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

Taxon	NOEC (ug/L) at times after 1st *lambda*-Cyhalothrin application					
	Day –1	Day 6	Day 20	Day 34	Day 54	Day 78
Cladocera						
Alona rectangular			(0.010)			
Chydorus spaericus		0.100	0.100	0.100	0.100	
Daphnia gr galeata		0.025			(0.100)	(0.025)
Daphnia pulex(1)						(0.100)
Graptoleberis testidinaria						0.010
Macrothrix laticornis				0.100		
Simocephalus vetulus		0.025			0.025	
Copepoda						
Cyclopoidea		0.025	0.100	0.100		
Nauplii		0.100	0.100	0.100	0.100	
Rotifera						
Keratella cochlearis(1,2)	<0.010					
Keratella quadrata				(0.100)	(0.100)	
Lecane gr lunaris				(<0.010)		
Lecane gr luna			0.100			
Lecane quadridentata		(0.100)	(0.100)	(0.025)		
Lepadella patella			(0.010)			
Polyarthra remata			(0.025)		0.100	
Trichocerca longiseta		0.100		0.025		

(1) low abundance
(2) irregular abundance
NOEC values in parentheses are significant increases
Gaps indicate that species-date combinations were not statistically significant

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Overall, the copepods (nauplii and Cyclopoida) and the Cladoceran (*Chydorus sphaericus*) showed the most consistent treatment related population reductions. Although several rotifer populations occasionally showed a treatment related decrease, significant increases in population densities were more common in this group (indirect effects). The fact that the PRC analysis showed a $NOEC_{community}$ of 0.010 µg/L on day 6 is in line with the observation that on day 6 one NOEC of 0.010 ug/L and several NOECs of 0.025 ug/L were observed for individual populations. Overall, the treatment related effects on zooplankton were considered to be negligible at the lowest treatment regime (0.010 ug *lambda*-Cyhalothrin/L). For most zooplankton taxa that showed significant population responses, a NOEC of 0.100 ug/L was observed. This is in line with the PRC analysis and the observation that several sensitive zooplankton populations (e.g. daphnids) showed a fast recovery, generally within a week of the final treatment, at most treatment levels except the highest one (0.250 ug/L). **Responses of phytoplankton and periphyton** A total of 37 phytoplankton taxa were identified. A clear treatment-response relationship could not be demonstrated. There was a trend of an increase at the community level, however this was not significant. Only a few isolated incidences of transient increases in abundance at the population level reached significance. Phytoplankton chlorophyll-a was significantly	

higher at highest two treatment levels (0.100 ug/L and 0.250 ug/L) compared to controls after five *lambda*-Cyhalothrin applications. Periphyton, measured by chlorphyll-*a* did not show any significant response during the experiment. Mean periphyton chlorphyll-*a* did not show any significant response with treatment rate. Mean levels were 0.11 ± 1 ug/cm^3. **Conclusions:** In the opinion of the study authors, effects after 5 weekly treatments of *lambda*-Cyhalothrin (plus other pesticides) to isolated test aquatic systems at 0.010 ug/L were negligible. At 0.025 ug/L, after repeated applications, effects were not pronounced and only transient.	

	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	

98/8 Doc IIIA section No.	7.4.3/04	Effects on aquatic organisms, further studies
91/414 Annex Point addressed		

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Reference point (location) in dossier	7.4.3/04	
Title:	Effects of *lambda*-Cyhalothrin in two ditch microcosm systems of different trophic status.	X1
Project/Report number:	TMJ 4971B	
Author(s):	Roessink I, Arts G H P, Belgers D, Brasen F, Maund S J and Brock T C	
Date of report:	2004	
Published:	See below	
Testing facility:	Alterra, Wageningen and Wageningen University and Research Centre, the Netherlands	
Test substance:	100 g/L capsule suspension (CS) formulation of *lambda*-Cyhalothrin (KARATE™)	
Study dates	Not stated	
GLP:	No	
Deficiencies:	None	
Reliability indicator	1	

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This work was performed at Alterra, Wageningen and Wageningen University and Research Centre, the Netherlands as part of a project undertaken to investigate the ecological impacts of realistic, worst case pesticide contamination of aquatic environments with differing eutrophication levels. The work was largely funded by the Dutch Ministry of Agriculture, with some additional funding from Syngenta to cover the costs of additional fate investigations. This work is being reported in three separate papers for publication as follows:	
Roessink I, Arts G H P, Belgers D, Maund S, Bransen F and Brock T C M. Effects and fate of *lambda*-Cyhalothrin on the aquatic communities of two ditch microcosm systems of different trophic status. Alterra/Syngenta joint accepted by Ecotoxicology and Chemistry, August 2003.	X2
Schroer A F W, Belgers D, Brock T C M, Matser A M, Maund S J and Van den Brink P J. (2004b). Comparison of laboratory single species and field population-level effects of the pyrethroid insecticide *lambda*-Cyhalothrin on freshwater invertebrates. Alterra/Syngenta joint paper in Arch. Environ Contam Toxicol. 46, 324-335.	X3
Van Wijngaarden R P A, Brock T C M, Belgers D, Shroer A F W and Maund S J. Ecologic effects of spring and late summer applications of *lambda*-Cyhalothrin on freshwater mesocosms. Alterra/Syngenta joint paper in Arch. Environ Contam Toxicol. 49, 1-22.	X3
Materials and method: The field ditch enclosure and *in situ* bioassay experiments were performed with the „Karate" with Zeon Technology formulation, with 100 g *lambda*-Cyhalothrin per L as a capsule suspension (CS). The laboratory acute toxicity tests were performed with „Karate" as an	

emulsion concentrate, with 50 g *lambda*-Cyhalothrin/L. **Field Experiments** The studies were conducted in experimental ditches at the Sinderhoeve Experimental Station, Renkum, The Netherlands. The ditches had been subjected to different regimes of macrophyte growth and nutrient supply (eutrophication) over several years to produce distinctive, stable ecosystems. Three of the experimental ditches were used in the work described here: Ditch 2: Non-eutrophicated, macrophyte dominated. Spring applications. Ditch 19: Eutrophicated, plankton dominated. Spring applications. Ditch 8: Non-eutrophicated, macrophyte dominated. Late summer applications. To obtain replicate systems within each ditch, fourteen polycarbonate enclosures (cylinders: diameter 1.05 m, height 0.9 m, water volume 0.43 m^3) were placed in each ditch. In each ditch, twelve enclosures were used for effects assessment and two for fate determinations. <u>*Lambda*-Cyhalothrin treatments</u> Two replicate enclosures in each ditch were dosed with *lambda*-Cyhalothrin, as an aqueous solution of „Karate" with Zeon Technology, at nominal concentrations of active substance of 10, 25, 50, 100, and 250 ng/L. These concentrations correspond to 0.2%, 0.5%, 1%, 2% and 5% spray drift emission at the label recommended rate for tulips (15 g ai/ha) and evenly distributed to a water depth of 30 cm (standard EU ditch scenario). The application was poured into the water column in each enclosure in a total water volume of 4 L, and the surface water gently mixed to aid distribution of the dose. Additionally, two enclosures in each ditch were used as controls and dosed with 4 L of water in the same manner. Each enclosure was dosed three times at one-week intervals. The *lambda*-Cyhalothrin applications were made to the enclosures in Ditches 2 and 19 on 16th, 23rd and 30th May 2000 and to the enclosures in Ditch 8 on 15th, 22nd and 29th August 2000. Concentrations of *lambda*-Cyhalothrin in the water column of the enclosures were measured in all the test enclosures one hour after application. Additional samples of water sediment and macrophytes were taken at intervals for analysis of *lambda*-Cyhalothrin from the fate enclosures, which were treated on the same days as the effects enclosures. <u>Physico-chemical Assessments</u> Physico-chemical parameters in the microcosms (pH, electrical conductivity, temperature and alkalinity) were measured about a week before the first of the pesticide applications and at weekly intervals throughout the study. <u>Biological assessments in the enclosures</u> Biological assessments included phytoplankton, periphyton, zooplankton, macrophytes and macrofauna. Initial assessments were made about two weeks before the first of the pesticide applications to establish baseline conditions. Subsequently, macro-invertebrates were sampled from each enclosure once during each treatment period and twice during the recovery period by means of artificial substrates and litter bags. Macroinvertebrates present on the substrates were identified and counted alive, and then returned into the corresponding enclosure. At the end of the experiment and after identifying and counting the macroinvertebrates, the animals were preserved and their identification checked. Phytoplankton and zooplankton were sampled weekly. For the qualitative and quantitative analysis of the zooplankton community at least 6 depth-integrated water samples were randomly collected in each enclosure on each sampling day. One week before the first application in each ditch and the day after the last application, the	X4 X5

biomass of aquatic macrophytes was determined by harvesting the macrophyte material in two representative plots (0.25 x 0.25 m) outside the enclosures. After all effects measurements were completed, the total macrophyte material was also harvested from each experimental enclosure. The macrophytes were cut close to the sediment, any roots were removed, and the material dried at 35°C before determining the dry weight.

In situ bioassays

In situ bioassays were used to assess the acute effects of *lambda*-Cyhalothrin of selected macroinvertebrate species and the recovery potential of these species in ditches with two distinctly different trophic states. Bioassays were performed with *Asellus aquaticus, Daphnia pulex* and *Chaoborus obscuripes* during the applications in the spring and with *Asellus aquaticus, Chaoborus obscuripes* and *Proasselus coaxalis* during the applications made during the late summer. The test animals originated from those gathered from the ditches one month before the start of the experiment and maintained in aquaria in the laboratory. One week before exposure, the organisms to be tested were acclimatised in the ditches in the bioassay cages. For the *Asellus* and *Chaoborus* bioassays, cages constructed of stainless steel gauze 0.5 mm, length 33 cm, diameter 6 cm, volume 930 cm^3 were used. For the *Asellus* bioassays, twenty-five adult, sexually mature individuals (average size 6.0 mm) were used. For the *Chaorborus* bioassays, thirty 4th instars were used. *Populus* sp. leaves were included in the cages with the *Asellus* and *Proasellus* to provide shelter and food. For the *Daphnia* bioassay a glass container (water volume 100 mL), sealed with a 55 um gauze was used with twenty adult females (7 days old) in each bioassay.

Two types of in situ bioassays were performed in the enclosures treated in the spring:

1) Acute – to investigate the effects occurring immediately after the first application of *lambda*-Cyhalothrin. Single cages containing *Chaoborus obscuripes* and *Asellus aquaticus* were introduced to the enclosures on the day before the application, while cages containing *Daphnia pulex* were introduced on the day of application. These bioassays lasted for 6 days and affected and unaffected individuals were counted at 1, 2, 3 and 6 (except for *Daphnia*, which was not counted on day 3).
Acute bioassays were also performed following the *lambda*-Cyhalothrin treatments in late summer and on these occasions three replicate cages were placed in each enclosure.

2) Recovery – to assess recovery potential by investigating effects on animals exposed in the enclosures at intervals after the last application of *lambda*-Cyhalothrin. In these bioassays fresh bioassay cages containing *Chaorborus* and *Asellus* were introduced into the enclosures on three occasions: day –1, day 4 and day 8 after the last application in the spring. Cages incubated on day –1 and day 4 remained *in situ* for four days, while those incubated at day 8 were retrieved after 6 days.

To ensure that the water in the bioassay cages *in situ* matched that of the bulk water column in the enclosures as far as possible, the cages were moved up and down through the water column once a day. The endpoint for all the bioassays was immobility and mortality for calculating median effective (EC_{50}) and lethal (LC_{50}) values.

Leaf-litter decomposition

Litter bags were used to investigate the decomposition of oarticulate organic matter (POM). The POM used consisted of *Populus x canadensis* leaves, which had been leached three times for 2 days to remove soluble humic compounds. The leached leaves were dried for 72 hours at 60 °C and stored before use. The litter bags were comprised of a glass Petri dish (diameter 11.6 cm) closed with a cover of stainless steel wire (mesh size 0.7 x 0.7 mm), in which 2 holes (0.5 cm) were punched to allow most invertebrates to enter. In each litter bag were place 2 g dry weight of *Populus* leaves. Two litter bags were placed in each enclosure at the sediment surface for a period of 2 weeks, after which time the bags were removed and replaced with

fresh litter bags. On retrieval, the litter bags were gently washed in the overlying water of the microcosm to remove adhering sediment particles. Each litter bag was emptied into a white tray to separate the POM and the macroinvertebrates. The organic plant material was dried at 105 °C for 24 hours. The decomposition over each 2-week period was expressed as % remaining organic material.	
Single Species Laboratory Acute Toxicity Tests Single species toxicity tests were performed to enable comparisons to be made between effects in the laboratory tests, the *in situ* bioassays in the field enclosures and on free-living organisms in the enclosures. Sixteen different invertebrate species were tested. *Daphnia galeata*, *Simocephalus vetulus* and *Proasellus coxalis* were obtained from laboratory cultures that originated from individuals collected in shallow freshwater ecosystems in the vicinity of Wageningen, the Netherlands. The other species were obtained directly from the field. The species, life stages and sizes used in the tests are given in Table 8.2-40. All species were acclimated under laboratory conditions for at least 2 days. The tests were performed in a variety of vessels and the test unit volumes used for each species is shown in Table 8.2-40.	X6
Ten individuals were generally used per test vessel. In the case of the *Daphnia galeata* and *Simocephalus vetulus* tests where 25 individuals were used per test unit. With *Notonecta glauca*, *Erytrhromma viridulum* and *Sialis lutaria* only 9 individuals were tested per test unit and to reduce stress, these animals were tested under (semi-)individual conditions by using partitioned aquaria.	X7

Taxonomic group and stage of the species used in the laboratory acute toxicity tests and the test system volumes

(SUB)CLASS, order, *genus* and *species*	Stage and size (mean±sd, n≥10)	Test Unit Volume (L)
(MACRO-)CRUSTACEA		
Isopoda		
Asellus aquaticus Linnaeus	(sub)adult 8.8 ±0.8 mm	1.8
Proasellus coxalis Dollfus	(sub)adult 4.6 ±0.5 mm	1.8
Amphipoda		
Gammarus pulex Linnaeus	(sub)adult 11.6 ±1.4 mm	1.8
(MICRO-)CRUSTACEA		
Cladocera		
Daphnia galeata Richard.	(sub)adult 0.7±0.08 mm	0.6
Simocephalus vetulus Müller	(sub)adult 1.7±0.3 mm	0.6
INSECTA		
Ephemeroptera (Mayflies)		
Cloeon dipterum Linnaeus	larvae 4.1±0.9 mm	1.8
Caenis horaria Linnaeus	larvae 4.6±0.7 mm	1.8
Hemiptera (True bugs)		
Sigara striata Linnaeus	adult 7.4±0.8 mm	1.8
Notonecta glauca Linnaeus	adult 14.4±1.7 mm	3.0[(1)]
Diptera (True flies)		
Chaoborus obscuripes Van der Wulp	Larvae[(2)] 1.9 ±0.1mm[(3)]	1.8
***Macropelopia sp.* Thienemanns**	larvae 7.6 ±1.7 mm	0.65
Zygoptera (Dragonflies)		
Erythromma viridulum Charp	larvae 17.3±2.0 mm	3.0[(1)]
Megaloptera (Alderflies)		
Sialis lutaria Linnaeus	larvae 17.8±4.2 mm	3.0[(1)]
MOLLUSCA (Molluscs)		
Gastropoda (Snails)		
Lymnaea stagnalis Linnaeus	(sub)adult 24.5±2.6 mm	1.8
Bithynia tentaculata Linnaeus	(sub)adult 9.7±0.8 mm	1.8
TURBELLARIA (Flatworms)		
Polycelis nigra/tenuis	adult (na)	1.8

[(1)] Aquaria with compartments
[(2)] Instar 3-4
[(3)] Head length

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The laboratory tests were performed with *lambda*-Cyhalothrin formulated as an emulsion concentrate (50 g *lambda*-Cyhalothrin/L). A stock solution of the test material was prepared in distilled water and the test media were prepared by diluting the stock solution with filtered (55 um) water collected from a pond at the experimental ditches site at Renkum, the Netherlands. This pond water contained 0.4-2.0 mg/L NO_3^-, 0.4-0.5 mg/L PO_4-P, 0.08-0.3 mg/L NH_4^-, 4.9-7.8 mg/L chloride and had an alkalinity of 0.60-0.88 meq/L. The tests were performed with 6 *lambda*-Cyhalothrin concentration levels, except for those non-arthropod species that were known to be particularly insensitive (*Lymnaea stagnalis*, *Bithynia tentaculata* and *Polycelis nigra/tenuis*) for which 4 concentration levels were used. Selected concentration intervals in the test ranges differed by a factor of 3. Control vessels were set up for each species and were treated with blank media only. All the single species tests were set-	

up as static tests, in duplicate, in a temperature-controlled room (20°C) with a light/dark regime of 14 h light, 10 h dark. No aeration of test media took place during the tests.

Determination of the initial *lambda*-Cyhalothrin test concentrations was based on measured concentrations in subsamples of the stock solutions. *Lambda*-Cyhalothrin concentrations in the test vessels were determined in subsamples taken at 1, 4, 24, 48 and 96 hours after dosing.

Immobility (sublethal) and mortality (lethal) effects were monitored at 24, 48 and 96 hours after application, except for the zooplankton species for which the test duration only 48 hours. In general, tests were not considered valid when sublethal effects or mortality in the controls were higher than 20 % for the specific test period (48 or 96 hour).

Data Analyses

Data from the field enclosures

EC_{10} and EC_{50} values for field populations were calculated from the data from artificial substrate samples collected 9 + 1 days after the first application of *lambda*-Cyhalothrin for the macroinvertebrates and from the densities of populations in the water column of the enclosures at 13 days post first application for the zooplankton. Application of *lambda*-Cyhalothrin to enclosures occurred two times in this period (days 0 and 7). Prior to analysis, the macroinvertebrate data were $\ln(2x+1)$ transformed, where x is the abundance value. This was done to down-weight high abundance values and approximate a log-normal distribution of the data. In contrast to single species tests and bioassays, the densities of free-living individuals were not known *a priori* and could have been different between replicates. Therefore, these data from the field were fitted to the concentration-response model shown below, where d represents the expected *number* in the control enclosures. The model gives a sigmoid concentration-response curve for ln(concentration). Numbers were assumed to be quasi-Poisson distributed. The EC_{50}s and the EC_{10}s of the field enclosures were defined as the concentration at which numbers were reduced to the abundance in the controls by 50% and 10%, respectively. The regression models were programmed in GENSTAT, version 5.3.1. The concentration-effect model for ditch enclosure data was as follows:

X8
X9
X10
X11

$$Expected\ \ Number = \frac{(d)}{\left(1+\exp^{-b[\ln(concentraton)-a]}\right)}$$

a = ln (EC_{50})
b = slope of parameter
d = expected number in the control enclosures

No Observed Effect Concentration (NOEC) calculations at parameter or taxon level were derived using the Williams test (ANOVA). The analyses were performed with a Community Analysis computer programme, resulting in an summary of NOECs in each sampling week for the data analysed. The threshold level for P was 0.05 for all statistical analyses.

The effects of the *lambda*-Cyhalothrin treatments at the community level for macroinvertebrates and zooplankton in the field were analysed by the Principal Response Curves method (PRC), performed using the CANOCO software package, version 4.02. The significance of the PRC diagram in terms of displayed treatment variance was tested by Monte Carlo permutation of the enclosures to test the significance of the treatment regime, ecosystem structure, and their interaction.

The treatments which differed significantly from the control were identified in order to derive the No Observed Effect Concentration at the community level ($NOEC_{community}$) for each sampling date. The Williams test can be applied to a multivariate data set if the data set is

reduced to a single variable. The first principle component of a Principal Component Analysis suits this purpose. The NOEC calculations were therefore done by applying the Williams test to the sample scores of the first principal component of each sampling date in turn.

In situ bioassays and single species laboratory data

The $E(L)C_{10}$ and $E(L)C_{50}$ from the field bioassay data and the single species laboratory tests and their confidence limits were calculated by a log concentration-logit effect regression model as follows:

$$Expected\ \ Affected\ \ Fraction = \frac{(1-c)}{\left(1+\exp^{-b[\ln(concentraton)-a]}\right)}$$

a = ln (EC_{50})
b = slope of parameter
c = *fraction* of affected individuals in controls

Species sensitivity distribution analyses

Species sensitivity distribution (SSD) analyses were carried out with the single species acute toxicity data (48 hour EC_{50} values) using the computer program ETX-temporary version 1. Calculations were made of the HC_5 and HC_{50} and their 95% confident limits of toxicity data. The model assumes a log-normal distribution of toxicity data according to the following formula (Gaussian-curve): X12

$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} * \exp\left(\frac{-0.5*(x-\mu)^2}{\sigma^2}\right)$$

x = ln(EC_{50})
μ= median EC_{50} = ln (HC_{50})
σ= standard deviation of ln (EC_{50})

In this study the SSD is defined as the cumulative frequency distribution of toxicity data, according to the formula:

$$F(x) = \int_{\infty}^{x} f(x)ds$$

Tests for log-normality were performed by means of Anderson-Darling goodness-of-fit test, a standard statistic output of the computer program ETX –version 1.403. Normality of toxicity data was assumed when P was ≥ 0.05. A two-sample F-test was used to assess significant differences in the variances of SSDs. To determine significant differences in SSDs (mutual distance of two SSDs), t-tests were performed. Both tests were performed for 'full' curve comparison.

Findings:

This is a summary of the data and statistical analyses currently available and reported in the submitted papers by Roessink *et al.* and Shroer *et al.* Further evaluation of the study data obtained during the late summer applications is on going and will be reported in the paper for scientific publication by Van Wijngaarden *et al.*

FIELD MICROCOSMS

Chemical analyses

The measured concentrations of *lambda*-Cyhalothrin in the „Karate" with Zeon Technology application mixtures used in the spring (macrophyte and phytoplankton dominated ditches) were between 96 and 112% of nominal and in the macrophyte ditches in late summer were between 93 and 111% of nominal. The concentrations of *lambda*-Cyhalothrin determined in water column samples taken from the enclosures one hour after each application of *lambda*-Cyhalothrin are shown in the following table. These values show that initial *lambda*-Cyhalothrin concentrations in the water column were close to nominal in the ditch treated in the late summer. In the ditches treated in the spring, the one-hour water column concentrations tended to be higher than nominal. As the *lambda*-Cyhalothrin concentrations in the application mixtures were found to be close to nominal, these high initial water column values are considered to have arisen because the dose had not mixed completely through the 0.5 m water column in the enclosures by the time these samples were taken. Biodegradability data indicates that the concentration of *lambda*-Cyhalothrin in the water column in the enclosures rapidly declined to levels close to the limit of determination (2 ng/L) within seven days.	X13

Mean measured concentrations of *lambda*-Cyhalothrin in the water column of the ditch enclosures approximately one hour after application[1]

Nominal Application rate ng/L	Enclosure No.	Macrophyte-dominated Ditch (No. 2)		
		16th May	23rd May	30th May
0	6	0.000	0.000	0.000
0	14	0.001	ND	0.003
10	1	0.005	0.026	0.025
10	10	0.010	0.011	0.019
25	7	0.024	0.086	0.044
25	12	0.032	0.058	0.048
50	4	0.069	ND	0.077
50	8	0.078	0.092	ND
100	5	0.113	0.132	0.171
100	11	0.115	0.151	0.102
250	3	0.318	0.337	0.300
250	13	ND	0.269	0.233

[1] Mean values based on duplicate analyses reported in ***Zweers et al., 2001***. LOD 2 ng/L.
ND not determined

Mean measured concentrations of *lambda*-Cyhalothrin in the water column of the ditch enclosures approximately one hour after application[1] (continued)

Nominal Application rate ng/L	Enclosure No.	Phytoplankton-dominated Ditch (No. 19)		
		16th May	23rd May	30th May
0	6	0.000	0.000	0.000
0	14	0.002	0.003	0.005
10	1	0.013	0.018	0.028
10	10	0.014	0.020	0.016
25	7	0.014	0.055	0.054

25	12	0.022	0.046	ND
50	4	0.050	0.077	0.086
50	8	0.045	0.084	0.098
100	5	0.093	0.103	0.107
100	11	ND	0.126	0.143
250	3	0.287	0.228	0.355
250	13	ND	0.266	0.292

Mean measured concentrations of *lambda*-Cyhalothrin in the water column of the ditch enclosures approximately one hour after application[1] (continued)

Nominal Application rate ng/L	Enclosure No.	Macrophyte-dominated Ditch (No. 8)		
		15th August	22nd August	29th August
0	6	ND	ND	ND
0	14	ND	ND	0.004
10	1	0.010	0.009	0.011
10	10	0.019	0.011	0.011
25	7	0.026	0.025	0.025
25	12	0.028	0.031	0.026
50	4	0.052	0.045	0.044
50	8	0.052	0.050	0.048
100	5	0.099	0.082	0.084
100	11	0.091	0.076	0.091
250	3	ND	0.214	0.222
250	13	0.241	0.228	0.210

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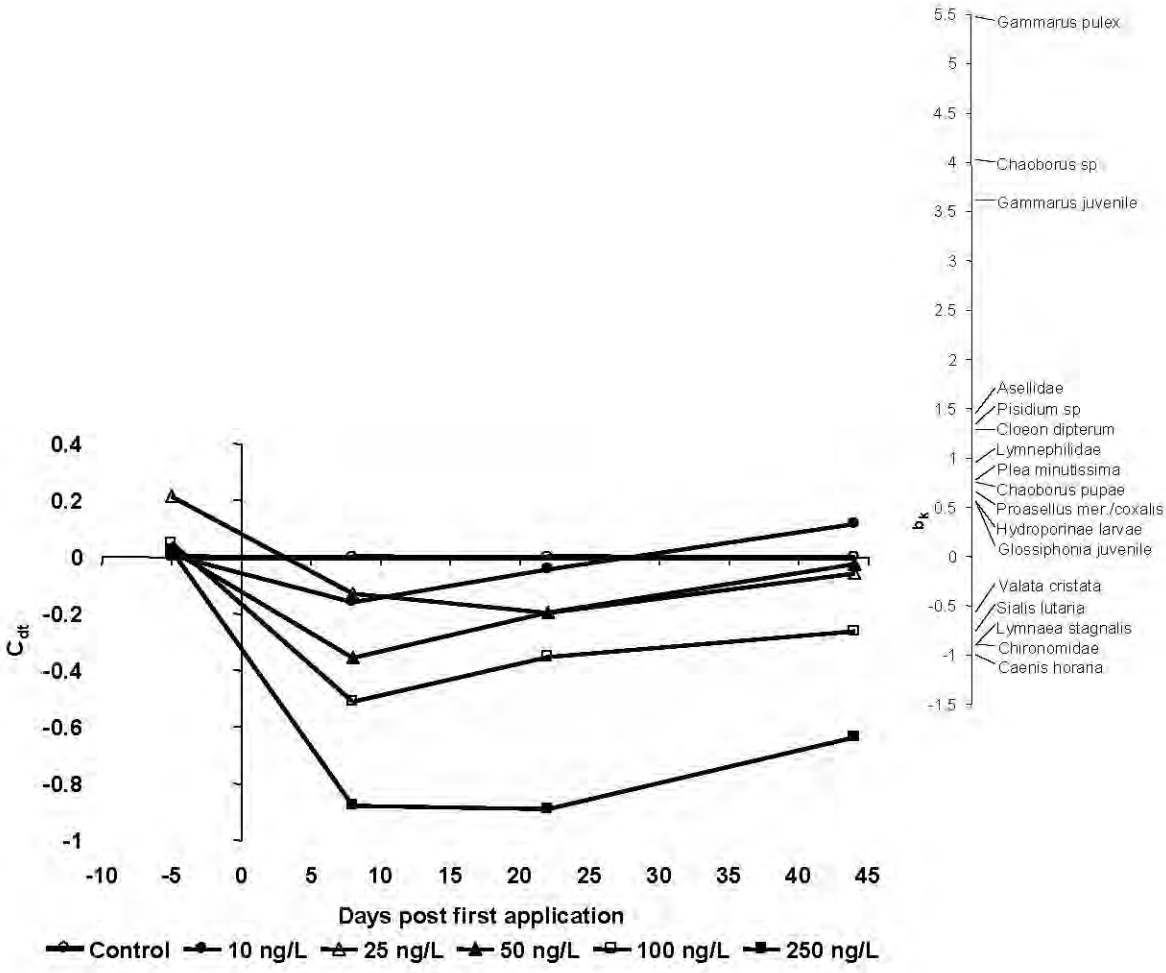
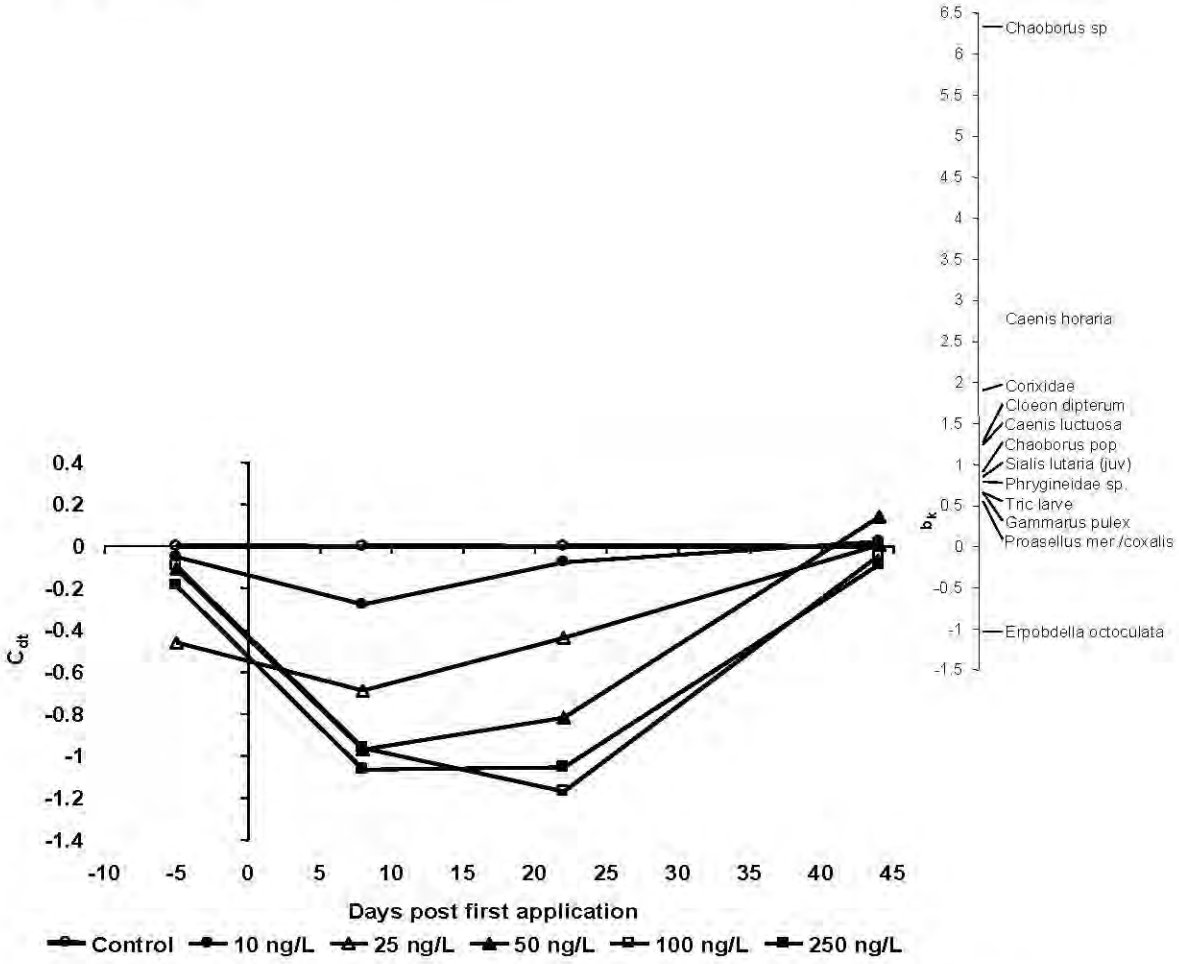
Biological assessments The study microcosms contained a diverse macroinvertebrate community. In total 47 taxa were sampled by means of the artificial substrates. In the macrophyte-dominated ditch, snails (particularly *Armiger cristata)*, midges (particularly *Chaoborus obscuripes)*, amphipods (particularly *Gammarus pulex*), and isopods (particularly *Asellus aquaticus*) dominated the community, whereas Heteroptera, bivalves, and tubellarians were less abundant. The Heteroptera were best represented by *Cloeon dipterum*, bivalves by *Pissidium sp.*, and tubellarians by *Polycelis* and *Mesostoma.* In the phytoplankton-dominated ditch, the most abundant groups were midges (mainly *Chaoborus obscuripes* and *Chironomidae*), Heteroptera (mainly *Cloeon dipterum*), snails (mainly *Bythinia tentaculata*) and oligochaeta (not determined to species level). Again, in this ditch the amphipod and isopod communities were represented by *Gammarus pulex* and *Asellus aquaticus,* respectively. Tubellarians in the ditches were dominated by *Polycelis niger/tenuis*. Leeches, except *Erpobdella octaculata,* were rare in the enclosures. Additionally, 39 zooplankton taxa were identified in the microcosms. In decreasing order of abundance, the communities of both types of enclosures were dominated by rotifers (mainly *Keratella cochlearis, Keratella quadrata*, and *Anureopsis fissa*), cyclopoid Copepoda (*nauplii*), nematocerans (*Chaoborus obscuripes*) and Cladocera (*Daphnia galeata*).	

Responses of Macroinvertebrates in the Ditches Multivariate analysis of the macroinvertebrate community of both the macrophyte and phytoplankton ditches enclosures revealed clear, but different concentration-responses. By the end of the experiment (21 days after the last application), the macroinvertebrate communities in the enclosures in the phytoplankton ditch treated at rates up to 250 ng/L has recovered. Recovery of communities in the macrophyte ditch enclosures was slower, however those treated at three times 100 ng/L had recovered compared to controls within the same period. In both types of ditch systems, the lowest community NOEC was observed on the first sampling day after treatment). Similar effects were evident on the community in the macrophyte ditch treated in the late summer.	

Principal response curve showing the effects of the *lambda*-Cyhalothrin treatments on the macroinvertebrate community in the macrophyte dominated ditch following applications in the spring



Principal response curve showing the effects of the *lambda*-Cyhalothrin treatments on the macroinvertebrate community in the phytoplankton dominated ditch following applications in the spring



NOECcommunity values based on data from the spring applications and nominal *lambda*-Cyhalothrin treatment rate

Date[(1)]	P-Value	$NOEC_{community}$ ng/L
Macrophyte dominated ditch		
7	< 0.001	<10
21	< 0.001	50
42	0.016	100
Phytoplankton dominated ditch		
7	< 0.001	<10
21	< 0.001	10
42	>0.05	>250

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

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The variance allocated in the macroinvertebrate PRC diagram and the statistical analysis of the community response is as follows: Macrophyte-dominated ditch	

Sampling date: 35% Treatment regime: 35% of which 27% is displayed in the diagram <u>Phytoplankton-dominated ditch</u> Sampling date: 31% Treatment regime: 40% of which 38% is displayed in the diagram Consistent treatment-related responses were predominantly comprised of effects on arthropods. However, of the various populations sampled in the enclosures in both the spring and in the late summer, only a limited number showed a clear concentration-response relationship indicative of a direct toxic effect (decrease in numbers). In total, short-term field EC_{50}s could be calculated for six taxa only and NOECs could be calculated for 13 taxa. The macroinvertebrate EC_{50}s and NOEC values from the data from the enclosures treated in the spring are shown in Table 8.2-43. Individual population EC_{50} values for the macrophyte ditch treated in late summer from Van Wijngaarden *et al.* (in preparation) are shown in the table below. In the case of *Chaorborus obscuripes*, where three field EC_{50} values could be calculated from the three different enclosure systems (macrophyte ditches treated in the spring and in the late summer, and the phytoplankton ditch treated in the spring), these values were very similar.	X14 X15

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

Ditch	Taxon	Day	NOEC (ng/L)	Based on
Macrophyte-dominated	*Armiger cristata*	8	100	Decrease
	Asellidae	44	100	Decrease
	Caenis horaria	8	100	Increase
	Chaoborus obscuripes	8	10	Decrease
		22	10	Decrease
	Cloeon dipterum	8	100	Decrease
	Gammarus pulex	8	100	Decrease
		22	25	Decrease
		44	50	Decrease
	Lymnea stagnalis	22	100	Increase
	Sialis lutaria	8	25	Increase
Phytoplankton-dominated	*Caenis horaria*	8	25	Decrease
		22	10	Decrease
	Caenis luctuosa	8	50	Decrease
	Ceratopogonidae	8	10	Increase
	Chaoborus obscuripes	8	<10	Decrease
		22	25	Decrease
	Cloeon dipterum	22	<10	Decrease
	Corixidae	22	50	Decrease
	Oligochaeta	44	50	Increase
	Sialis lutaria	44	<10	Decrease

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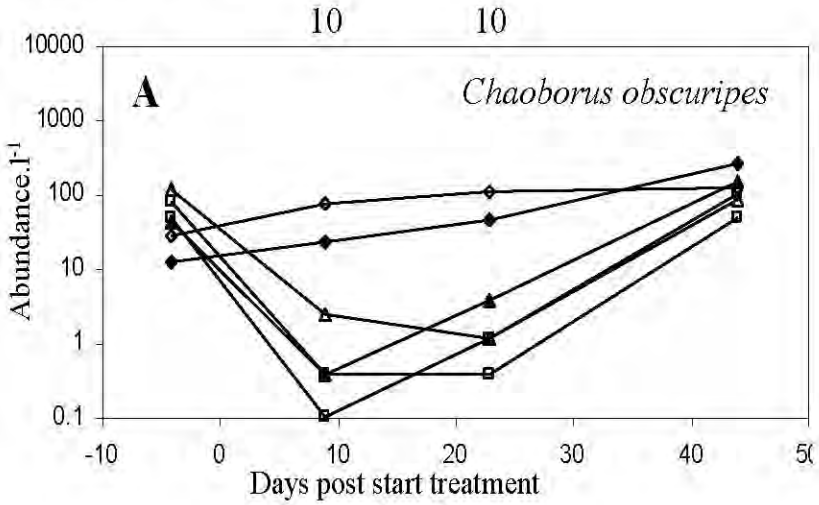
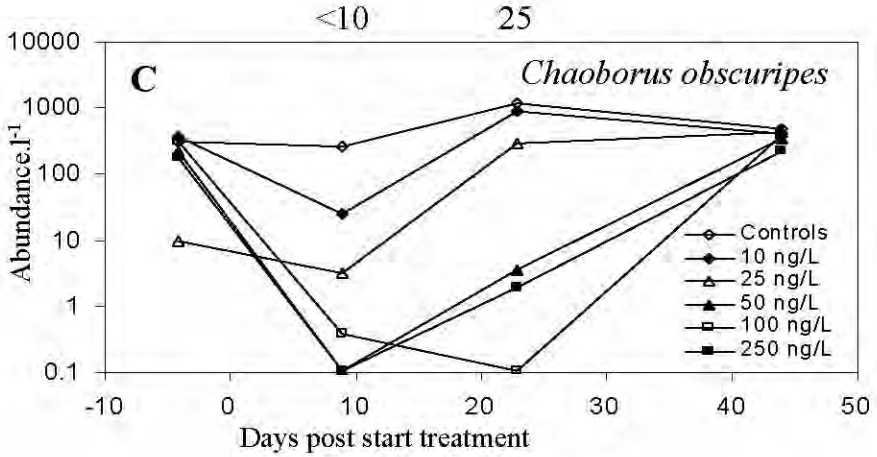
In the enclosures in the macrophyte ditch, reductions in the number of *Gammarus pulex* with treatment rate gave the highest weight to the PRC analysis, followed by reductions in *Chaoborus sp.* In the enclosures in the phytoplankton ditch, reductions in the number of *Chaoborus sp.* with treatment rate gave the highest weight to the PRC analysis, followed by reductions in *Caenis horaria.* The lower score for *Gammarus* in the PRC diagram of the phytoplankton ditch and that of *Caenis horaria* in the macrophyte enclosures are result of the lower abundance of these species in these enclosures and not an indication of a lower treatment-related responses in these test systems. Overall, the treatment-related responses of *Chaoborus obscuripes* between the two types of test systems were very similar, with a decline in the treatment period and recovery in the post-treatment period. The changes in abundance of this species in the macrophyte and phytoplankton ditches with time after application are presented in Figure 8.2-6. Although on day 8 post treatment the EC_{50} values for *Chaoborus* were lower in the enclosures in the phytoplankton ditch, the confidence limits of these values overlapped when comparing the two types of test systems.	

Effect of *lambda*-Cyhalothrin treatments on the numbers of *Chaoborus obscuripes* in the artificial substrate samplers in (A) the macrophyte-dominated and (B) the phytoplankton-dominated ditches after applications in the spring

NOECs plotted above the figures





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In the enclosures in the macrophyte ditches, *Gammarus pulex* showed a clear decline without recovery in the two highest treatments (100 and 250 ng/L). The lowest NOEC observed for *Gammarus* was 25 ng/L on day 22. Although *Gammarus pulex* showed a clear concentration-response relationship, EC_x values could not be calculated because there was only one concentration where a partial effect was observed (100 ng/L). In the enclosures in the phytoplankton ditch, *Caenis horaria* showed a pronounced decline during the treatment period. In the course of the experiment, this species declined in all enclosures (including controls), most probably due to emergence. The lowest NOEC observed for *Caenis horaria* was 10 ng/L on day 22. Evidence of indirect effects of *lambda*-Cyhalothrin on the ditch communities was observed. In the macrophyte ditch, *Caenis horaria*, *Lymnea stagnalis* and *Sialis lutaris* appeared to be positively correlated with the treatment rate (increase in numbers). In the phytoplankton ditch, the oligochaete worm *Stylaria lacustris* and Ceratopognidae appeared to be positively correlated with the treatment rate. <u>Responses of Zooplankton</u> Multivariate analysis of the zooplankton communities in the enclosures in both the macrophyte and phytoplankton ditches did not reveal clear concentration-dependent effects on the community structure. On the one and only sampling date at which the zooplankton community NOEC was significant compared to the control data, the percentage of the total variance due to treatment was smaller than that due to time. The results of univariate analysis of the zooplankton data are shown in Table 8.2-44. In both types of enclosures, indirect effects of *lambda*-Cyhalothrin were observed for planktonic communities. In the enclosures in the macrophyte ditch, a significant increase in abundance of the rotifer *Lecane lunaris* was observed and there were indications of higher densities of Cladocera in the 25, 50, and 100 ng/L treatments compared to the controls and the 250 ng/L treatments. A similar trend for Cladocera densities was evident in the enclosures in the phytoplankton ditch. Furthermore, in the enclosures in the phytoplankton ditch, densities of *nauplii* and *Keratella quadrata* increased at all treatment levels. These data, particularly the responses of *nauplii* and rotifers, indicate that the species involved in indirect effects differed between the macrophyte and phytoplankton dominated test systems.	

The increases in *Lecane lunaris* in the macrophyte ditch and *nauplii* and *Keratella quadrata* in the phytoplankton ditch and the overall higher densities of Cladocera in the 10, 25, 50, and 100 ng/L treatments are probably due to decreased predation by *Chaoborus*. The responses observed for Cladocera populations in both ditches can be explained by a combination of direct and indirect effects. The relatively low abundance of Cladocera in the controls was most probably caused by intense *Chaoborus* predation. With increasing treatment levels up between 50 and 100 ng/L, the predation pressure of *Chaoborus* decreased due to direct effects of *lambda*-Cyhalothrin on this species, resulting in higher Cladocera densities. At concentrations between 100 and 250 ng/L *lambda*-Cyhalothrin may have had direct toxic effects on the zooplankton (laboratory EC_{50} for *Daphnia galeata*, the dominant Cladocera is 117 ng/L), resulting in a decline in abundance. Phytoplankton chlorophyll-a concentrations in the phytoplankton ditch enclosures were also higher in the control and the highest treatment enclosures (particularly on the last sampling day), which is probably a reflection of the lower densities of cladocerans in these test systems. The indirect effects observed on rotifer populations (increases in abundance) are consistent with previous reports of the ecological impact of insecticides in aquatic systems.	

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

Ditch	Taxon	Day	NOEC (ng/L)	Based on
Macrophyte-dominated	*Anureopsis fissa*	20	100	Decrease
	Branchionus angularis	28	<10	Decrease
	Cephalodella gibba	6	100	Increase
	Lecane lunaris	13	100	Increase
		20	<10	Increase
	Lecane luna	20	<10	Increase
	Synchaeta spp	28	100	Increase
	Trichocerca capucina	20	50	Increase
	nauplius	6	100	Decrease
		13	25	Decrease
		20	100	Decrease
	Daphnia galeata	28	10	Increase
	Ostracoda	-7	<10	Decrease
	Chaoborus obscuripes	6	10	Decrease
		13	<10	Decrease
		20	100	Decrease
		28	100	Decrease
	Cladocera	20	100	Decrease
		28	<10	Increase
Phytoplankton-dominated	*Branchionus angularis*	20	<10	Increase
		28	10	Decrease
	Cephalodella gibba	20	50	Increase
	Keratella cochlearis	20	10	Increase
	Keratella quadrata	13	10	Increase
		20	<10	Increase
	Lecane lunaris	6	<10	Decrease
	Lepadella patella	20	100	Increase
	Polyarthra remata	13	10	Increase
	Synchaeta spp	20	<10	Increase
	Trichocerca capucina	20	25	Increase
	nauplius	20	<10	Increase
		28	<10	Increase
		41	10	Increase
	Chaoborus obscuripes	6	<10	Decrease
		13	<10	Decrease
	Ostracoda	20	100	Increase
		41	100	Increase

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Overall summaries of treatment-related effects on the macroinvertebrates and zooplankton observed in both types of ditch systems are presented in the table below.	X16

Summary of effects observed in the enclosures in the macrophyte and phytoplankton ditches treated with the lambda-Cyhalothrin three times at one-week intervals

	Treatment-related effects [1]				
	10 ng/L	25 ng/L	50 ng/L	100 ng/L	250 ng/L
Macrophyte ditch					
Macrocrustaceans	1	2↓	2↓	4↓	4↓
Insects	2	3↓	3↓	3↓	3↓
Other macroinvertebrates	1	1	1	1	2↑↓
PRC macroinvertebrates	2	2	3	3	4
Microcrustaceans	1	2↓	2↓	3↓	4↓
Rotifers	2↑	2↑↓	2↑↓	2↑↓	2↓; 3↑
PRC zooplankton	1	1	2	2	2
Algae	1	1	1	1	1
Macrophytes	1	1	1	1	1
Community metabolism	1	1	1	1	1
Phytoplankton ditch					
Macrocrustaceans	-*	-*	-*	-*	-*
Insects	2↓	3↓	3↓	3↓	3↓
Other macroinvertebrates	1	1	1	2↑	2↑
PRC macroinvertebrates	2	3	3	3	3
Microcrustaceans	2-3↑	4↑	4↑	4↑	4↑↓
Rotifers	2↑	3↑	3↑	3↑	3↑
PRC zooplankton	2	2	2	2	2
Algae	1	1	1	1	2↑**
Macrophytes	-	-	-	-	-
Community metabolism	1	1	1	1	1

1 = no effect, 2 = slight effect, 3 = clear short-term effects, full recovery observed (4-8 weeks), 4 = clear effects, no full recovery observed at the end of the experiment.
↑=increase, ↓=decrease, ↑↓=increase and decrease on species and/or sampling date.
* Low abundance of free-living population.
** Trend of an increase.

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Field Bioassays The NOEC values for *Chaoborus obscuripes*, *Asellus aquaticus* and *Daphnia pulex* determined from the "acute" and "recovery" bioassay data from the ditch enclosures treated in the spring are shown in Tables 8.2-46 and 8.2-47, respectively. EC_x values from the bioassay data for *Chaoborus*, *Asellus* and *Proasellus coxalis* from all the experimental ditches are shown in the following table. As expected from laboratory data, *Chaoborus* was the most sensitive of the species examined in the *in situ* bioassays and showed a clear and similar treatment-related response in all the experimental ditches. Furthermore, very similar responses for the animals in the cages in the macrophyte ditches were observed in the spring and summer experiments. The recovery assessments in the bioassay cages indicate that the potential for recovery of *Chaoborus* was somewhat slower in the macrophyte-dominated ditch. *Chaoborus* exposed *in situ* eight days after the last *lambda*-Cyhalothrin application were not affected compared to controls at all	X17

treatment rates in the phytoplankton ditch whereas, over the same period, treatment related effects were no longer observed at levels of 50 ng/L and below in the macrophyte-dominated ditch. These findings are consistent with the free-living macroinvertebrate data, which indicate somewhat faster recovery of *Chaoborus* in the phytoplankton ditch during the course of the study. *Asellus aquaticus* showed clear and similar treatment related responses in the bioassay cages in both types of ditches. As for *Chaoborus*, the "recovery" bioassay data from the spring experiment indicate that the potential for recovery of *Asellus* was somewhat faster in the phytoplankton ditch. Animals exposed *in situ* 8 days after the last *lambda*-Cyhalothrin application were not affected compared to controls at all treatment rates in the phytoplankton ditch, whereas, over the same period, treatment related effects were no longer observed at levels of 100 ng/L and below in the macrophyte-dominated ditch. It is evident that toxicity values for *Asellus* and *Proasellus* based on enclosure and *in situ* bioassay data are higher than the laboratory toxicity values. This may be due to the exposure conditions. In the bioassay the animals would have been able to shelter between the *Populus* leaves that were placed in the cages as a food source and free-living populations will have predominantly inhabited in the detritus layer on top of the sediment. In contrast, the laboratory tests were conducted in water only systems where exposure will have been more severe. *Daphnia* in the bioassay cages in the phytoplankton ditch also showed a treatment related response. However, bioassay data for *Daphnia* could not be obtained from the macrophyte ditches as in this system the caged *Daphnia* were preyed on by the Tubellarian, *Mesostoma sp.* *Mesostoma* was only observed in the macrophyte ditches.	

NOEC's calculated from the data obtained from the acute effects bioassays performed in the ditch enclosures treated with *lambda*-Cyhalothrin in the spring.

Ditch	Taxon	Day[1]	NOEC (ng/L)	Based on
Macrophyte-dominated	*Chaoborus obscuripes*	1	25	Decrease
		2	<10	Decrease
		3	<10	Decrease
		6	<10	Decrease
	Asellus aquaticus	1	10	Decrease
		2	25	Decrease
		3	25	Decrease
		6	50	Decrease
Phytoplankton-dominated	*Chaoborus obscuripes*	1	50	Decrease
		2	10	Decrease
		3	10	Decrease
		6	10	Decrease
	Asellus aquaticus	1	50	Decrease
		2	25	Decrease
		3	50	Decrease
		6	50	Decrease
	Daphnia pulex	1	50	Decrease
		2	25	Decrease
		6	-	

[1] Acute bioassays exposed for 6 days from day 0

NOEC's calculated from the data obtained from the recovery bioassays performed in the ditch enclosures treated with *lambda*-Cyhalothrin in the spring.

Ditch	Taxon	Period [1]	NOEC (ng/L)	Based on
Macrophyte-dominated	*Chaoborus obscuripes*	0-4	10	Decrease
		4-8	10	Decrease
		8-14	50	Decrease
	Asellus aquaticus	0-4	25	Decrease
		4-8	25	Decrease
		8-14	100	Decrease
Phytoplankton-dominated	*Chaoborus obscuripes*	0-4	<10	Decrease
		4-8	10	Decrease
		8-14	>250	Decrease
	Asellus aquaticus	0-4	25	Decrease
		4-8	100	Decrease
		8-14	>250	Decrease

[1] Recovery bioassays exposed for 4 or 6 days at 0, 4, or 8 days from the day of the last application

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Responses of primary producers Primary producers in the enclosures comprised phytoplankton (characterised by *chlorophyll*-a), macrophytes (characterised by biomass), and periphyton (characterised by *chlorophyll*). *Chlorophyll*-a levels at the start of the spring experiment were lower in the enclosures in the macrophyte ditch (approximately 20-130 ug/L) than in the phytoplankton ditch (approximately 110-210 ug/L). No clear treatment-related effects were observed, however, in the phytoplankton ditch enclosures at the end of the experimental period chlorophyll-a levels in the control and 250 ng *lambda*-Cyhalothrin/L treatment were higher than, but not statistically different to, the *chlorophyll*-a levels of the intermediate treatment levels. This difference was related to the abundance of Cladocerans, which were lowest in the control and 250 ng/L treatments. The macrophyte biomass harvested from the enclosures in the macrophyte ditch at the end of the experimental period ranged from approximately 70 to 150 g/m^2 and there were no treatment-related effects on macrophyte biomass. There were no macrophytes present in the enclosures in the phytoplankton ditch. Summaries of treatment-related effects on the primary producers observed in both types of ditch systems are presented in the following table. **Community metabolism and decomposition** During the experimental period following the spring applications, there were no treatment related effects observed on the community metabolism parameters measured (including pH, electrical conductivity, alkalinity, NH_4^+, NO_3^-, NO_2^- and PO_4^-). The residual dry weight of the *Populus* leaves from the litter bags amounted to approximately 68% and 72% in the macrophyte dominated and phytoplankton dominated enclosures, respectively. No significant treatment-related effect on litter decomposition was observed and there were no effects of treatment on the periphyton.	X18

Summaries of treatment-related effects on community metabolism observed in both types of ditch systems are presented below. X19

Single species laboratory acute toxicity tests

The results of the laboratory acute toxicity tests with single species are given below. *Chaoborus obscuripes* was the most sensitive species of all those tested in the laboratory systems and the amphipod *Hyallella azteca* showed similar sensitivity. Several macrocustaceans (*Asellus aquaticus, Proasellus coxalis, Gammarus pulex*) and larvae of the insect groups Ephemeroptera (*Caenis horaria, Cloeon dipterum*), Hemiptera (*Sigara striata, Notonecta glauca, Corixa sp.*) and Megaloptera (*Sialis lutaria*) were more sensitive than the microcrustaceans tested (including *Daphnia magna*). The toxicity to insect larvae of Zygoptera (*Ischnura elegans, Erythromma viridulum*) and Chironomidae (*Macropelopia, Chironomus riparius*) was in the range of EC_{50} values for microcrustaceans, with Ostracoda as the least sensitive arthropod taxon tested.

Of the non-arthropods tested, the flatworm (*Polycelis nigra/tenuis*) and snails (*Lymnaea stagnalis* and *Bithynia tentaculata*) can be characterised as non-sensitive species, since their EC_{50} values are above the solubility of *lambda*-Cyhalothrin. *Polycelis nigra/tenuis* and *Lymnaea stagnalis* showed no visible treatment-related response. *Bithynia tentaculata*, however, responded by means of closing the operculum (avoidance behaviour). After the test period, individuals with a closed operculum were placed in clean water. Within a day, they opened their operculum again and did not show any adverse negative effects.

As shown in the table below, the 48 hour EC_{50} values are all similar to previously reported values. X20

48 hour EC_{50} values from the single species laboratory toxicity tests and those reported previously

Species	EC50 48 hour (ng/L) (95% confidence limits)	
	Schroer *et al.* (2004a)	Maund *et al.* (1998)
Chaoborus obscuripes	2.8 (1.8-4.4)	
Chaoborus sp.		2.8 (1.0-7.8)
Hyalella azteca		2.3 (1.8-4.1)
Notonecta glauca	14.8 (10.0-21.9)	
Proasellus coxalis	17.7 (13.1-23.9)	
Caenis horaria	17.9 (12.8-25.1)	
Sigara striata	18.2 (9.2-36.1)	
Gammarus pulex	23.6 (16.0-34.9)	14 (9.1-19)
Asellus aquaticus	24.8 (18.4-33.4)	26 (18-36)
Cloeon dipterum	24.8 (17.2-35.8)	38 (23-93)
Corixa sp.		30 (21-42)
Hydracarina		47 (33-62)
Sialis lutaria	51.5 (30.3-87.7)	
Daphnia galeata	116 (86.6-157)	
Ischnura elegans		130 (92-190)
Macropelopia sp.	244 (183.2-326)	
Cyclops sp.		300 (200-460)
Daphnia magna		360 (280-460)
Erythromma viridulum	689 (479-992)	
Simocephalus vetulus	957 (707-1295)	

Chironomus riparius		2400 (1400-5200)
Ostracoda		3300 (2100-6600)
Lymnaea stagnalis	>5000 [1]	
Bithynia tentaculata	>5000 [1]	
Polycelis nigra/tenuis	>5000 [1]	

[1] EC_{50} above the water solubility of *lambda*-Cyhalothrin (5 ug/L)

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The table below shows the available comparisons of EC_x values for organisms in the laboratory tests with values from the spring and summer experiments for free-living populations in the enclosures and for organisms in the *in situ* bioassays.	

Comparison of short-term EC10 and EC50 values (ng/L) for arthropods derived from laboratory single species tests and outdoor ditch enclosures and acute bioassay studies

Note: In the three enclosure experiments EC_{10} or EC_{50} could not be calculated for some taxon due to very low abundances and/or lack of concentration related responses

Species	x	EC_x from Ditch experiments		EC_x from Laboratory test
		Enclosure	Bioassay	Schroer *et al.* (2004a)
Chaoborus obscuripes	10	2.4 (0.8-7.3)[a]	1.2 (0.3-5.0)[a]	0.6 (0.3-1.3)
	50	6.2 (3.5-10.9)[a]	4.9 (2.5-9.9)[a]	2.8 (1.8-4.4)
	10	0.5 (0.0-10.2)[a,d]		
	50	4.0 (0.8-20.6)[a,d]		
	10		1.7 (0.5-5.6)[b]	
	50		5.0 (2.7-9.3)[b]	
	10	1.5 (0.4-5.0)[c]	5.4 (3.2-8.9)[c]	
	50	3.9 (2.0-7.8)[c]	12.6 (9.5-16.7)[c]	
Gammarus pulex	10	2.5 (0.4-14.4)[b,c]		14.2 (7.4-27)
	50	9.0 (3.5-22.7)[b,c]	nt	23.6 (16.0-34.9)
Asellus aquaticus	10		30.3 (17.4-52.6)[a]	10.7 (6.6-17.6)
	50		71.9 (54.5-95.1)[a]	24.8 (18.4-33.4)
	10		10.4 (6.3-17.1)[b]	
	50		51.9 (40.9-65.8)[b]	
	10		18.5 (11.2-30.6)[c]	
	50		64.2 (50.4-81.7)[c]	
Proasellus coxalis	10		53.9 (35.6-81.6)[b]	13.0 (8.7-19.6)
	50		133 (108-164)[b]	17.7 (13.1-23.9)
Cloeon dipterum	10	8.3 (2.1-34.0)[b]		7.2 (3.7-14.0)
	50	24.0 (10.9-53.1)[b]	nt	24.8 (17.2-35.8)
Caenis luctuosa	10	5.0 (0.5-45.8)[c]		
	50	22.1 (7.2-67.4)[c]	nt	nt
Caenis horaria	10	5.3 (1.6-14.8)[c]		7.2 (3.7-14.0)
	50	14.3 (8.1-25.0)[c]	nt	17.9 (12.8-25.1)
Corixidae/Corixa	10	3.7 (0-361)[c]		
	50	27.2 (2.6-288)[c]	nt	

[a] macrophyte dominated system, spring
[b] macrophyte dominated system, summer

[c] plankton dominated system, spring
[d] Based on zooplankton sampling
[e] *Gammarus pulex* juvenile
nt = no test performed

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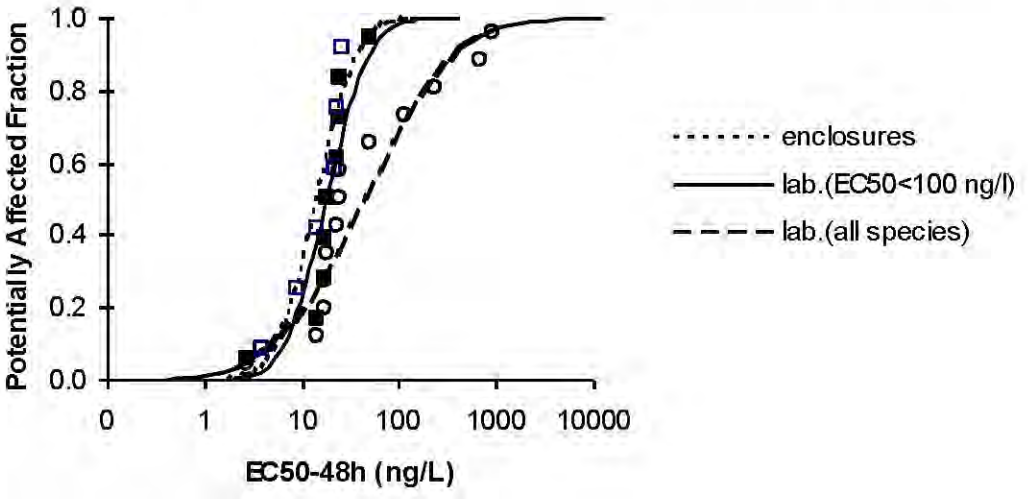
As only a limited number of species showed clear concentration-related responses in the field enclosure experiments, indicative of a direct toxic effect (decrease in numbers), comparisons between laboratory and field toxicity values could be made for 6 taxa only. In these cases, the laboratory EC_{50} values (based on the endpoint immobility) compare well with those derived from the field data (based on abundance), as shown in Table 8.2-49, for all species except *Gammarus*. Only *Gammarus pulex* was apparently more sensitive in the field, with a field population-level EC_{50} value of 9 ng/L (determined on day 8-10 after applications on days 0 and 7), compared to a laboratory value of 23.6 ng/L (however, 95% confidence limits overlapped). The apparent increase in sensitivity was probably due to differences in life stage. In the laboratory, adult organisms were used, whereas the field-derived EC_{50} was based on juvenile organisms. Juvenile *Gammarus* have been found to be approximately twice as sensitive as adults. Species sensitivity distribution (SSD) curves based on field and laboratory toxicity data were compared. Two SSD curves were prepared form the laboratory data, the first including all 13 arthropod species tested and the second excluding the 4 species with an EC_{50} higher than 100 ng/L, because in these cases the field EC_{50} values are unlikely to be accurate as the highest field treatment was only 250 ng/L. These SSD curves are compared with the SSD curve based on the field data in Figure 8.2-7. From this comparison it is evident that the field and laboratory SSDs for *lambda*-Cyhalothrin are very similar when based on the same sensitive taxonomic groups (insects and crustaceans) and when a similar range of exposure concentrations is taken into account.	

Comparison of Species Sensitivity Distribution curves for *lambda*-Cyhalothrin based on data from either laboratory tests or field ditch enclosures

Note: For the enclosures, only the EC_{50} values for macro-arthropods are available (□ …) . From the laboratory data, the curve based only on macro-invertebrate arthropods with an EC50≤100 ng/L (9 species) is denoted (■) and that based on all 13 arthropod species is denoted (○ - - -).



	Official use only
Conclusions: The data obtained from the two types of ditch enclosure systems and *in situ* bioassays in this study with three weekly applications of *lambda*-Cyhalothrin demonstrate that there are no great differences between the direct responses, and resulting critical effects threshold for shallow freshwater systems of differing nutrient status, despite these differences in community structure. The only differences in direct effects and recovery potential between test systems were due to the populations that predominantly occurred in one of the test systems only, particularly high abundance of Gammarus in the macrophyte-dominated relative to the phytoplankton-dominated systems. PRC analysis of the macroinvertebrate community in particular revealed that the observed treatment-related response was largely correlated with a decrease in arthropod populations. In the macrophyte ditch systems, *Gammarus* and *Chaoborus* had the most pronounced effect on the community response, while in the phytoplankton ditch systems it was *Chaoborus* and *Caenis*. The observed responses of free-living populations of arthropods in the enclosures over the study period were in agreement with acute toxicity data from the laboratory single species tests, at least when exposure conditions were similar. As *lambda*-Cyhalothrin dissipated very rapidly in the ditch enclosures, the observed similarities between the toxicity data from laboratory (over 48 hours) and field (over up to 3 weeks) indicate that symptoms of toxicity due to *lambda*-Cyhalothrin occur within a few hours after exposure. In the ditch enclosures treated three times at 10 ng/L at one week intervals, only slight and transient effects were observed in both the macrophyte and phytoplankton ditch systems, indicating that the threshold effect level for freshwater invertebrates is close to 10 ng/L. Following three treatments at 25 and 50 ng/L, effects in both ditch systems were mainly slight or short-term. The ditch study allowed the recovery of affected populations to be assessed. The PRC-diagrams indicated that following three applications of *lambda*-Cyhalothrin at 250 ng/L full recovery of the macroinvertebrate community occurred in the enclosures in the phytoplankton ditch within 21 days after the last *lambda*-Cyhalothrin application. In the macroinvertebrate ditch, a full recovery was observed in the same period after three applications at 100 ng/L. The recovery of the community in the macrophyte ditch was influenced by the significant contribution of *Gammarus* pulex to the PRC analysis and the lack of recovery for these populations in the ditch enclosures. As *Gammarus pulex* does not have non-aquatic life-stages, the potential for this species to recolonise isolated systems, such as these ditch enclosures is very limited. Data from the *in situ* bioassays, including those for the most sensitive species tested (*Chaoborus obscuripes*), are consistent with the observed recovery of the free-living populations and clearly indicate that recovery of is possible within one to a few weeks of the last *lambda*-Cyhalothrin application.	

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

[redacted]

[redacted]

Results and discussion	[redacted]

[redacted]

Conclusion	[redacted]

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

98/8 Doc IIIA section No.	7.4.3.1 01	Prolonged toxicity to an appropriate species of fish
91/414 Annex Point addressed	II 8.2.2.2/01	Fish early life stage toxicity test

		Official use only
Reference point (location) in dossier	**7.4.3.1/01**	
Title:	PP321: Determination of the chronic toxicity to sheepshead minnow (*Cyprinodon variegatus*) embryos and larvae	
Project/Report number:	BL/B/2677	
Author(s):	[redacted]	
Date of report:	1985	
Published:	Not published.	
Testing facility:	[redacted]	
Test substance:	Technical *lambda*-Cyhalothrin (P321), purity [redacted]	
Study dates	18 March - 28 April 1985	
GLP:	Yes	
Deficiencies:	None.	
Reliability indicator	1.	

	Official use only
Materials and methods: Sheepshead minnow (*Cyprinodon variegatus*) embryos obtained within 36 hours of fertilisation were used to initiate the test. Test concentrations were nominally, 0.10, 0.18, 0.32, 0.56 and 1.0 µg/L, plus solvent and untreated controls. Approximately 30 embryos were introduced into each replicate test chamber, 15 in each of two incubation cups. After hatch, embryos were fed 2 or 3 times daily with brine shrimp nauplii. From 11 days post-hatch, feeding was supplemented with a proprietary fish food. Numbers of live and dead eggs were recorded daily, together with total hatch. Exposure continued until 28 days post-hatch, assessing fry mortality, behaviour and appearance daily. At the end of the exposure period surviving larvae were individually wet weighed and their lengths measured	X1
Findings: Measured throughout the test, dissolved oxygen was in the range 6.0-7.6 mg/L, pH 8.2-8.3, temperature 24.1-26.2°C and salinity 23.5-26.7‰. The biological results are summarised in the table below. There was no significant effect on hatchability, larval survival or length at any test concentration. Larval weight was affected only at the highest mean measured concentration of 0.38 µg/L. The NOEC, based on mean measured concentration was therefore 0.25 µg/L.	X2 X3

Effects on the early life stages of sheepshead minnow following embryo and 28-day post-hatch exposure to *lambda*-Cyhalothrin

Mean Measured Conc.(ug/L)	Rep.	No. Embryos at Start	No. of Fry Hatched	Larvae Surviving (28 days)	Average Length (mm)	Average Weight (g)
Control	A	30	28	26	18.4	176.1
	B	31	26	26	18.3	168.0
Solvent control	A	30	27	24	18.1	177.0
	B	29	27	25	18.6	186.4
0.04	A	30	30	28	18.7	184.4
	B	31	29	28	17.6	163.3
0.07	A	29	24	22	18.6	190.5
	B	29	25	24	18.5	186.1
0.14	A	32	26	25	18.5	172.4
	B	30	29	27	17.7	163.3
0.25	A	30	26	25	18.5	177.3
	B	29	27	25	18.6	182.6
0.38	A	30	27	26	17.7	154.8*
	B	30	29	28	18.1	161.0*

* level of significance from control values

	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.	**7.4.3.1/02**	**Prolonged toxicity to an appropriate species of fish**
91/414 Annex Point addressed	**II 8.2.2.3/01**	**Fish life cycle test**

		Official use only
Reference point (location) in dossier	**7.4.3.1/02**	
Title:	*Lambda*-Cyhalothrin (Karate PP321): Determination of chronic toxicity to fathead minnow (*Pimephales promelas*) full lifecycle	
Project/Report number:	BL/B/3476	
Author(s):	[redacted]	
Date of report:	1990	
Published:	Not published.	
Testing facility:	[redacted]	
Test substance:	*Lambda*-Cyhalothrin technical, purity: [redacted]	
Study dates	14 March 1988 –March 1990	
GLP:	Yes	
Deficiencies:	None.	
Reliability indicator	1.	

	Official use only
Materials and methods: *Lambda*-Cyhalothrin technical (non-radioactive), purity: [redacted] w/w *lambda*-Cyhalothrin. ^{14}C-methine labelled *lambda*-Cyhalothrin; radiochemical purity [redacted] and specific gravity 1.73 GBq/mmol. The radiolabelled and non-radiolabelled test materials were mixed in triethylene glycol to provide appropriate specific activities for dosing. Fathead minnow (*Pimephales promelas*) embryos obtained within 24 hours of fertilisation were exposed to maintained concentrations of *lambda*-Cyhalothrin in a freshwater flow-through test system at 25±1°C, with exposure continuing for 300 days through a complete life-cycle. Test concentrations were nominally, 0.03, 0.06, 0.12, 0.25 and 10.50 µg/L and corresponding specific activities were 174, 364, 799, 1667, 3492 Bq/µg. A dechlorinated dilution water control and solvent control (containing 12.5 µL triethylene glycol/L) were also included in the study. Actual test concentrations were measured regularly throughout the study using liquid scintillation counting (LSC). The test exposure was initiated by placing two incubation cups, each containing 20 eggs, into duplicate tanks (giving a total of 40 eggs per duplicate and 80 eggs per concentration). The numbers of live and dead eggs were recorded daily and dead eggs and dead larvae were discarded. When the hatch was complete the number of live, deformed, and dead fish in each	X1

duplicate tank was recorded. The percentage hatch was then calculated. Twenty-five larvae, selected at random from each of the two batches in each progeny tank (two tanks per replicate) were then released into the progeny tanks. The "hatch day" was determined to be that day on which the greatest number of fish larvae hatched, this was exposure day 4. Daily observations of fish larvae mortality, behaviour and appearance were made and any abnormal effects recorded. On exposure days 32 and 60 (post-hatch days 28 and 56 respectively) all surviving fish were photographed to determine standard length (snout to base of tail). The fish were transferred to their respective spawning tanks (adult tanks) on exposure day 60 (post-hatch day 56). On exposure day 90, 3 days after viable eggs has first been noticed in the tanks, five spawning tiles were introduced into each adult tank. These were checked daily and any eggs present were collected for quantification and observation using a microscope. On exposure day 134 each tank was divided into four equal sized breeding compartments. On exposure day 149 the fish were individually examined and were randomly paired (1 male and 1 female per breeding chamber). One spawning tile was also introduced into each chamber. These were checked daily and any eggs present were collected for quantification and observation using a microscope. For each duplicate tank, hatchability and early life stage studies were performed (for the F_1 generation) using spawnings of 50 or more eggs. In these studies, the eggs were checked daily and any dead eggs were noted and discarded. When the hatch was complete, the F_1 generation fish larvae from the hatchability trials were discarded, but those for the early life stage studies were transferred to the corresponding progeny tanks. The F_1 generation early life stage tests terminated on post-hatch day 56 and measurements of individual fish larvae weight and length were recorded. The fish larvae from all the early life stage studies from each replicate tank were frozen after measurement and stored for subsequent residue analysis. The adult fish (F_0 generation) exposure was terminated on exposure day 300. Each fish was sexed, weighed and measured. All fish were then frozen and stored for residue analysis. The termination of the last embryo larval test was day 345. **Findings:** Actual *lambda*-Cyhalothrin concentrations measured by LSC were overall 68% of nominal. As *lambda*-Cyhalothrin epimerises in water to the enantiomer pair, *cis* A, both the *cis* A and *cis* B pairs were determined by gas chromatography (GC) analysis. The overall mean measured concentration of the combined *cis* A and *cis* B pairs was 60% of nominal, consistent with the LSC results (overall mean 68%). *Lambda*-Cyhalothrin consists of the *cis* B isomer pair, which amounted to 77% of the total for *cis* A and *cis* B. The measured concentrations determined by LSC were corrected for 77% *cis* B to estimate the actual concentration of *lambda*-Cyhalothrin. None of the 51 tests for inter-replicate differences on hatch, survival and length data showed any significant (P=0.05) differences. Therefore it was deemed acceptable for the purposes of subsequent analysis to pool the replicates of each concentration. **F_0 and F_1 generation survival** Endpoints based on mortality observed at intervals during the study are presented below.	X2 X3

Mortality endpoints for fathead minnow following exposure to *lambda*-Cyhalothrin during a life-cycle test

Generation	Time (days post-hatch)	LC_{50} value (µg/l) based on mean measured concentrations	95% Confidence Limits
F_0	4	0.360	0.252 - 0.765
	7	0.130	0.114 - 0.151
	14	0.128	0.112 - 0.148
	21	0.121	0.108 - 0.138
	28	0.114	0.101 - 0.130
	56	0.108	0.095 - 0.124
F_1	56	0.059	0.052 - 0.067

The lowest observed effect concentrations (LOECs) and no observed effect concentrations (NOECs), based on corrected mean measured concentrations are summarised in the table below. The overall NOEC and LOEC values were 0.031 and 0.062 µg *lambda*-Cyhalothrin/L respectively, based on corrected mean measured concentrations, the critical parameter being survival of F_1 generation larvae to 56 days post-hatch. There was however no significant effect (P = 0.05) on survival to 56 days post-hatch of F_0 generation larvae at this concentration. The overall LOEC and NOEC values for fathead minnow (*P. promelas*) following exposure of newly fertilised eggs to maintained concentrations of *lambda*-Cyhalothrin in a freshwater flow-through test system through out a complete life-cycle, were 0.062 and 0.031 µg *lambda*-Cyhalothrin/L, respectively (based on corrected mean measured concentrations).	X4 X5

LOEC and NOEC values for adverse effects on fathead minnow following exposure to *lambda*-Cyhalothrin during a life-cycle test

Generation	Days	Parameter	LOEC (µg a.s./L)[a]	NOEC (µg a.s./L)[a]
F_0	5	Hatch	>0.273	≥0.273
	28	Survival	0.139	0.062
	56	Survival	0.139	0.062
	28	Length	>0.139 (1)	≥0.139 (1)
	56	Length	>0.139 (1)	≥0.139 (1)
	150-300	Survival	>0.139	≥0.139
	300	Length	>0.139	≥0.139
	300	Weight	>0.139 (1)	≥0.139
	150-300	Egg production	>0.139 (1)	0.062 (2)
F_1	3-5	Hatch	0.139	0.062
	56	Survival	0.062	0.031
	56	Length	>0.139 (1)	>0.139 (1)
	56	Weight	>0.139 (1)	>0.139 (1)
Overall			0.062	0.031

a Based on corrected mean measured concentrations.
(1) Significant increases in egg production relative to the controls occurred at lower concentrations.
(2) Significant increases in length or weight relative to the controls occurred at lower concentrations.

Accumulation of *lambda*-Cyhalothrin

After 300 days exposure to the test solutions, the overall mean tissue concentrations determined in fish from the four test solutions (0.03, 0.06, 0.12 and 0.25 µg *lambda*-Cyhalothrin/L), were 75.9, 131, 245 and 930 µg/kg wet weight respectively. These values represented bioconcentration factors (BCF) ranging from 3952 (in the 0.12 µg/L nominal exposure concentration) to 6691 (in the 0.25 µg/L nominal exposure concentration). The overall mean BCF was 4982 (standard deviation 1233).

At 56 days post-hatch the ^{14}C-radiochemical content of the adult F1 generation larvae in the dilution water and solvent control early life stage tests was found not to be significantly different from background.

The residues measured in the F1 generation larvae from the early life stage tests on the four test concentrations (0.03, 0.06, 0.12 and 0.25 µg *lambda*-Cyhalothrin/L) were 57.8, 107, 326 and 644 µg *lambda*-Cyhalothrin/kg (wet weight), respectively. These values represented bioconcentration factors of 3853, 3452, 5258 and 4633, respectively, with an overall mean of 4299 (standard deviation 806). X6

	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	No remarks

98/8 Doc IIIA section No.	7.4.3.3	Bioaccumulation in an aquatic organism *(headline)*

98/8 Doc IIIA section No.	7.4.3.3.1/1	Bioaccumulation in an appropriate species of fish
91/414 Annex Point addressed	II 8.2.5/01	bioaccumulation

		Official use only
Reference point (location) in dossier	**7.4.3.3.1/01**	
Title:	PP563 (Cyhalothrin) : Accumulation in fish (carp) in a flow-through water system	
Project/Report number:	MITES/58-367	
Author(s):	[redacted]	
Date of report:	1984	
Published:	Not published.	
Testing facility:	[redacted]	
Test substance:	^{14}C-cyclopropane labelled Cyhalothrin (PP563), purity: [redacted]	
Study dates	8 September 1983 - 31 March 1984	
GLP:	Yes	X1
Deficiencies:	None.	
Reliability indicator	1.	

	Official use only
Materials and methods: Carp (*Cyprinus carpio*), were exposed in a flow-through test system to ^{14}C-Cyhalothrin for an exposure phase of 28 days (Cyhalothrin contains ~50% *lambda*-Cyhalothrin and therefore the results are relevant to the assessment of *lambda*-Cyhalothrin). An untreated control system was included. Test vessels were 100 litre glass tanks dosed with the test material in acetone and dilution water at 250 ml/min. Water was sampled daily throughout the exposure period for total 14C-residues. In addition, ^{14}C-residues were extracted from water for characterisation of the radioactivity on days 0, 1, 3, 7, 14, 21 and 28 of exposure. Two fish were sampled for determination of total ^{14}C-residues on each of days 1, 3, 7, 14, 21 and 28. After exposure, remaining fish were transferred to separate depuration tanks, 100 litre glass tanks dosed with untreated water at 250 ml/min. Fish were sampled on days 1, 3 7, 14, 21 and 28 of the 28-day depuration period to monitor elimination of accumulated residues. **Findings:** Mean measured concentration in the water based on total radioactivity was 0.0203 ug Cyhalothrin equivalents/Litre. Measured concentrations of radioactivity in the fish during the exposure phase reached a plateau after 1-2 weeks. Maximum bioconcentration factors were 850, 7340 and 2240 in muscle, viscera and whole fish, respectively. During depuration approximately 80% of the accumulated radioactivity was eliminated from the fish tissues.	X2 X3 X4 X6

	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.	7.4.3.3.1/2	Bioaccumulation in an appropriate species of fish
91/414 Annex Point addressed	II 8.2.5	Bioaccumulation

		Official use only
Reference point (location) in dossier	**7.4.3.3.1/02**	
Title:	Cyhalothrin : The accumulation of Cyhalothrin and its degradation products by channel catfish and *Daphnia magna* in a soil/water system	
Project/Report number:	RJ0427B	
Author(s):	[redacted]	
Date of report:	1985	
Published:	Not published.	
Testing facility:	[redacted]	
Test substance:	^{14}C-cyclopropane labelled Cyhalothrin, radiochemical purity [redacted]	
Study dates	May 1983 – November 1984	
GLP:	Yes	
Deficiencies:	None.	
Reliability indicator	1.	

	Official use only
Materials and methods: *Daphnia magna* and channel catfish (*Ictalurus punctatus*) were exposed to ^{14}C-Cyhalothrin in test vessels containing soil and water. The test vessels were circular stainless steel tanks, to which 130 kg (dry weight) loamy sand soil (pH 5.2, OM 1.7%, sand 80%, silt 10%, clay 10%) was added. The soil added to one tank was treated with ^{14}C-labelled Cyhalothrin (application rate was 122 µg/kg dry weight soil, approximately equivalent to a 50 g/ha rate mixed into the top 3 cm soil). The soil was incubated for 21 days at ambient temperature, and was kept moist by watering. At the end of the incubation period, approximately 1400 litres water was added to give a total soil/water depth of 48 cm. Untreated control systems were included. The water was aerated and the system allowed to equilibrate for 3 days before the addition of 150 channel catfish (*Ictalurus punctatus*) and 1600 *Daphnia*, separated into groups of 100 in floating traps with a mesh bottom. The fish were fed pelleted food daily, *Daphnia* were not given supplemental food. Following an exposure period of 31 days, 60 of the remaining fish and 7 traps of *Daphnia* were transferred to separate flow-through systems for depuration periods of 42 and 7 days respectively. Soil, water, fish and *Daphnia* were sampled throughout the exposure period. Soil was extracted for total ^{14}C-residues. Seven fish were taken at each sample time, of which three were analysed whole and the remaining four separated into viscera and muscle prior to	X1