98/8 Doc IIIA section No.	7.4.1.4/02	Inhibition of microbiological activity
91/414 Annex Point addressed	II 8.7/02	Effects on biological methods for sewage treatment

		Official use only
Reference point (location) in dossier	**7.4.1.4/02**	
Title:	*Lambda*Cyhalothrin: Determination of the toxicity to *Pseudomonas putida*.	
Project/Report number:	BL/B/3467	
Author(s):	Mather, J.I. and Tapp, J.F.	
Date of report:	1989	
Published:	Not published.	
Testing facility:	Brixham Environmental Laboratory, AstraZeneca UK Limited, Brixham, UK	
Test substance:	*Lambda*-Cyhalothrin technical, Purity [redacted]	
Study dates	24 May 1988 to 28 February 1989	
GLP:	Yes	
Deficiencies:	None.	
Reliability indicator	1.	

	Official use only
Materials and methods: *Lambda*-Cyhalothrin technical purity [redacted] w/w (Analytical Reference [redacted], preparation reference [redacted]). The test methodology was based on that of ***Bringman and Kuhn (1980)*** and modified by ***Slabbert (1986)***. This procedure measures the degree of inhibition of growth of a pure culture of *Pseudomonas putida* during a 6 hour period when the cells are in the logarithmic growth phase, using optical techniques. Four experimental test groups, each containing 3 replicate flasks, were set up containing *lambda*-Cyhalothrin technical at nominally 1 mg/L in 0.1% v/v methanol (1), solvent control (0.1% v/v methanol) (2), reference toxic standard (18 mg/L 3,5-dichlorophenol) (3) and untreated control (4). In addition, 3 uninoculated flasks were set up with 1 mg *lambda*-Cyhalothrin/L in 0.1% v/v methanol (5), solvent only (6) and untreated (7) to run in parallel with the corresponding inoculated flasks. Treatments (5), (6) and (7) served as blank and chemical controls to compensate for any background colour or turbidity. After shaking at 150 rpm for 6 hours at 25°C on an incubated shaker, the optical density of each flask was measured at 600 nm. **Findings:** The mean optical densities and mean percent inhibition for the treatment groups are presented in the table below.	X1 X2 X3 X4

Effects of *lambda*-Cyhalothrin on the growth of *P. putida*

Treatment	Mean Optical Density at 600 nm	Corrected for Uninoculated Blank	% Inhibition
1 mg/L *lambda*-Cyhalothrin in 0.1% v/v methanol (1)	0.625	0.607	0
1 mg/L *lambda*-Cyhalothrin in 0.1% v/v methanol uninoculated (7)	0.018	-	-
Solvent control (2)	0.632	0.616	0
Solvent control uninoculated (6)	0.016	-	0
Untreated control (4)	0.574	0.549	-
Untreated control uninoculated (5)	0.025	-	-
Toxic reference standard (3)	0.032	-	94

The EC_{10} and EC_{50} values of *lambda*-Cyhalothrin technical with respect to growth of *Pseudomonas putida* were both >1 mg/L (nominal). The 18 mg/L solution of 3,5-dichlorophenol gave 94% inhibition, indicating that the *P. putida* culture was responding normally to the reference toxicant.

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	7.4.2/01	Bioconcentration
91/414 Annex Point addressed	II 8.2.3	Bioconcentration in fish

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [×] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [×] Other justification [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Not relevant	
Evaluation of applicant's justification	[redacted]	
Conclusion		
Remarks		

98/8 Doc IIIA section No.	**7.4.3/01 Effects on aquatic organisms, further studies**
91/414 Annex Point addressed	

		Official use only
Reference point (location) in dossier	7.4.3/01	
Title:	Evaluation of the impact of run-off and spray-drift on aquatic mesocosms, using USA experimental ponds.	
Project/Report number:	RJ0614B	
Author(s):	[redacted]	
Date of report:	1988	
Published:	Yes, a summary has been published in: Freshwater Field Tests for Hazard Assessment of Chemicals. CRC Press, Inc. pp. 345-360	X1
Testing facility:	[redacted]	
Test substance:	12% w/v emulsifiable concentrate (EC) formulation of *lambda*-Cyhalothrin (GFU 383-C)	
Study dates	1985-1986	
GLP:	Yes (certified laboratory)	
Deficiencies:	None	
Reliability indicator	1	

	Official use only
Report also refers to [redacted] (1992), *Lambda*-cyhalothrin: Addendum to RJ0614B. [redacted]; unpublished report No. RJ0614B Addendum; study dates October 1989 to June 1992. **Materials and method:** 12% w/v emulsifiable concentrate (EC) formulation of *lambda*-Cyhalothrin (GFU 383-C). Measured concentration of *lambda*-Cyhalothrin in the formulation was 13.77% (w/w, specific gravity 0.921 g/cm^3). The study was carried out in North Carolina in 1985-86. A total of 16 mesocosms were utilised, 4 replicates for each one of 3 test concentrations and 4 serving as controls. Each mesocosm was 15 x 30 m (0.04 ha), with a total water volume of 450 m^3. Water depth was from 15 cm at the shallow end to 2 m at the deepest point. Each mesocosm contained a layer of clay (15 cm deep), and this was covered with a layer of sandy loam soil (10 cm deep). The soil pH was 6.1, and its organic matter content 1.1%. Natural pond water containing aquatic organisms was added to the mesocosms. Macrophytes were planted in the mesocosms and additional macroinvertebrates were added. Twenty-five adult bluegill sunfish (*Lepomis macrochirus*), 11-16 cm long, were added to each mesocosm.	

Once a week for twelve weeks, commencing on the 10th or 11th June 1986, applications simulating spray drift were made to the mesocosms. Applications of a soil slurry containing *lambda*-Cyhalothrin (simulating run-off) were made on 6 occasions with two-week intervals commencing on the 13th or 14th June 1986, with the first one three days after first spray drift application. A spray-boom was used for application of spray and run-off slurry. The application rates were: high: 12 x 1.7 g a.s./ha (spray-drift) + 6 x 5 g a.s./ha (run-off slurry); mid: 12 x 0.17 g a.s./ha (spray-drift) + 6 x 0.5 g a.s./ha (run-off slurry); low: 12 x 0.017 g a.s./ha (spray-drift) + 6 x 0.05 g a.s./ha (run-off slurry) 0.017 g a.s./ha was equivalent to 0.0017 µg/L assuming mixing throughout the water column, 0.17 g a.s./ha was equivalent to 0.017 µg/L and 1.7 g a.s./ha was equivalent to 0.17 µg/L. The actual amounts of *lambda*-cyhalothrin applied as spray drift to the ponds were calculated from the weight of the test solution applied and the concentration of *lambda*-Cyhalothrin in the test solution, determined by gas-liquid chromatography (GC). The actual amounts of *lambda*-cyhalothrin applied as run-off slurry to the ponds were determined by analysis of sub-samples of the mixing tank preparations. These rates were considered to be acceptable for the study.	X2
Concentrations of *lambda*-Cyhalothrin in the mesocosms treated at the medium and high rates were determined in water samples taken at intervals after each application in the shallow and deep zones of each mesocosm. The sampling intervals are shown in Table 8.2-30. Samples were taken from 25 cm below the water surface in the shallow zone, and at 25 cm below the water surface and 25 cm above the sediment in the deep zone. The residues of *lambda*-Cyhalothrin in the water were analysed by GC. No samples were taken from the low rate ponds as residues of *lambda*-Cyhalothrin in these mesocosms were expected to be below the limit of determination (generally 0.001 ug/L for *lambda*-Cyhalothrin and its epimer pair A, individually). Sediment core samples were taken from two of the medium rate and two of the high rate mesocosms for analysis of *lambda*-Cyhalothrin residues at approximately two-week intervals throughout the study period. Samples were taken from the shallow and deep zones of each pond. The sediment cores were frozen and sectioned into the top 2.5 cm, the 2.5-5.0 cm layer and the 5.0-7.5 cm layer. The sections were extracted separately and analysed by GC. No samples were taken from the low rate ponds as residues of *lambda*-Cyhalothrin in these mesocosms were expected to be below the limit of determination (0.004 ug/kg). During the course of the study measurements were taken *in situ* in the microcosm water of temperature, dissolved oxygen, pH, turbidity, conductivity, alkalinity and nutrients (nitrogen, phosphorus, total carbon and total organic carbon). Regular biological assessments were also made of zooplankton (integrated depth), phytoplankton (integrated depth), macroinvertebrates (benthic and surface substrates, emergence traps and visual assessments), algal chlorophyll a (integrated depth), periphyton (artificial substrates), filamentous algae and macrophytes (percentage surface area). From the start of the applications (May 1986) until the end of the study (November 1986), sampling was conducted at 2-3 weeks interval, with some samples (visual observations, emergence traps sampling) made more often. **Findings:** **Water and Sediment Residue Data**	X3
Residue analysis of the treated pond water indicated that *lambda*-Cyhalothrin, comprised of the enantiomer pair with the stereochemical arrangement (*Z*)-(1*S*)-*cis R* α-cyano and (*Z*)-(1*R*)-*cis S* α-cyano, and referred to as pair B, had under gone a degree of epimerisation to form the isomer pair A. This finding was not surprising as *lambda*-Cyhalothrin is susceptible to isomerisation under alkaline conditions and the pH of the pond water during the study was above pH 7. Both *lambda*-cyhalothrin and its epimer pair A were quantified in the water	X4

samples and the ratio of B:A isomers was approximately 1:1 on all occasions. All other isomer forms of *lambda*-Cyhalothrin were below the limit of determination. The residues in the replicate high rate ponds were very similar at all times and there was no evidence of cross contamination in any of the ponds during the study. Only trace amounts of *lambda*-Cyhalothrin and the isomer pair A were found in the mid rate ponds at any sampling time and no residues of *lambda*-cyhalothrin were detected in samples taken throughout the study from the control ponds. The average water residues, determined for the samples taken at various depths in the water column and the total for *lambda*-Cyhalothrin and isomer pair A are summarised in the following two tables, for two of the four replicate high and mid rate ponds, respectively. Subsequent work was carried out to analyse water samples from the remaining two replicate high and mid rate ponds, and two of the low rate ponds and plus additional samples from the high and mid rate ponds previously sampled. This work is reported in [redacted] ***(1992)***. The residue values determined for the high and mid rate ponds were consistent with those values previously reported. Water residues in the low rate ponds were at or below the limit of determination at all sampling intervals. Both *lambda*-Cyhalothrin and its epimer pair A were detected in the sediment, generally in the ratio 2:1. Greater than 80% of the sediment residue recovered was found in the top 2.5 cm of the sediment layer. Residues in samples taken throughout the study from the control ponds were below the limit of determination.	

Average total *lambda*-Cyhalothrin plus isomer pair A residues in water samples from the high rate mesocosms following up to twelve spray drift applications and 6 runoff applications

Application	Date [1]	Sampling time [2]	Total Residue in Pond Water ug/L		Total Residue in Pond Sediment ug/kg			
			Pond 2B	Pond 3B	Pond 2B		Pond 3B	
					cm		cm	
Pre app			<LOD [3]	<LOD [3]	0-2.5	<LOD [4]	0-2.5	<LOD [4]
Drift 1	10/6/86	+ 1 d	<0.010	<0.010				
Runoff 1	13/6/86	+ 1 d	0.020	0.030				
		+ 3 d	0.003	0.004	0-2.5 2.5-5	5.0	0-2.5 2.5-5	5.5 <LOD [4]
Drift 2	17/6/86							
Drift 3	24/6/86							
Runoff 2	27/6/86	+ 3 d	0.005	0.004	0-2.5 2.5-5	15 <1.5	0-2.5 2.5-5	12 <0.5
Drift 4	1/7/86							
Drift 5	8/7/86							
Runoff 3	11/7/86	+ 3 d			0-2.5 2.5-5	9.8 <2.6	0-2.5 2.5-5	15 <1.0
Drift 6	15/7/86							
Drift 7	22/7/86							
Runoff 4	25/7/86	+ 1 d	0.090	0.098				
		+ 3 d	0.018	0.023	0-2.5 2.5-5	22 <LOD [4]	0-2.5 2.5-5	21 <LOD [4]
Drift 8	29/7/86	+ 1 d	0.027	0.033				
		+ 3 d	0.005	0.014				
Drift 9	5/8/86							
Runoff 5	8/8/86	+ 3 d	0.013	0.012	0-2.5 2.5-5	34 <0.5	0-2.5 2.5-5	33 0.4
Drift 10	12/8/86							
Drift 11	19/8/86							
Runoff 6	22/8/86	+ 3 d	0.018	0.034	0-2.5 2.5-5	40 0.7	0-2.5 2.5-5	27 <1.6
Drift 12	26/8/86	+ 13 d	0.007	0.004	0-2.5 2.5-5	26 <LOD [4]	0-2.5 2.5-5	18 <1.0
		+ 27 d	0.016	0.04	0-2.5 2.5-5	23 2.5	0-2.5 2.5-5	35 <1.1
		+ 41 d			0-2.5 2.5-5	31 4.2	0-2.5 2.5-5	20 4.0
		+ 62 d	0.002	<LOD [3]	0-2.5 2.5-5	21 2.8	0-2.5 2.5-5	27 <3.2

[1] Date of application to ponds 2B, ponds 3B was treated 1 day later on each occasion.
[2] Time relative to nearest application date
[3] Limit of determination for total *lambda*-Cyhalothrin and isomer pair A in the pond water was 0.002 ug/L
[4] Limit of determination for *lambda*-Cyhalothrin in the sediment was 0.4 ug/kg

Average total *lambda*-Cyhalothrin plus isomer pair A residues in water samples from the mid rate mesocosms following up to twelve spray drift applications and 6 runoff applications

Application	Date [1]	Sampling time [2]	Total Residue in Pond Water ug/L		Total Residue in Pond Sediment ug/kg			
			Pond 3A	Pond 7B	Pond 3A		Pond 7B	
					cm		cm	
Pre app			<LOD [3]	<LOD [3]	0-5	<LOD [4]	0-5	<LOD [4]
Drift 1	10/6/86							
Runoff 1	13/6/86	+ 1 d	0.002	<0.003				
		+ 3 d	<LOD [3]	<LOD [3]	0-5	<0.7	0-5	<LOD [4]
Drift 2	17/6/86	+ 3 d						
Drift 3	24/6/86							
Runoff 2	27/6/86	+ 3 d	<LOD [3, 5]	<LOD [3, 5]	0-5 5-10	0.9 <0.5	0-5 5-10	0.7 1.7
Drift 4	1/7/86							
Drift 5	8/7/86							
Runoff 3	11/7/86	+ 3 d	<LOD [3, 5]	<LOD [3, 5]	0-5 5-10	3.2 <LOD [4]	0-5 5-10	2.8 <LOD [4]
Drift 6	15/7/86							
Drift 7	22/7/86							
Runoff 4	25/7/86	+ 1 d	0.010	0.010				
		+ 3 d	<LOD [3]	0.002	0-5	1.4	0-5	0.8
Drift 8	29/7/86	+ 1 d	0.002	0.004				
Drift 9	5/8/86							
Runoff 5	8/8/86	+ 3 d	<LOD [3, 5]	0.003 [5]	0-5	1.0	0-5	0.9
Drift 10	12/8/86							
Drift 11	19/8/86							
Runoff 6	22/8/86	+ 3 d	0.004 [5]	0.002 [5]	0-5	1.1	0-5	1.8
Drift 12	26/8/86	+ 13 d	<LOD [3, 5]	<LOD [3, 5]	0-5	0.7	0-5	1.9
		+ 27 d			0-5	<0.8	0-5	1.3
		+ 41 d			0-2.5 2.5-5	2.8 <LOD [4]	0-2.5 2.5-5	3.6 <0.5
		+ 62 d			0-5	<0.6	0-5	0.9

[1] Date of application to ponds 2B, ponds 3B was treated 1 day later on each occasion.
[2] Time relative to nearest application date
[3] Limit of determination for total *lambda*-Cyhalothrin and isomer pair A in the pond water was 0.002 ug/L
[4] Limit of determination for *lambda*-Cyhalothrin in the sediment was 0.4 ug/kg
[5] Analysis reported in [redacted] *(1992)*.

Water Physicochemical Parameters	Official use only
There were no statistical differences in the water physiochemical parameters due to *lambda*-Cyhalothrin applications at any rate. Highest dissolved oxygen levels (16 mg/L) and pH (9.5) were recorded in May. Values for both parameters dropped during the summer to DO 4-5 mg/L and pH 7, before rising again in September. Water temperatures were mainly between 4°C and 10°C in all pond zones. The maximum recorded temperature in the water was 34°C in	

July. Water conductivity increased to a maximum of 240 to 270 uS/cm at the end of July and then declined. Alkalinity was highest in July (80-90 mg $CaCO_3$/L) and then declined to about 80 mg $CaCO_3$/L by the end of the study. Turbidity rose from about 1.5 NTU at the start of June to maximum levels of 9 to 11 NTU by the end of June. From then on, turbidity levels remained generally about 4 NTU. **Sediment Characteristics** There was no difference between treatments for any of the physico-chemical parameters measured in the pond sediment. Sediment organic matter content was approximately 1.3 %. The sediment pH was approximately 7.1 at the start of the study in June and 6.6 at the end in October. Counts of microorganisms within the sediment indicated that microbial populations were high and that the sediment was microbially active. There were no statistically significant differences in microbial parameters during the study, however in general numbers of aerobic bacteria, actinomycetes and fungi were found to be somewhat higher in samples taken in June, compared to those in samples taken in October/November. **Biological Measurements** A summary of the treatment related effects on the various biological parameters monitored are given below.	X4
Primary production by planktonic or periphytic algae was unaffected at all *lambda*-Cyhalothrin treatment rates. In samples after the first application, numbers of crustacean zooplankton were reduced in both the treated mesocosms and the controls. This was probably due to fish predation or the inhibiting effect of the run-off slurries on the filter feeders. The zooplankton population densities showed no overall effect with all of the *lambda*-Cyhalothrin treatments. Among the macroinvertebrate taxa, the groups most affected were those with species that move over the surface, swim within the water profile, or inhabit the water surface. Invertebrates living in the sediment were generally unaffected (see results table). As with the crustacean zooplankton, many of the macroinvertebrates were reduced in all ponds (including controls) in June due to fish predation. The numbers of fish collected at the end of the study (November 1986) ranged from 14,000 to 22,000 (7-14 kg wet weight) per mesocosm. There were no significant differences between the control and treated groups in either number or biomass of total, female or male adult fish. Extremely large numbers of young fish were collected in all mesocosms, and no treatment related differences were observed. However, 17-20% more fish were present in the treated mesocosms compared to the control. The biomass of young fish was also 28-38% less than in the control, and this difference was statistically significant. The findings were also reflected in measures of fish condition ***(Hill et al, 1994)***[1], as calculated from total weight and numbers in each size group. There were no differences between condition factors for adult fish or for young fish (≤ 10 cm) in any of the treatment groups or the control. However, the factor for the young fish was somewhat below the optimum value, indicating overcrowding in all mesocosms. Since there was no dose-related response in these effects, it is not considered that these differences were treatment-related. The high numbers of young fish could have been due to the large beds of macrophytes present in the treated mesocosms, providing extra refuge from adult predation. Another possible explanation is that competition from tadpole populations (which varied a lot but were highest in the treated mesocosms) may have contributed to the reduced biomass of the young fish.	X5 X6

[1] Hill *et al.* (1994). Effects of *lambda*-cyhalothrin on aquatic organisms in large scale mesocosms. In Freshwater Field Tests for Hazard Assessment of Chemicals. Hill IR, Heimbach F, Leeuwangh P and Matthiessen P (eds.) pp345-360.

Summary of effects of low, mid and high rate *lambda*-Cyhalothrin treatments in US mesocosm study

Parameter	Effect in comparison to control mesocosm [1]		
	low rate[2]	low rate[2]	low rate[2]
Physico-chemical parameters	-	-	-
Microbial populations (sediment)	-	-	-
Phytoplankton & Periphyton			
- cell numbers, volume, biomass	-	-	-
- taxonomic groups	-	-	-
- activity	-	-	-
Filamentous algae	-	-	-
Macrophytes (mainly *Ludwigia uraguaiensis*)	(+)	(+)	(+)
Zooplankton (148 taxa)			
Protozoa (60 taxa)	-	-	-
Rotifera (64 taxa)	-	-	-
Crustacea (24 taxa)	-	-	++(r)
Macroinvertebrates			
Turbellaria	-	-	-
Mollusca	-	-	-
Oligochaeta	-	-	-
Hydracarina (water mites)	-	+(-)	++(-)
Ephemeroptera - Baetidae (mayfly)	+(R)	++(r)	++(r)
Ephemeroptera - Caenidae (mayfly)	-	++(r)	++(r)
Odonata - Anisoptera (dragon-flies)	-	-	-
Odonata - Zygoptera (damsel-flies)	-	-	+(r)
Hemiptera - Belostomatidae	-	-	++(-)
Hemiptera - Gerridae	-	++(r)	++(nr)
Hemiptera - Notonectidae (back-swimmer)	-	-	++(-)
Hemiptera - Veliidae	-	++(-)	++(-)
Coleoptera - Hydrophilidae	-	-	+(-)
Coleoptera - Haliplidae	-	-	++(-)
Trichoptera - Leptoceridae	-	+(R)	++(nr)
Diptera - Ceratopogonidae	-	-	+(R)
Diptera - Chironominae (sedentary Chironomid)	-	-	-
Diptera - Tanypodinae (free-living Chironomid)	-	+(R)	++(r)
Fish (*Lepomis macrochirus*)			
- activity	-	-	-
- numbers	(+)	(+)	(+)
- weight	(+)	(+)	(+)

[1] NOVA; if F-test was significant, then pooled estimate of error was used to calculate two-sided Student"s t-test. The latter was used to compare mean response in control group with mean response in each treated group. Null hypothesis: no difference between treatment groups and control. Level of significance 5%.

[2] Low rate 12 x 0.017 g a.s./ha as spray-drift + 6 x 0.05 g a.s./ha as run-off slurry.

[3] Mid rate 12 x 0.17 g a.s./ha as spray-drift + 6 x 0.5 g a.s./ha as run-off slurry.

[4] High rate 12 x 1.7 g a.s./ha as spray-drift + 6 x 5 g a.s./ha as run-off slurry

-	no effect	(R)	full recovery by study end
+	minor effect	(r)	partial recovery
++	major effect	(nr)	no recovery
(+)	different from control, but no dose-response		
(-)	not possible to judge recovery (unlikely to have been caused by *lambda*-Cyhalothrin)		

Conclusions:	Official use only
There were no significant effects compared to the controls on the fish population as a whole, or on female or male adult fish with any of the treatment rates, including the high treatment with 12 spray applications equivalent to 1.7 g a.s./ha and 6 runoff applications equivalent 5.0 g a.s./ha. At the lowest treatment rate, with 12 spray applications equivalent to 0.017 g a.s./ha and 6 runoff applications equivalent 0.05 g a.s./ha, freshwater plant and animal life were not adversely affected.	X7
At the mid rate, there were some effects on aquatic invertebrates, however these were minor and transient. At the mid rate, represented by 12 spray applications equivalent to 0.17 g a.s./ha and 6 runoff applications equivalent 0.5 g a.s./ha, *lambda*-Cyhalothrin is unlikely to cause adverse effects on populations or productivity in aquatic ecosystems.	X8

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and Methods	[redacted]
Results and discussion	[redacted]

	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

98/8 Doc IIIA section No.	7.4.3/02	Effects on aquatic organisms, further studies
91/414 Annex Point addressed		

		Official use only
Reference point (location) in dossier	7.4.3/02	
Title:	*Lambda*-Cyhalothrin and cypermethrin: Evaluation and comparison of the impact of multiple drift applications on aquatic ecosystems (experimental ponds)	
Project/Report number:	RJ0571B	
Author(s):	Farmer D., Coulson J.M., Runnalls J.K., Hill S.E., McIndoe E.C. and Hill I.R.	
Date of report:	1993	
Published:	No	
Testing facility:	ICI Agrochemicals, Jealott's Hill Research Station, UK	
Test substance:	12% w/v emulsifiable concentrate (EC) formulation of *lambda*-Cyhalothrin (GFU 383-C)	
Study dates	January 1986 to October 1991	
GLP:	Yes (certified laboratory)	
Deficiencies:	None	
Reliability indicator	1	

	Official use only
Materials and method: 12% w/v emulsifiable concentrate (EC) formulation of *lambda*-Cyhalothrin (GFU 383-C). Measured concentration of *lambda*-Cyhalothrin in the formulation was 130 g/L. The study was conducted in outdoor experimental ponds constructed of reinforced concrete. Each pond was 5 m x 5 m and 1.25 m deep, and contained water to a depth of approximately 1 m over a minimum depth of 15 cm sediment. Each ponds contained flora and fauna representative of a natural aquatic ecosystem, except that no fish were included. *Lambda*-Cyhalothrin as the EC formulation was applied at two rates: 1.7 and 0.17 g a.s./ha. Four surface applications of *lambda*-Cyhalothrin were made to each treated pond with a spray-boom to simulate spray drift at 14-day intervals, from the 2nd June to the 5th August. Nominal concentrations in the water column, estimated from the volumes of water in each pond (approximately 25,000 L), and assuming instantaneous complete mixing (which from the residue data shown in Table 8.2-22 was unlikely) were 0.17 and 0.017 ug a.s./L at the low and high rates, respectively. Two replicate ponds were used for each treatment rate and two untreated ponds were used for control assessments. This application regime represents exaggerated exposure, as the proposed use pattern for *lambda*-Cyhalothrin is two or three applications, rather than the four applications used in this study. The actual amounts of *lambda*-Cyhalothrin applied to the ponds were calculated from the weight of the test solution applied and the concentration of *lambda*-Cyhalothrin in the test	Official use only X1

solution, determined by gas-liquid chromatography (GC). The measured application rates and percentage of the nominal amounts are shown in Table 8.2-25. These rates were considered to be acceptable for the study.	X2

Lambda-Cyhalothrin (GFU 383-C): Mean nominal and measured rates of _lambda_-Cyhalothrin applied to the study microcosms

Nominal Rate g a.s./ha	Measured Rate g a.s./ha	Rate as % Nominal
1st Application (24/06/86)		
1.7	1.53	90
0.17	0.13	76
2nd Application (08/07/86)		
1.7	2.22	130
0.17	0.20	118
3rd Application (22/07/86)		
1.7	1.48	87
0.17	0.16	97
4th Application (05/08/86)		
1.7	1.60	94
0.17	0.16	91

Concentrations of *lambda*-Cyhalothrin in the treated ponds were determined in water samples taken at intervals after each application, as indicated in the following table. To assess the distribution of *lambda*-Cyhalothrin with depth, samples were taken from 15 cm below the surface ("shallow") and 15 cm above the sediment ("deep"), and each water sample was comprised of three sub-samples taken from different positions in the pond and bulked. The residues of *lambda*-Cyhalothrin in the water were analysed by GC. Sediment core samples were taken for analysis of *lambda*-Cyhalothrin residues 8 days after each application and additionally at two-week intervals for 3 months after the 4th application. The sediment cores were frozen and sectioned into the top 2.5 cm, the 2.5-5.0 cm layer and the 5.0-7.5 cm layer. The sections were extracted separately and analysed by GC. During the course of the study measurements were taken *in situ* in the microcosm water of temperature, dissolved oxygen, pH, turbidity, conductivity, alkalinity and nutrients (nitrogen, phosphorus, total carbon and total organic carbon). Regular biological assessments were also made of zooplankton (integrated depth), phytoplankton (integrated depth), macroinvertebrates (benthic and surface substrates, emergence traps and visual assessments), algal chlorophyll a (integrated depth), periphyton (artificial substrates), filamentous algae and macrophytes (percentage surface area). Sampling continued for approximately 3 months after treatment (until November). One-way analysis of variance was used to assess any effects in the treatment groups, and where treatment effects were found to be significant ($P<0.05$ for the variance ratio of the treatment effects), treatment means were compared with the control using a two-sided t-test. **Findings:** In one of the two mesocosms treated at high rate, sticklebacks accidentally colonised during the study. As this affected both chemical/physical parameters as well as the biology of the mesocosms the results from it were omitted from the analysis. However, no effects were observed on the sticklebacks. ***Lambda*-Cyhalothrin Water Residue Data** Water residues in samples taken from shallow and deep locations in the treated ponds are shown in the table below:	Official use only X3 X4

Lambda-Cyhalothrin residues (ug/L) in water samples from treated ponds following up to four applications

Application Date	Sampling Time [1]	Nominal Rate 0.17 g a.s./ha		Nominal Rate 1.7 g a.s./ha	
		Shallow [2]	Deep [3]	Shallow [2]	Deep [3]
	- 8 d	<LOD [4]	<LOD	NA [5]	NA
24/06/86	+ 2 d	NA	NA	0.028	0.019
	+ 6 d	NA	NA	≤0.004	≤0.006
08/07/86	+ 2 d	NA	NA	0.013	0.009
	+ 6 d	NA	NA	<LOD	<LOD
22/07/86	+ 1 h	≤2.5	<LOD	0.094	0.023
	+ 1 d	≤LOD	≤LOD	0.0385 [6]	0.0385 [6]
	+ 3 d	<LOD	<LOD	<LOD	<LOD
	+ 6 d	<LOD	<LOD	<LOD	<LOD
05/08/86	+ 2 d	<LOD	<LOD	0.012	0.009
	+ 6 d	NA	NA	0.002	<LOD
	+ 20 d	NA	NA	<LOD	<LOD

(1) Time relative to nearest application date
(2) Samples taken 15 cm below the water surface
(3) Samples taken 15 cm above the sediment
(4) Limit of determination for *lambda*-Cyhalothrin in the pond water was 0.002 ug/L
(5) Not analysed. Samples following the application on 22 July were analysed first and based on this data, residues >LOD were not expected in these samples.
(6) Mean residue for shallow and deep samples combined

The water residue data indicate that *lambda*-Cyhalothrin, applied as a spray to the surface of the water, dissipated rapidly from the water column in the treated ponds. The concentration of *lambda*-Cyhalothrin in the pond water was below that limit of detection (LOD 0.002 ug/L) in the ponds treated at 0.17 g a.s./ha within one day, and in the ponds treated at 1.7 g a.s./ha water concentrations had fallen to the LOD following three of the four applications within 3 to 6 days. These data indicate that, even at high spray drift rates (up to 23% of the maximum proposed use rates), negligible amounts of *lambda*-Cyhalothrin will remain in the water from previous applications prior subsequent applications. Residue analysis of the treated pond water indicated that *lambda*-Cyhalothrin, comprised of the enantiomer pair with the stereochemical arrangement (*Z*)-(1*S*)-*cis R* α-cyano and (*Z*)-(1*R*)-*cis S* α-cyano, and referred to as pair B, epimerised in the pond water to form the isomer pair A. This finding was not surprising as *lambda*-Cyhalothrin is susceptible to isomerisation under alkaline conditions and the pH of the pond water during the daytime was between pH 8 and 10 during the water-sampling period. The ratio of isomer pairs B:A isomers approached 1:1 after approximately 48 hours. As the biological activity resides with the B isomer pairs, conversion of isomer pair B to the 1:1 mixture of A and B isomers, will effectively reduce the ecotoxic exposure concentration of *lambda*-Cyhalothrin. Stratification of exposure in the water column following these surface applications was evident with the higher treatment rate only. One hour after application at the higher rate, *lambda*-Cyhalothrin concentrations in the shallow water sample were approximately 4 x	Official use only X5

higher than those in the deeper water samples. Two days after the applications the concentrations in the shallow samples were about 1.3 x higher, and complete mixing appeared to take 3 to 6 days. ***Lambda*-Cyhalothrin Sediment Residue Data** Both *lambda*-Cyhalothrin and its epimer pair A were detected in the sediment, generally in the ratio 2:1. Sediment residues in samples taken from shallow and deep locations in the treated ponds are shown in the table below.	X6

***Lambda*-Cyhalothrin residues (ug/Kg) in pond sediment samples following up to four applications**

Application Date	Sampling Time [1]	Nominal Rate 0.17 g a.s./ha	Nominal Rate [3] 1.7 g a.s./ha		
		0-2.5 cm [2]	0-2.5 cm	2.5-5.0 cm	5.0-7.5 cm
24/06/86	+ 8 d	<LOD [4]	1.2	≤LOD	≤LOD
08/07/86	+ 8 d	≤0.5	4.2	0.8	-
22/07/86	+ 8 d	0.7	7.0	1.9	0.6
05/08/86	+ 8 d	≤LOD	7.3	3.9	1.2
	+ 22 d	≤LOD	5.0	5.3	0.6
	+ 36 d	≤LOD	4.4	2.5	0.5
	+ 50 d	≤0.5	4.2	5.6	1.8
	+ 64 d	≤0.5	5.0	5.1	2.3
	+ 78 d	≤LOD	2.3	4.1	0.7

[1] Time relative to nearest application date
[2] Residues below the limit of determination in 2.5-7.5 cm sediment layers. Only the samples from one of the replicate ponds were analysed as residues were generally <LOD
[3] Mean values for replicate ponds given
[4] Limit of determination for *lambda*-Cyhalothrin in the sediment was 0.4 ug/kg

Water Physicochemical Parameters All water chemistry measurements were within the range commonly reported in the literature for natural water bodies. There were few statistically significant differences in physicochemical parameters detected compared to control. A summary of the treatment related effects on the physicochemical parameters is given in the table below. None of these differences in water physiochemical parameters were considered to be ecologically relevant.	Official use only X7

Number of statistical differences in water physicochemical parameters between treatments and control (higher or lower than control) in the UK mesocosm study

Parameter	Application Rate 4 x 0.17 g a.s/ha		Application Rate 4 x 1.7 g a.s./ha	
	higher	lower	higher	lower
Temperature/pH/condition				
Alkalinity	1	1	1	1
Turbidity	3		2	1

Sediment Characteristics The sediment pH was neutral, with little change throughout the study and the organic matter content was approximately 7 to 10%. The sediment was classified as a sandy-clay-loam. Counts of microorganisms within the sediment indicated that microbial populations were high and that the sediment was microbially active. There were no statistically significant differences in microbial parameters during the study, except that fungal populations were found to increase in the ponds treated at the higher rate between the start and end of the study. **Biological Measurements** A summary of the treatment related effects on the various biological parameters monitored is given in the table below.	Official use only X8 X9

Number of statistical differences in biological measurements between treatments and control (higher or lower than control) in the UK mesocosm study

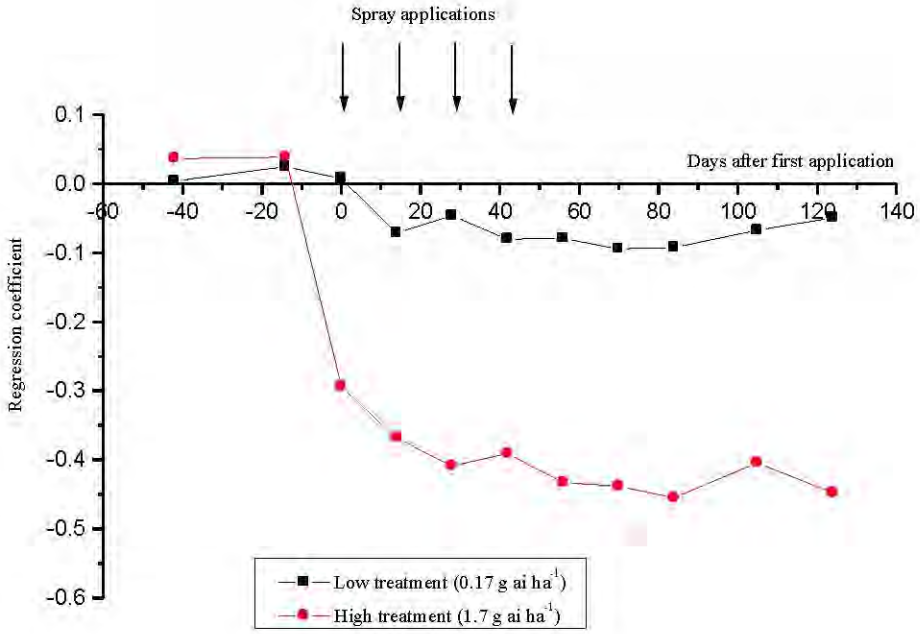
Parameter	Application Rate 4 x 0.17 g a.s./ha		Application Rate 4 x 1.7 g a.s./ha	
	higher	lower	higher	lower
Phytoplankton				
Gross photosynthesis				
Productivity	1		1	
Total numbers				
Total biomass			3	
Chlorophyll *a*				
Periphyton				
Total numbers			2	
Total biomass	1		2	
Chlorophyll *a*				
Filamentous algae				
Macrophyta				
Phytoplankton				
Gross photosynthesis				
Zooplankton				
Rotifera				
Copepod adults				
Copepod naupli				
Daphniidae				
Chydoridae	1		1	
Total numbers				
Substrates				
Tubellaria			1	
Gastropoda				
Oligochaeta				
Hirudinea				
Asellidae				23
Gammaridae		7		22
Baetidae	7			2
Coleoptera		1		1
Chironomidae	3	1	5	1
Emergence traps				
Baetidae	5			1
Chaoboridae		1		1
Chironomidae	2			
Quadrat visual observation				
Planorbidae		1		1
Lymnaeidae				
Notonectidae				

There were very few significant treatment effects on the phytoplankton, periphyton, filamentous algae or macrophytes populations at either treatment rate. At the higher application rate (1.7 g a.s./ha), there were initial transient effects on certain insect species, but recovery was rapid. There were also effects on amphipods and isopods from which little recovery had occurred by the end of the study. At the lower rate (0.17 g ai/ha), there were negligible effects on insects or isopods, but there were still effects on the Gammaridae. Although recovery of the Gammaridae had not occurred by the end of the study, the closed-off nature of the test system did not include the possibility for recovery by normal life-history processes (e.g. drift and recolonisation by swimming and scrambling). Under more natural ecological conditions, recovery at this treatment rate would have been expected through recolonisation. Further analysis of this study data has been made on the invertebrate community structure, (see figure below) using the recently developed Principle Response Curves (PRC) method **(Maund *et al,* 1998)**[2]. This method generates a statistic that is derived from abundances of all species. Comparison of the statistic from control and treatments allows the determination of effects at the community level. The PRCs for the low and high *lambda*-Cyhalothrin treatment rates are shown in Figure 8.2-1. PRC analysis showed only minimal differences in community structure at the lower treatment rate, but more substantial changes in community structure at the higher treatment rate. This suggests that the overall impact on the ecosystem at the lower treatment rate (4 x 0.17 g ai/ha) is small. The no observed adverse ecological effect concentration (NOAEC) from this study was therefore judged to be that resulting from a spray drift entry equivalent to 0.17 g ai/ha (equivalent to 0.017 µg a.s./L). This pond study was designed to simulate spray drift entry and the deposition rate on the surface water is expressed in g ai/ha. The treatment rates can be related to drift from agricultural applications using the percentage spray drift deposition rates published by the BBA in 2000. However, care is needed in correlating the effects observed in the pond study with exposure concentrations in the pond water. To consider nominal concentrations, based on the total volume of water in the study ponds, assumes that the test spray mixture distributed evenly and completely through the water column in a very short period of time. However, water residue data indicate that there was a degree of stratification in the concentration of *lambda*-Cyhalothrin over up to 3 to 6 days. Therefore, basing a NOAEC on nominal concentrations of *lambda*-Cyhalothrin in this study would lead to an underestimate.	Official use only X10 X11 X12

[2] Maund *et al.* (1998). Aquatic ecotoxicology of the pyrethroid insecticide *lambda*-cyhalothrin: considerations for higher-tier aquatic risk assessment. Pestic. Sci., 54, 408-417

Principal response curves for low and high treatments of *lambda*-Cyhalothrin in the UK mesocosm study.

The X-axis represents the control community structure, and deviations from the axis for the treatment plots indicates a difference in community structure.



Conclusions: The biological results, together with those from the physico-chemical data and *chlorophyll a* analyses, indicate that *lambda*-Cyhalothrin had no effect on overall phytoplankton community levels or algal productivity in the water at any of the treatment levels. At the lower treatment rate, there were negligible effects on insects or on isopods, whereas the most sensitive group to *lambda*-Cyhalothrin were the Gammaridae. From analysis of the effects on community structure, the *lambda*-cyhalothrin no observed adverse ecological effect concentration (NOAEC) for freshwater zooplankton and macroinvertebrates is considered to be 0.17 g ai/ha. Since in this study four applications of a worst-case spray drift event were simulated and the proposed use patterns of *lambda*-Cyhalothrin cover only two or three applications, this NOAEC is likely to be a conservative estimate.	Official use only X13

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and Methods	[redacted]

[redacted]

[redacted]

[redacted]

Results and discussion

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Conclusion

	[redacted]
	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

98/8 Doc IIIA section No.	**7.4.3/03**	**Effects on aquatic organisms, further studies**
91/414 Annex Point addressed		

		Official use only
Reference point (location) in dossier	7.4.3/03	
Title:	Ecological risk assessment of a package of pesticides (including *lambda*-Cyhalothrin) used in tulip fields (Teeltox). An interim report on responses of invertebrates in freshwater microcosms.	X1
Project/Report number:	TMJ4657B	X1
Author(s):	Brock, T. C. M.	X1
Date of report:	2001	X1
Published:	Van Wijngaarden, RPA, Cuppen, JGM, Arts, GHP, Crum, SJH, van den Hoorn, MW, van den Brink, PJ and Brock, TCM (2004). Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: A microcosm study. Published in Ecotoxicology & Chemistry, 23(6), 1479-1498	X2
Testing facility:	Alterra, Wageningen UR, P.O. Box 47, 6700 Wageningen, The Netherlands	
Test substance:	50 g/L emulsifiable concentrate (EC) formulation of *lambda*-Cyhalothrin (KARATE™)	X3
Study dates	July 1999 to July 2000	
GLP:	Yes	X4
Deficiencies:	None	X5
Reliability indicator	1	

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Materials and method: 50 g/L emulsifiable concentrate (EC) formulation of *lambda*-Cyhalothrin (KARATE™). The treatment regimes included formulated applications of the fungicide fluazinam as the 500 g/L suspension concentrate (SC) formulation Shirlan™, and the herbicides asulam as the 400 g/L SL formulation Asulox™ and metamitron as Goltix™ (70% a.s.). This study was performed at Alterra, Wageningen University and Research Centre, The Netherlands as part of this project undertaken to investigate the ecological impacts of the pesticides, including *lambda*-Cyhalothrin, used in normal agricultural practice in the tulip crop. The work was fully funded by the Dutch Ministry of Agriculture Twelve indoor microcosms simulating the aquatic community typical of Dutch drainage ditches were prepared. Each microcosm was 110 cm long, 110 cm wide and 70 cm high, with a sediment layer (sandy loam) of 10 cm, a water column of 50 cm, and a water volume of	

approximately 0.6 m^3. The microcosms were planted with 25 *Elodea nuttallii* shoots in each to stimulate the development of macrophyte-dominated ecosystems. Plankton and macro-invertebrates, collected from outdoor experimental ditches, were introduced to develop a representative freshwater community. The microcosms were situated in a climate room with a daily photoperiod of 14 h and a constant temperature of approximately 20 ± 1°C and acclimatised for 2 months, during which time the water was circulated through all 12 systems. Small doses of inorganic nitrogen (as NH_4NO_3) and phosphorus (as KH_2PO_4) were added to each microcosm each week to support plant growth. The experimental pesticide treatments were designed to simulate an application scenario based on tulip bulb production practices. Multiple applications of *lambda*-Cyhalothrin and the three other pesticides were applied to the microcosms at four application rates, equivalent to spray drift entry of 0.2%, 0.5%, 2% and 5% of the label recommended use rates and at the recommended frequencies for each pesticide. Three microcosms were designated as controls. A fourth microcosm, intended as a control, had to be discarded due to accidental application of pesticides. The target application rates and schedules are shown in the tables below.	

Application rates and target initial water concentrations for *lambda*-Cyhalothrin and the fungicide fluazinam and the herbicides, asulam and metamitron.

Application Rate (spray drift %)	Pesticide[1]	Rate (g a.s./ha)	Target Initial Water Concentration (ug/L)
1 (0.2%)	*Lambda*-Cyhalothrin (I)	0.05	0.01
	Fluazinam (F)	1.34	0.27
	Asulam (H)	2.68	0.54
	Metamitron (H)	2.34	0.47
2 (0.5%)	*Lambda*-Cyhalothrin (I)	0.125	0.025
	Fluazinam (F)	3.35	0.67
	Asulam (H)	6.70	1.34
	Metamitron (H)	5.85	1.17
3 (2%)	*Lambda*-Cyhalothrin (I)	0.50	0.10
	Fluazinam (F)	13.4	2.70
	Asulam (H)	26.8	5.40
	Metamitron (H)	23.4	4.70
4 (5%)	*Lambda*-Cyhalothrin (I)	1.25	0.25
	Fluazinam (F)	33.5	6.70
	Asulam (H)	67.0	13.4
	Metamitron (H)	58.5	11.7

[1] I insecticide, F fungicide, H herbicide

Number of applications, intervals and timing used for the treatments of *lambda*-Cyhalothrin, fluazinam, asulam and metamitron.

Pesticide	Number of Applications	Application Interval (days)	First Application
Lambda-Cyhalothrin	5	7	Day 0
Fluazinam	5	7, 7, 14, 14	Day -14
Asulam	3	7, 28	Day - 7
Metamitron	2	28	Day 0

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Concentrations of *lambda*-Cyhalothrin in the water column of the microcosms were measured at intervals during the study. The limit of determination for the analysis of *lambda*-	

Cyhalothrin residues in the microcosm water was set at 0.005 ug/L for the samples taken over the first 10 days from the first application, and then lowered to 0.002 ug/L for the remaining samples. Physico-chemical parameters in the microcosms (pH, electrical conductivity, temperature and alkalinity) were measured about two weeks before the first of the pesticide applications and at weekly intervals throughout the study.

Biological assessments included phytoplankton, zooplankton, macrophytes and macrofauna. The first of these assessments were made about two weeks before the first of the pesticide applications to establish baseline conditions. Subsequently, macro-invertebrates were sampled from each microcosm on -20, 8, 35, and 78 days after the first lambda-Cyhalothrin application by means of artificial substrates and litter bags. Macroinvertebrates present on the substrates were identified and counted alive, and then returned into the corresponding microcosm. Zooplankton were sampled weekly to two-weekly from each microcosm (in total 16 sampling dates). For the qualitative and quantitative analysis of the zooplankton community at least 6 depth-integrated water samples were randomly collected in each microcosm on each sampling day, until an 8-litre sample had been obtained. These samples were passed through a 40 um mesh net to collect the plankton, which were then preserved with formalin (approximately 4% in the final volume).

To assess treatment effects on the invertebrate communities, the datasets were assessed using multivariate statistics (Principal Response Curves, PRC). Univariate analysis was used to assess treatment related responses for individual populations of macroinvertebrates and statistically significant ($P<0.05$) deviation from the controls was calculated with the Williams test.

Findings:
Analysis of the microcosm water one hour after each application of *lambda*-Cyhalothrin indicates that the microcosms were dosed successfully at close to the nominal concentrations, see tables below. Concentrations of *lambda*-Cyhalothrin in the water column rapidly declined and were at, or close to, the limit of determination within seven days or less for all treatment rates.

Measured concentrations of *lambda*-Cyhalothrin in (ug/L) in the water column of the microcosms

Day	Application / Sampling	Nominal Treatment Rate					
		Control			0.010 ug/L		
		Cosm 2	Cosm 6	Cosm 8	Cosm 12	Cosm 5	Cosm 10
0	Pre App 1	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
0	App 1 + 1 h	<0.005	<0.005	<0.005	<0.005	0.008	0.007
3	App 1 + 3 d	<0.005		<0.005			
7	App 1 + 7 d	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
7	App 2 + 1 h	<0.005	<0.005	<0.005	<0.005	0.008	0.008
10	App 2 + 3 d	<0.005		<0.005			
14	App 2 + 7 d	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
14	App 3 + 1 h	<0.002	<0.002	<0.002	0.072[1]	0.007	0.008
17	App 3 + 3 d	<0.002		<0.002	-		
21	App 3 + 7 d	<0.002	<0.002	<0.002	-	<0.002	<0.002
21	App 4 + 1 h	<0.002	<0.002	<0.002	-	0.007	0.008
24	App 4 + 3 d	<0.002		<0.002	-		
28	App 4 + 7 d	<0.002	<0.002	<0.002	-	<0.002	<0.002
28	App 5 + 1 h	<0.002	<0.002	<0.002	-	0.009	0.008
31	App 5 + 3 d	<0.002		<0.002	-		

35	App 5 + 7 d				-		

(1) Control microcosm 12 dosed in error and discarded.

Measured concentrations of *lambda*-Cyhalothrin in (ug/L) in the water column of the microcosms (continued)

Day	Application / Sampling	Nominal Treatment Rate					
		0.025 ug/L		0.10 ug/L		0.25 ug/L	
		Cosm 3	Cosm 7	Cosm 4	Cosm 11	Cosm 9	Cosm 9
0	Pre App 1	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
0	App 1 + 1 h	0.016	0.017	0.071	0.091	0.190	0.178
3	App 1 + 3 d	<0.005	<0.005			0.140	0.008
7	App 1 + 7 d	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
7	App 2 + 1 h	0.018	0.018	0.062	0.068	0.233	0.197
10	App 2 + 3 d	<0.005	<0.005			<0.005	lost
14	App 2 + 7 d	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
14	App 3 + 1 h	0.022	0.014	0.061	0.079	0.231	0.164
17	App 3 + 3 d	<0.002	<0.002			0.015	0.014
21	App 3 + 7 d	<0.002	<0.002	<0.002	<0.002	0.003	0.002
21	App 4 + 1 h	0.016	0.020	0.063	0.063	0.201	0.174
24	App 4 + 3 d	<0.002	<0.002			0.026	0.012
28	App 4 + 7 d	<0.002	<0.002	<0.002	<0.002	0.004	<0.002
28	App 5 + 1 h	0.020	0.019	0.072	0.071	0.209	0.186
31	App 5 + 3 d	0.004	<0.002			0.029	0.016
35	App 5 + 7 d	<0.002	<0.002			0.004	0.002

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The study microcosms developed a diverse macro-invertebrate community. In total 77 taxa were sampled by means of the artificial substrates. A few populations apparently dominated the community in that they were sampled from the artificial substrates in relatively high numbers (generally > 10 individuals in controls). These dominant taxa were oligochaetes, turbellarians, snails, crustaceans (mainly *Gammarus pulex*, *Asellus aquaticus* and their juveniles), and mayflies (*Cloeon dipterum*). Midges of the family Chironomidae were relatively numerous at the start of the experiment. The midge, *Corynoneura scutellata* agg., occurred frequently an in high numbers at the end of the experiment. Additionally, at least 34 zooplankton taxa were present in the microcosms. The most dominant taxa comprised the rotifers *Keratella quadrata* and *Polyarthra remata*, copepod nauplii, ostracods and the daphnids *Alonella exigua* and *Chydorus sphaericus*. Of the four pesticides applied, *lambda*-Cyhalothrin appeared to have the largest initial impact on invertebrates. Results are therefore presented in this summary principally in relation to the *lambda*-Cyhalothrin treatment regime. However, from the data available, it is not possible to completely discount effects of the other compounds, particularly those which may have resulted in more subtle or indirect effects (e.g. small effects on primary productivity). RESPONSES OF MACROINVERTEBRATES Evaluation of the community level indicates that immediately after treatment there were effects on the macroinvertebrate community structure at concentrations of 0.025, 0.100 and 0.250 ug *lambda*-Cyhalothrin/L. However, by 35 days after the first *lambda*-Cyhalothrin	

application there was no longer any effect at the community level at 0.025 ug/L, indicating that recovery had occurred.

The results of the four sampling dates (days –20, 8, 35 and 78 after the first *lambda*-Cyhalothrin application) were analysed using PRC. Day –20 represents the pre-treatment period. In total 3 fluazinam applications, 2 *lambda*-Cyhalothrin, 2 asulam applications, and 1 metamitron application had occurred by day 8. After day 28 all applications had taken place (5x fluazinam; 5x *lambda*-Cyhalothrin, 3x asulam; 2x metamitron). PRC diagram for the macroinvertebrates sampled in the microcosms is shown in Figure 8.2-2. The variance allocated in the PRC diagram and the statistical analysis of the community response is as follows:

Sampling date:		29 %
Differences between replicates:	34 %	
Treatment regime:		37 % of which 40% is displayed in the diagram

The PRC diagram indicates that the two highest treatment levels in particular deviate from the control microcosms. The macroinvertebrate $NOEC_{community}$ values, based on nominal *lambda*-Cyhalothrin concentration (in ug/L) at the assessment times during the study from this statistical analysis are given in the table below.

Macroinvertebrate $NOEC_{community}$ values based on target *lambda*-Cyhalothrin concentration (in ug/L)

Date[1]	P-Value	$NOEC_{community}$ ug/L
-20	> 0.05	> 0.250
8	< 0.005	0.010
35	< 0.005	0.025
78	< 0.005	0.025

[1] Days after the first *lambda*-Cyhalothrin application

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The PRC diagram shows that the responses of the arthropods *Gammarus pulex*, *Asellus aquaticus*, *Corynoneura scutellata agg.* and *Cloeon dipterum* are negatively correlated with the treatment regime (higher positive weight, decrease in numbers). In contrast, the oligochaete worm *Stylaria lacustris*, cf. *Nais sp.* and Tubificidae, and the leech *Helobdella stagnalis* appear to be positively correlated with the treatment regime (increase in numbers).

The crustaceans *Asellus* and *Gammarus* have been observed to be the most sensitive organisms in other studies with *lambda*-Cyhalothrin. There were clear effects on these taxa after treatment at 0.100 and 0.250 ug/L. At 0.025 ug/L there were only occasional, partial effects on *Gammarus* and significant reductions in *Asellus* populations. However, after five application of *lambda*-cyhalothrin at 0.025 ug/L, neither taxon was eliminated, and under more realistic ecological and exposure conditions in the field, recovery processes would have been faster than in these enclosed test systems. After five applications at 0.010 ug/L, there were no significant effects on either group.

Univariate analysis of the treatment related responses for individual populations of macro-invertebrates are consistent with the community effects seen in the PRC diagram. For 30 species, statistically significant ($P<0.05$) deviations from the controls were found on various sampling days. The *lambda*-Cyhalothrin NOEC values for the more abundant of these taxa

are summarised in the table below.	

Summary of the *lambda*-Cyhalothrin NOEC values for macroinvertebrates at the population level from univariate analysis at various times post-treatment

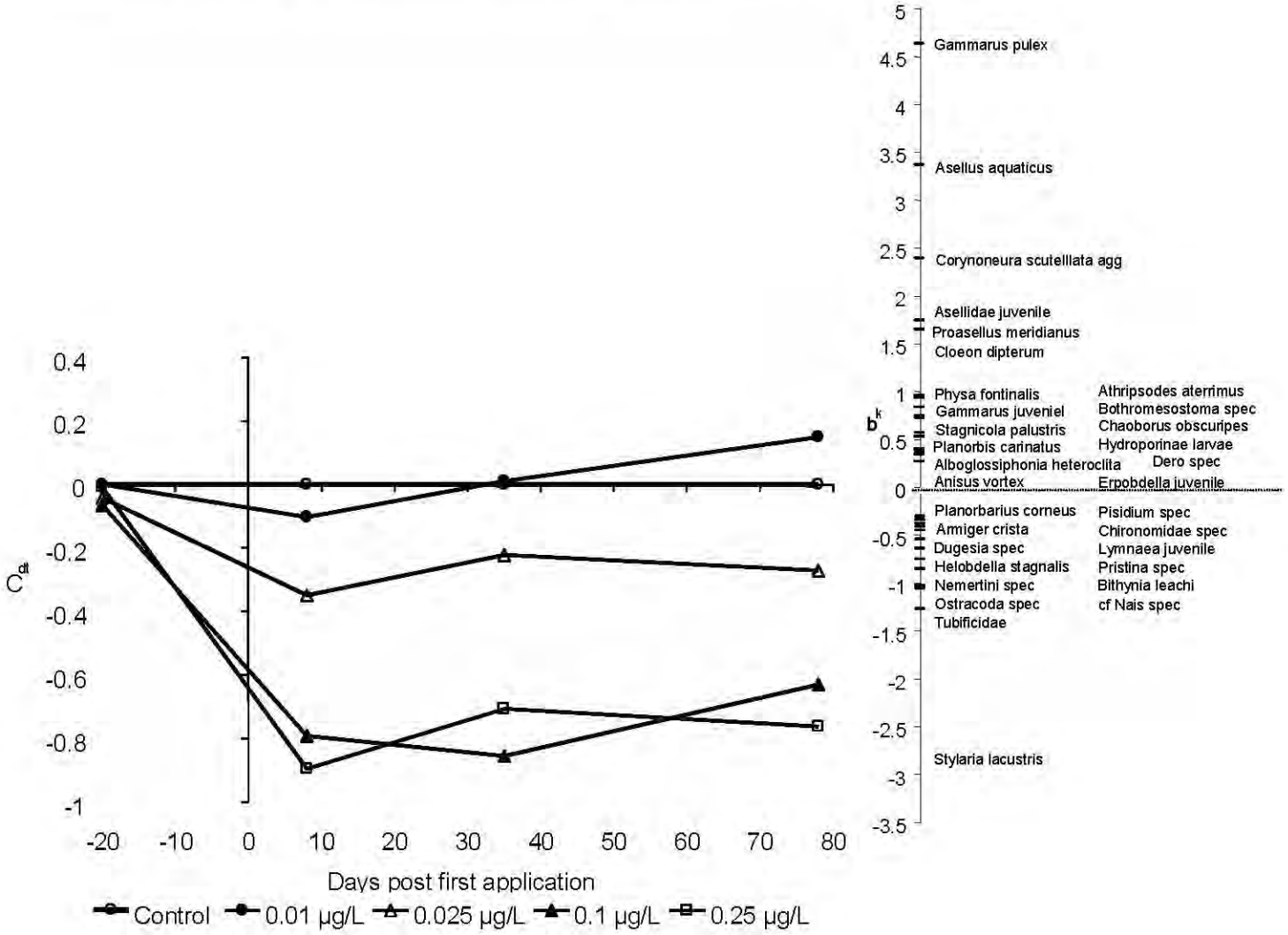
Taxon	NOEC (ug/L) at times after 1st *lambda*-Cyhalothrin application		
	Day 8	Day 35	Day 78
Crustacea			
Asellus aquaticus	0.010	0.010	0.010
Gammarus pulex	0.025	0.025	0.010
Proasellus meridianus(1,2)	0.010	<0.010	
Insecta			
Athripsodes aterrimus(2)		<0.010	
Chaoborus obscuripes	<0.010		
Chironomidae sp. (1,2)	(0.010)	(0.010)	
Cloeon dipterum	0.100	0.025	
Coryoneura scuttellata agg(1)	<0.010	0.025	0.025
Oligochaeta			
Dero sp.			0.025
Cf *Nais* sp.		(0.025)	
Stylaria lacustris	(0.025)		
Tubificidae(2)			(0.010)
Tricladia			
Polycelis tenuis		0.100	
Hirudinea			
Erpobdella octoculata(2)			0.010
Helobdella stagnalis(1,2)		(0.010)	(0.010)
Gastropoda			
Bithynia tentaculata		<0.010	
Gyraulus albus		(0.010)	

(1) consistent response
(2) low abundance

NOEC values in parentheses are significant increases
Empty cells indicate that species-date combinations were not statistically significant

PRC diagram showing the effects of the pesticide treatments on the macroinvertebrate community in the microcosms

Sampling on days –20, 8, 35 and 78 after the first *lambda*-Cyhalothrin application. 3 fluazinam applications, 2 *lambda*-Cyhalothrin and asulam applications, and 1 metamitron application had occurred on day 8. By day 35 all applications had taken place (5x fluazinam; 5x *lambda*-Cyhalothrin, 3x asulam; 2x metamitron).



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Responses of Zooplankton Analysis of the zooplankton data presented in this interim report show that at least 34 zooplankton taxa were present in the microcosms. The most dominant, comprising 20 of these taxa, were the rotifers. In abundance, rotifers were followed by Cladocera and Copepoda. The PRC diagram for the zooplankton sampled in the microcosms is shown below. The responses of Copepoda (*nauplii, Cyclopoida*) particularly show positive weights with the treatment regime (decrease in numbers). Representatives of Rotatoria show both positive weight (e.g. *Lecane* group *luna* and *Lepadella patella*) and negative weight (e.g. *Keratella quadrata*) with the treatment regime. A similar phenomenon could be observed for Cladocera of which *Chydorus sphaericus* and *Simocephalus vetulus* show positive weights with the treatment regime, and *Daphnia* group *galeata* an overall negative weight.	

Multivariate analysis (PRC) of the zooplankton data indicates that the highest treatment level in particular deviates from the control microcosms. The lowest treatment regime (including 0.010 ug/L *lambda*-Cyhalothrin) had little effect on the zooplankton community and the treatment including 0.025 ug/L *lambda*-Cyhalothrin resulted in only transient, short term effects. More pronounced treatment related direct effects and/or indirect effects (e.g. the increase in some rotifer taxa) were observed at the two highest treatment levels (including 0.100 and 0.250 ug/L *lambda*-Cyhalothrin).

The variance allocated in the zooplankton PRC diagram and the statistical analysis of the community response is as follows:

Sampling date: 44 %
Differences between replicates: 29 %
Treatment regime: 27 % of which 22 % is displayed in the diagram

From the statistical analysis, the $NOEC_{community}$ values based on nominal *lambda*-Cyhalothrin concentration (in ug/L) at the assessment times during the study were:

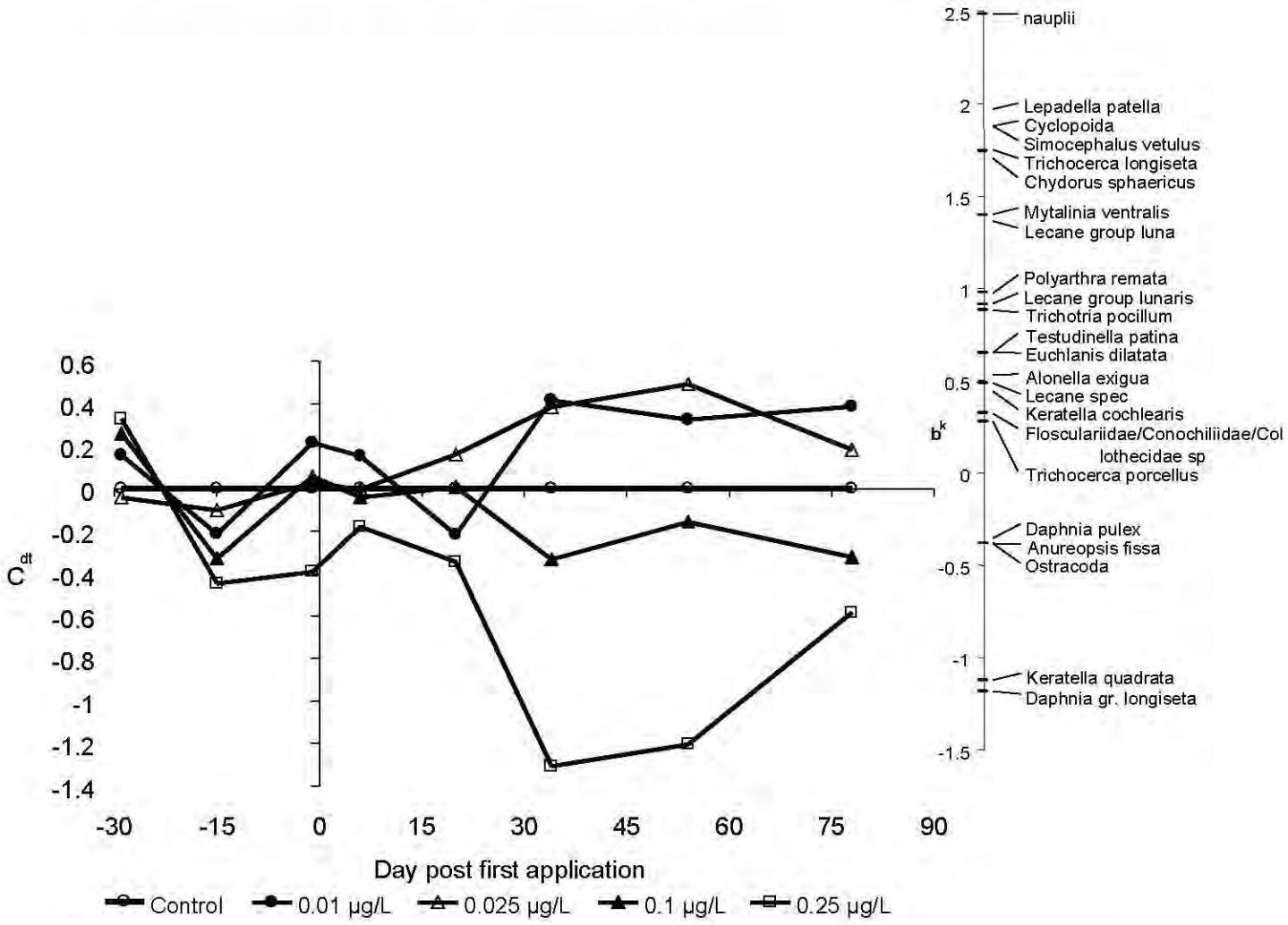
Zooplankton $NOEC_{community}$ values based on target initial *lambda*-Cyhalothrin concentration (in ug/L)

Date[1]	P-Value	$NOEC_{community}$ ug/L
-29	> 0.05	> 0.25
-15	>0.05	>0.25
-1	> 0.05	> 0.25
6	< 0.005	0.010
20	<0.005	0.100
34	< 0.005	0.100
54	0.020	0.100
78	0.015	0.025

[1] Days after the first *lambda*-Cyhalothrin application

PRC diagram showing the effects of the pesticide treatments on the zooplankton community in the microcosms

Sampling on days –29, -15, -1, 6, 20, 34, 54 and 78 after the first *lambda*-cyhalothrin application. 3 fluazinam applications, 2 *lambda*-cyhalothrin and asulam applications, and 1 metamitron application had occurred on day 8. By day 35 all applications had taken place (5x fluazinam; 5x *lambda*-cyhalothrin, 3x asulam; 2x metamitron).



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Statistical testing on the basis of the total zooplankton data set suggests that applications of fluazinam and asulam did not result in adverse effects, since on day –1 relative to the first *lambda*-Cyhalothrin application, fluazinam had already been applied twice and asulam once. On day 6, a $NOEC_{community}$ for the treatment regime with 0.010 ug/L *lambda*-Cyhalothrin was calculated. However, the zooplankton community showed a high capacity to recover at all treatment levels except the highest, since on days 34 and 54 a $NOEC_{community}$ was calculated for the treatment regime including 0.100 ug/L *lambda*-Cyhalothrin (plus other pesticides). The results of univariate analysis of the zooplankton data are consistent with those of the multivariate PRC analysis. For 17 species, statistically significant ($P<0.05$) deviations from the controls were found on various sampling days. The *lambda*-Cyhalothrin NOEC values for the more abundant of these taxa are summarised in the table below.	