_1_2_2-4: Test system recovery of added ^{14}C-permethrin, Acid Label (Flask 1)

Cycle (Day)	Permethrin added (ppm)	Recovery of radioactivity[a]				
		Supernatant	Solids	Volatiles	CO_2	Total % recovery
1	0.1	**b**	**b**	**b**	**b**	**b**
2	0.5	**22.7**	**72.6**	**<0.1**	**<0.1**	**95.3**
3	1.0	**18.8**	**86.6**	**<0.1**	**<0.1**	**105.4**
4	5.0	**7.7**	**80.4**	**<0.1**	**<0.1**	**88.1**
5	10.0	**7.9**	**95.2**	**<0.1**	**<0.1**	**103.1**
6	20.0	**4.5**	**94.3**	**<0.1**	**<0.1**	**98.8**
7	40.0	**3.2**	**84.7**	**<0.1**	**<0.1**	**87.9**
8	60.0	**2.4**	**67.2**	**<0.1**	**<0.1**	**69.6**
9	80.0	**2.2**	**67.9**	**<0.1**	**<0.1**	**70.1**
10	100.0	**2.1**	**73.7**	**<0.1**	**<0.1**	**75.8**

a Values given in % of dose
b Below limit of quantification

Table A7_1_2_2-5: Test system recovery of added ^{14}C-permethrin, Acid Label (Flask 2)

Cycle (Day)	Permethrin added (ppm)	Recovery of radioactivity[a]				
		Supernatant	Solids	Volatiles	CO_2	Total % recovery
1	0.1	**b**	**b**	**b**	**b**	**b**
2	0.5	**9.6**	**77.5**	**<0.1**	**<0.1**	**87.1**
3	1.0	**14.9**	**79.5**	**<0.1**	**<0.1**	**94.4**
4	5.0	**9.3**	**44.5**	**<0.1**	**<0.1**	**53.8[c]**
5	10.0	**9.3**	**99.1**	**<0.1**	**<0.1**	**108.4**
6	20.0	**5.4**	**87.1**	**<0.1**	**<0.1**	**92.5**
7	40.0	**3.2**	**81.8**	**<0.1**	**<0.1**	**85.0**
8	60.0	**2.5**	**70.8**	**<0.1**	**<0.1**	**73.3**
9	80.0	**2.3**	**74.4**	**<0.1**	**<0.1**	**76.7**
10	100.0	**2.5**	**62.4**	**<0.1**	**<0.1**	**64.9**

a Values given in % of dose
b Below limit of quantification
c Value not included in further summaries

Table A7_1_2_2-6: Test system recovery of added ^{14}C-permethrin, Alcohol Label (Flask 3)

Cycle (Day)	Permethrin added (ppm)	Recovery of radioactivity[a]				
		Supernatant	Solids	Volatiles	CO_2	Total % recovery
1	0.1	**b**	**b**	**b**	**b**	**b**
2	0.5	**13.4**	**70.2**	**<0.1**	**<0.1**	**83.6**
3	1.0	**12.3**	**72.5**	**<0.1**	**<0.1**	**84.8**
4	5.0	**4.7**	**84.6**	**<0.1**	**<0.1**	**89.3**
5	10.0	**5.3**	**86.8**	**<0.1**	**<0.1**	**92.1**
6	20.0	**3.7**	**95.6**	**<0.1**	**<0.1**	**99.3**
7	40.0	**2.1**	**76.2**	**<0.1**	**<0.1**	**78.3**
8	60.0	**1.6**	**70.0**	**<0.1**	**<0.1**	**71.6**
9	80.0	**1.3**	**85.7**	**<0.1**	**<0.1**	**87.0**
10	100.0	**0.9**	**74.3**	**<0.1**	**<0.1**	**75.2**

a Values given in % of dose
b Below limit of quantification

Table A7_1_2_2-7: Test system recovery of added ^{14}C-permethrin, Alcohol Label (Flask 4)

Cycle (Day)	Permethrin added (ppm)	Recovery of radioactivity[a]				
		Supernatant	Solids	Volatiles	CO_2	Total % recovery
1	0.1	**b**	**b**	**b**	**b**	**b**
2	0.5	**39.6**	**87.4**	**<0.1**	**<0.1**	**127.0[c]**
3	1.0	**10.8**	**62.6**	**<0.1**	**<0.1**	**73.4**
4	5.0	**5.2**	**92.1**	**<0.1**	**<0.1**	**97.3**
5	10.0	**5.9**	**84.2**	**<0.1**	**<0.1**	**90.1**
6	20.0	**3.6**	**86.5**	**<0.1**	**<0.1**	**90.1**
7	40.0	**2.1**	**77.9**	**<0.1**	**<0.1**	**80.0**
8	60.0	**1.3**	**82.6**	**<0.1**	**<0.1**	**83.9**
9	80.0	**1.0**	**82.2**	**<0.1**	**<0.1**	**83.2**
10	100.0	**0.8**	**78.5**	**<0.1**	**<0.1**	**79.3**

a Values given in % of dose
b Below limit of quantification
c Value not included in further summaries

Table A7_1_2_2-8: Recovery of scraped TLC platesof cycle 10 ^{14}C-permethrin (Acid and Alcohol Label) solids and filtrate extracts

	Permethrin		DCVA		Phenoxybenz-		Recovery of radiocarbon
Sample	*Cis-*	*Trans-*	*Cis-*	*Trans-*	acid	alcohol	
Solids							
Flasks 1-2 (acid label)	**37.7**	**55.5**	**0.70**	**0.50**	**0.50**	**0.20**	**105.9**
% of dose	**36.46**	**53.67**	**0.68**	**0.48**	**0.48**	**0.19**	
Flasks 3-4 (alcohol label)	**36.5**	**58.0**	**1.6**	**0.30**	**0.30**	**0.10**	**104.8**
% of dose	**36.10**	**57.36**	**1.58**	**0.30**	**0.30**	**0.10**	
Filtrates							
Flasks 1-2 (acid label)	**17.0**	**24.1**	**0.70**	**8.4**	**13.4**	**3.3**	**128.6**
% of dose	**0.58**	**0.82**	**0.02**	**0.29**	**0.46**	**0.11**	
Flasks 3-4 (alcohol label)	**17.5**	**27.8**	**2.0**	**1.2**	**7.6**	**0.10**	**148.5**
% of dose	**0.19**	**0.31**	**0.02**	**0.01**	**0.08**	**0.001**	

Table A7_1_2_2-9: Test system parameters after addition of ^{14}C-permethrin, Acid Label (Flask 1)

						Plate Counts		
Cycle (Day)	**Permethrin added (ppm)**	**pH**	**Temp. (°C)**	**DO (ppm)**	**Suspended solids (mg/1)**	**A**	**B**	**C**
0	0.0	7.79	23.0	6.8	1,387	7.6×10^5	1.1×10^6	1.0×10^5
1	0.1	7.69	23.0	6.8	1,569	5.8×10^5	1.6×10^6	4.4×10^4
2	0.5	6.14	22.0	7.3	1,617	7.9×10^6	1.2×10^6	6.2×10^4
3	1.0	7.60	21.6	6.5	1,745	1.1×10^6	2.4×10^5	1.3×10^4
4	5.0	7.78	21.0	7.6	1,681	3.1×10^6	2.5×10^5	1.8×10^4
5	10.0	7.63	23.0	5.8	1,939	3.3×10^6	1.4×10^5	1.1×10^5
6	20.0	7.71	21.5	7.3	1,957	5.3×10^6	1.0×10^6	8.9×10^4
7	40.0	7.59	20.5	5.9	2,179	7.0×10^6	6.3×10^5	1.8×10^4
8	60.0	7.37	21.5	5.1	2,151	6.1×10^6	7.6×10^5	7.4×10^4
9	80.0	6.97	21.5	2.8	2,116	7.7×10^6	4.7×10^5	3.9×10^4
10	100.0	5.46	21.1	4.4	2,123	3.4×10^6	6.5×10^5	4.0×10^4
11	100.0	5.51	20.2	6.0	2,216	7.1×10^6	4.1×10^5	3.6×10^4
12	100.0	4.69	19.1	8.1	2,027	2.0×10^7	1.5×10^6	6.9×10^4
13	100.0	5.10	20.4	8.1	2,352	9.9×10^6	7.8×10^5	4.8×10^4
14	100.0	4.15	22.9	6.7	2,162	1.3×10^7	1.3×10^6	8.5×10^4
15	100.0	4.57	20.0	6.4	2,224	2.1×10^7	1.3×10^6	6.6×10^4
16	100.0	3.80	19.0	7.0	2,213	9.7×10^6	1.2×10^6	1.0×10^5
17	100.0	3.97	21.0	6.5	2,197	5.1×10^6	6.3×10^5	4.7×10^4

Table A7_1_2_2-10: Test system parameters after addition of ^{14}C-permethrin, Acid Label (Flask 2)

Cycle (Day)	Permethrin added (ppm)	pH	Temp. (°C)	DO (ppm)	Suspended solids (mg/1)	Plate Counts		
						A	B	C
0	0.0	7.72	23.0	6.4	1,435	9.5×10^7	1.0×10^8	2.5×10^6
1	0.1	7.70	23.0	6.7	1,610	5.7×10^6	1.2×10^7	6.5×10^4
2	0.5	9.13	21.9	7.3	1,641	6.0×10^6	9.6×10^6	1.2×10^7
3	1.0	7.67	21.6	7.1	1,706	9.5×10^5	1.1×10^5	4.0×10^4
4	5.0	7.73	21.0	7.4	1,737	1.1×10^6	1.4×10^5	2.0×10^6
5	10.0	7.59	23.0	6.0	1,830	6.5×10^6	6.4×10^4	9.6×10^3
6	20.0	7.71	21.5	7.4	1,831	2.7×10^7	6.7×10^5	8.9×10^4
7	40.0	7.73	20.5	6.5	1,957	1.6×10^6	5.5×10^5	6.7×10^4
8	60.0	7.58	21.1	6.8	1,968	4.6×10^6	1.6×10^6	6.3×10^4
9	80.0	7.47	21.5	5.6	2,010	8.8×10^6	5.5×10^5	4.0×10^4
10	100.0	7.09	22.0	4.5	1,904	1.6×10^7	6.8×10^5	4.9×10^4
11	100.0	6.94	20.2	4.4	2,045	5.8×10^6	1.5×10^5	6.6×10^3
12	100.0	7.24	19.2	3.2	2,117	1.6×10^7	1.0×10^6	4.9×10^4
13	100.0	6.38	20.4	2.1	2,410	7.9×10^5	3.7×10^5	1.2×10^4
14	100.0	6.24	23.0	0.3	1,895	7.1×10^6	1.1×10^6	TNC[1]
15	100.0	6.63	20.2	3.4	1,922	1.7×10^7	2.6×10^6	2.9×10^5
16	100.0	4.52	19.2	5.8	1,896	5.7×10^6	1.1×10^6	1.1×10^6
17	100.0	4.18	21.0	7.7	1,978	3.1×10^6	7.8×10^5	1.2×10^5

1 Too Numerous to Count

Table A7_1_2_2-11: Test system parameters after addition of ^{14}C-permethrin, Alcohol Label (Flask 3)

Cycle (Day)	Permethrin added (ppm)	pH	Temp. (°C)	DO (ppm)	Suspended solids (mg/1)	Plate Counts		
						A	B	C
0	0.0	7.79	23.0	6.9	1,452	7.7×10^7	5.9×10^6	1.5×10^5
1	0.1	7.67	22.5	7.0	1,607	4.5×10^6	5.7×10^5	1.4×10^4
2	0.5	8.15	22.0	7.2	1,646	4.4×10^6	1.0×10^6	4.6×10^4
3	1.0	7.63	21.6	7.1	1,720	7.3×10^5	1.7×10^5	3.9×10^4
4	5.0	7.79	21.0	8.0	1,813	2.0×10^6	4.5×10^4	1.9×10^4
5	10.0	7.59	22.8	6.5	1,947	9.5×10^6	7.6×10^4	1.2×10^4
6	20.0	7.71	21.5	7.5	1,854	1.6×10^6	3.4×10^5	2.1×10^4
7	40.0	7.62	20.5	6.1	2,053	1.4×10^6	1.4×10^5	1.9×10^4
8	60.0	7.36	21.1	5.3	2,140	1.0×10^7	4.4×10^5	3.7×10^4
9	80.0	7.23	21.5	4.2	2,124	4.9×10^6	7.5×10^4	1.2×10^4
10	100.0	6.56	22.0	3.9	1,986	8.7×10^6	1.08×10^5	6.7×10^3
11	100.0	6.28	20.2	4.1	2,129	4.4×10^6	7.4×10^4	5.8×10^3
12	100.0	5.55	19.5	5.5	1,958	7.3×10^6	6.1×10^5	3.3×10^4
13	100.0	6.57	20.4	8.2	2,140	1.1×10^6	8.4×10^4	9.8×10^3
14	100.0	4.63	23.0	5.8	2,061	1.5×10^7	4.8×10^5	1.5×10^5
15	100.0	4.56	20.5	6.3	2,200	2.5×10^6	1.4×10^5	7.4×10^4
16	100.0	4.30	19.5	6.1	2,250	1.1×10^7	3.1×10^5	7.5×10^3
17	100.0	4.25	21.0	6.4	2,232	1.5×10^6	5.4×10^4	3.8×10^3

Table A7_1_2_2-12: Test system parameters after addition of ^{14}C-permethrin, Alcohol Label (Flask 4)

Cycle (Day)	Permethrin added (ppm)	pH	Temp. (°C)	DO (ppm)	Suspended solids (mg/1)	Plate Counts		
						A	B	C
0	0.0	7.76	23.0	6.7	1,427	1.1×10^7	2.0×10^6	2.4×10^5
1	0.1	7.68	22.5	6.9	1,541	9.1×10^5	6.5×10^5	4.7×10^4
2	0.5	8.11	21.9	7.4	1,630	4.8×10^6	3.8×10^5	3.1×10^4
3	1.0	7.66	21.8	7.0	1,651	7.1×10^5	1.1×10^5	9.2×10^3
4	5.0	7.74	21.0	7.8	1,719	1.5×10^6	1.3×10^5	1.5×10^4
5	10.0	7.58	21.0	6.0	1,842	5.7×10^6	5.8×10^4	1.1×10^4
6	20.0	7.74	21.5	7.6	1,817	1.3×10^7	7.2×10^5	5.8×10^4
7	40.0	7.55	20.8	5.9	1,928	9.5×10^5	3.5×10^5	4.7×10^4
8	60.0	7.28	21.1	5.3	1,925	4.8×10^6	2.1×10^5	4.0×10^3
9	80.0	7.12	21.8	3.9	1,968	1.0×10^7	3.7×10^5	1.3×10^4
10	100.0	6.19	22.0	3.6	1,904	7.4×10^6	1.2×10^5	7.7×10^3
11	100.0	5.95	20.2	5.2	1,988	5.5×10^6	2.9×10^5	7.3×10^3
12	100.0	5.00	19.9	6.5	1,006	1.3×10^7	1.9×10^5	1.6×10^4
13	100.0	4.65	20.4	9.2	2,140	4.7×10^6	6.9×10^4	9.7×10^3
14	100.0	4.57	23.0	5.5	2,075	8.1×10^6	4.9×10^5	1×10^4
15	100.0	4.37	20.5	6.6	2,149	1.6×10^7	1.2×10^4	1.2×10^4
16	100.0	4.21	19.9	4.7	2,199	1.0×10^7	6.1×10^5	1.4×10^4
17	100.0	4.14	21.2	6.3	2,169	5.5×10^6	5.4×10^5	5.0×10^3

Table A7_1_2_2-13: Test system parameters after addition DMSO (Control Flask 11)

Cycle (Day)	Permethrin added (ppm)	pH	Temp. (°C)	DO (ppm)	Suspended solids (mg/1)	Plate Counts		
						A	B	C
0	0.0	7.75	23.0	6.8	1,455	1.3×10^8	7.1×10^6	1.4×10^5
1	0.0	7.66	22.6	7.3	1,601	6.0×10^6	6.3×10^5	6.1×10^3
2	0.0	8.12	22.9	7.1	1,699	5.2×10^6	1.4×10^6	7.3×10^4
3	0.0	7.55	22.0	8.1	1,707	7.2×10^5	1.0×10^5	8.5×10^3
4	0.0	7.76	21.9	8.1	1,831	1.4×10^6	9.0×10^4	1.6×10^4
5	0.0	7.59	23.0	6.7	1,900	8.5×10^5	1.0×10^5	1.1×10^4
6	0.0	7.57	22.5	7.1	1,992	1.7×10^6	3.9×10^5	5.9×10^4
7	0.0	7.58	21.0	7.2	1,905	7.3×10^6	7.5×10^5	4.3×10^4
8	0.0	7.46	21.9	6.9	1,850	4.3×10^6	7.8×10^5	3.1×10^4
9	0.0	7.35	21.8	4.8	1,871	2.2×10^6	8.2×10^4	5.5×10^3
10	0.0	6.24	22.9	3.5	1,782	4.9×10^6	1.2×10^5	5.3×10^3
11	0.0	5.25	20.5	7.8	1,775	1.2×10^6	8.7×10^4	4.0×10^3
12	0.0	4.00	20.0	9.0	1,469	6.1×10^6	6.8×10^5	6.6×10^3
13	0.0	5.08	20.6	9.2	1,787	1.1×10^6	5.6×10^5	8.6×10^3
14	0.0	4.67	23.1	6.4	1,680	2.8×10^7	1.1×10^6	6.0×10^3
15	0.0	4.41	20.8	7.3	1,617	2.6×10^7	8.2×10^4	5.2×10^3
16	0.0	4.00	20.0	6.7	1,589	1.2×10^7	4.2×10^5	3.8×10^3
17	0.0	4.49	20.0	6.9	1,618	7.8×10^6	3.4×10^5	3.6×10^3

Table A7_1_2_2-14: Test system parameters after addition DMSO (Control Flask 12)

Cycle (Day)	Permethrin added (ppm)	pH	Temp. (°C)	DO (ppm)	Suspended solids (mg/1)	Plate Counts		
						A	B	C
0	0.0	7.70	23.0	6.1	1,441	1.6×10^7	6.4×10^7	1.3×10^5
1	0.0	7.69	22.8	7.0	1,569	1.1×10^6	4.3×10^5	4.1×10^4
2	0.0	8.09	22.8	7.0	1,591	4.9×10^6	7.0×10^5	5.7×10^4
3	0.0	7.64	22.0	8.0	1,625	7.7×10^5	6.0×10^4	9.0×10^3
4	0.0	7.74	22.0	7.3	1,869	7.8×10^5	1.6×10^3	1.8×10^4
5	0.0	7.47	23.0	6.2	1,938	2.0×10^6	5.8×10^5	1.3×10^4
6	0.0	7.53	22.5	6.5	1,976	2.2×10^6	2.4×10^5	8.1×10^4
7	0.0	7.60	21.2	7.4	1,885	1.1×10^6	4.9×10^5	9.5×10^3
8	0.0	7.44	22.0	5.9	1,926	2.9×10^6	4.5×10^5	1.4×10^4
9	0.0	7.36	22.0	4.5	1,851	3.3×10^5	4.6×10^5	8.5×10^3
10	0.0	6.75	23.0	3.3	1,830	8.1×10^6	4.5×10^5	6.0×10^3
11	0.0	5.50	20.5	6.8	1,831	8.0×10^5	8.0×10^4	5.9×10^3
12	0.0	4.92	20.0	8.4	1,637	6.2×10^6	9.7×10^5	1.0×10^4
13	0.0	5.18	21.0	7.6	1,877	4.8×10^6	4.5×10^5	5.2×10^3
14	0.0	4.82	23.2	5.8	1,628	1.8×10^7	2.3×10^5	6.1×10^3
15	0.0	4.94	21.0	6.5	1,538	2.1×10^7	5.5×10^4	5.9×10^3
16	0.0	4.62	20.0	6.9	1,618	4.4×10^6	4.1×10^5	3.9×10^3
17	0.0	5.26	21.5	5.1	1,514	7.1×10^6	3.8×10^5	2.9×10^3

Table A7_1_2_2-15: Floculation parameters and protozoa (^{14}C-permethrin, Acid Label Flask 1)

Cycle (Day)	**Permethrin added (ppm)**	Settled Solids (%)	Protozoa (+/-)
0	0.0	**11.0**	+
1	0.1	**16.0**	+
2	0.5	**18.5**	+
3	1.0	**21.0**	+
4	5.0	**23.0**	+
5	10.0	**24.0**	+
6	20.0	**27.5**	+
7	40.0	**26.5**	+
8	60.0	**28.0**	+
9	80.0	**22.0**	+
10	100.0	**28.0**	+
11	100.0	**32.0**	+
12	100.0	**28.0**	-
13	100.0	**23.5**	+
14	100.0	**22.9**	-
15	100.0	**23.0**	+
16	100.0	**20.0**	+
17	100.0	**19.5**	+

Table A7_1_2_2-16: Floculation parameters and protozoa (^{14}C-permethrin, Acid Label Flask 2)

Cycle (Day)	**Permethrin added (ppm)**	Settled Solids (%)	Protozoa (+/-)
0	0.0	**12.0**	+
1	0.1	**17.0**	+
2	0.5	**20.0**	+
3	1.0	**20.5**	+
4	5.0	**21.0**	+
5	10.0	**21.0**	+
6	20.0	**19.0**	+
7	40.0	**24.0**	+
8	60.0	**27.0**	+
9	80.0	**22.0**	+
10	100.0	**25.0**	+
11	100.0	**28.5**	+
12	100.0	**30.0**	+
13	100.0	**29.0**	+
14	100.0	**31.0**	-
15	100.0	**24.5**	+
16	100.0	**20.0**	+
17	100.0	**19.0**	+

Table A7_1_2_2-17: Floculation parameters and protozoa (^{14}C-permethrin, Alcohol Label Flask 3)

Cycle (Day)	Permethrin added (ppm)	Settled Solids (%)	Protozoa (+/-)
0	0.0	**10.0**	+
1	0.1	**16.0**	+
2	0.5	**18.0**	+
3	1.0	**21.0**	+
4	5.0	**22.0**	+
5	10.0	**24.0**	+
6	20.0	**27.0**	+
7	40.0	**29.0**	+
8	60.0	**30.0**	+
9	80.0	**23.5**	+
10	100.0	**29.0**	+
11	100.0	**34.0**	+
12	100.0	**28.5**	+
13	100.0	**23.0**	+
14	100.0	**19.0**	-
15	100.0	**22.0**	+
16	100.0	**14.0**	+
17	100.0	**17.0**	+

Table A7_1_2_2-18: Floculation parameters and protozoa (^{14}C-permethrin, Alcohol Label Flask 4)

Cycle (Day)	**Permethrin added (ppm)**	Settled Solids (%)	Protozoa (+/-)
0	0.0	**10.0**	+
1	0.1	**16.0**	+
2	0.5	**18.0**	+
3	1.0	**20.0**	+
4	5.0	**21.0**	+
5	10.0	**22.0**	+
6	20.0	**19.0**	+
7	40.0	**24.0**	+
8	60.0	**25.0**	+
9	80.0	**21.0**	+
10	100.0	**25.0**	+
11	100.0	**34.5**	+
12	100.0	**25.0**	+
13	100.0	**22.0**	+
14	100.0	**21.0**	-
15	100.0	**19.5**	+
16	100.0	**12.5**	+
17	100.0	**16.0**	+

Table A7_1_2_2-19: Floculation parameters and protozoa (Control Flask 11)

Cycle (Day)	**Permethrin added (ppm)**	Settled Solids (%)	Protozoa (+/-)
0	0.0	**11.0**	+
1	0.0	**16.0**	+
2	0.0	**18.0**	+
3	0.0	**19.0**	+
4	0.0	**19.5**	+
5	0.0	**20.0**	+
6	0.0	**23.0**	+
7	0.0	**22.0**	+
8	0.0	**23.0**	+
9	0.0	**21.0**	+
10	0.0	**27.0**	+
11	0.0	**32.0**	+
12	0.0	**21.0**	+
13	0.0	**18.5**	+
14	0.0	**12.0**	+
15	0.0	**15.0**	+
16	0.0	**13.0**	+
17	0.0	**12.0**	+

Table A7_1_2_2-20: Floculation parameters and protozoa (Control Flask 12)

Cycle (Day)	**Permethrin added (ppm)**	Settled Solids (%)	Protozoa (+/-)
0	0.0	**11.0**	+
1	0.0	**16.0**	+
2	0.0	**18.5**	+
3	0.0	**20.0**	+
4	0.0	**21.0**	+
5	0.0	**23.0**	+
6	0.0	**26.5**	+
7	0.0	**24.5**	+
8	0.0	**26.0**	+
9	0.0	**23.0**	+
10	0.0	**29.0**	+
11	0.0	**35.5**	+
12	0.0	**25.0**	+
13	0.0	**20.0**	+
14	0.0	**19.5**	+
15	0.0	**17.5**	+
16	0.0	**13.5**	+
17	0.0	**10.0**	+

Figure A7_1_2_2-1: Recovery of radioactivity and compartment association

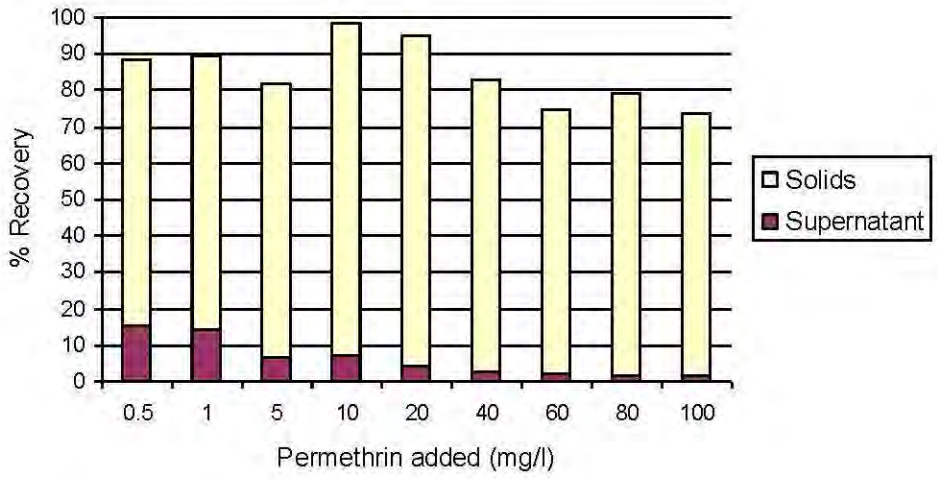

Figure A7_1_2_2-2: pH measurements

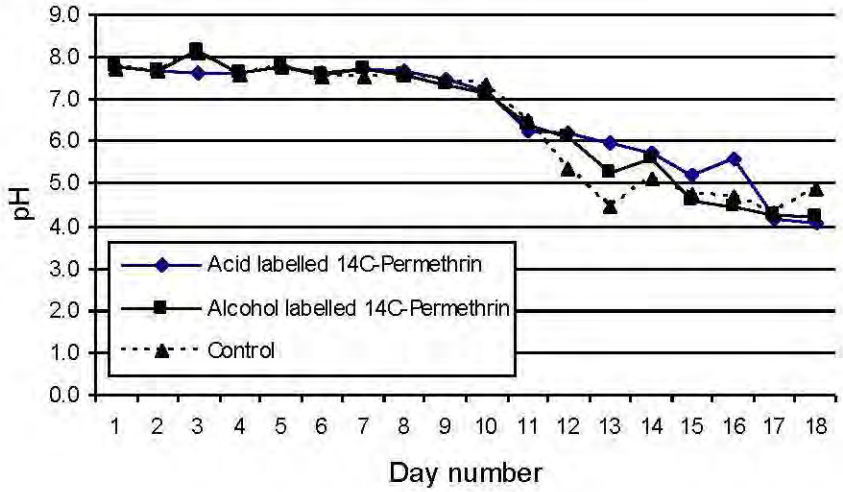

Figure A7_1_2_2-3: Plate counts: Universal agar

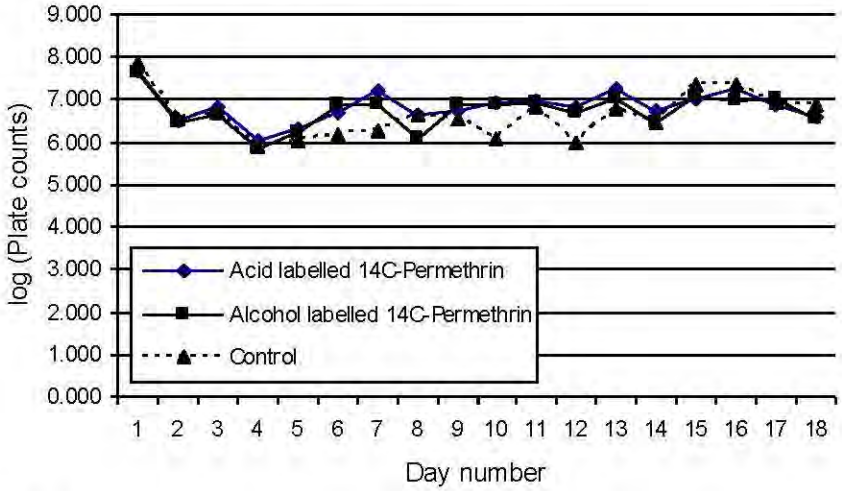

Figure A7_1_2_2-4: Plate counts: Actinomycete isolation agar

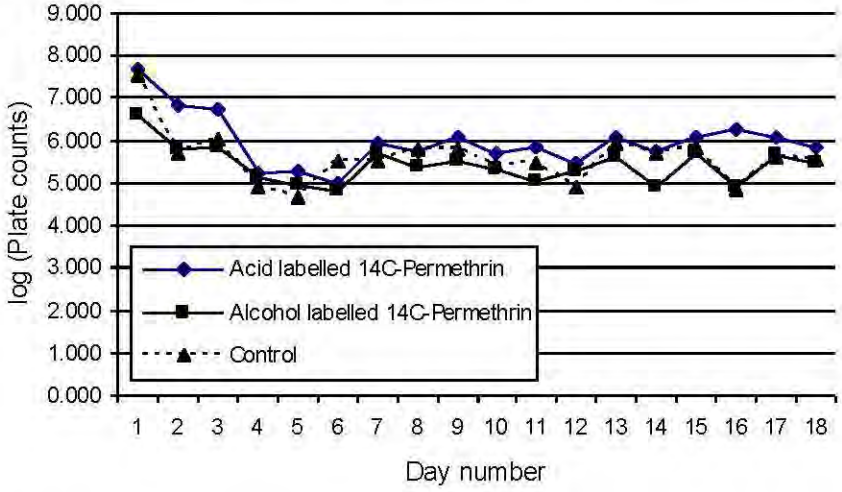

Figure A7_1_2_2-5: Plate counts: Yeast malt

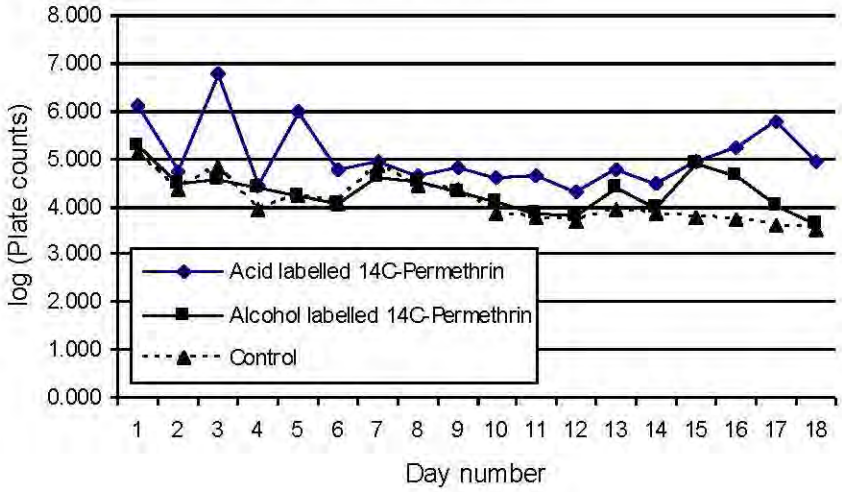

Figure A7_1_2_2-6: Suspended solids

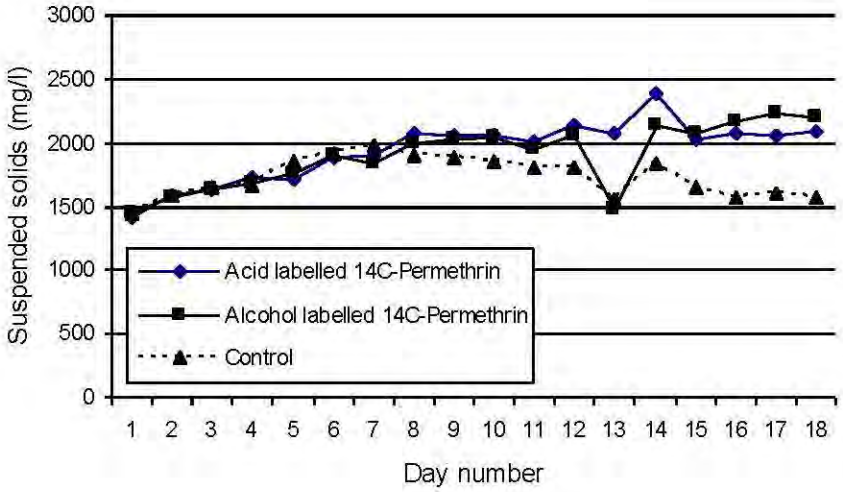

Figure A7_1_2_2-7: Settled solids