Arch. Environ. Contam. Toxicol. 46, 324–335 (2004)
DOI: 10.1007/s00244-003-2315-3

ARCHIVES OF
Environmental
Contamination
and Toxicology

Comparison of Laboratory Single Species and Field Population-Level Effects of the Pyrethroid Insecticide λ-Cyhalothrin on Freshwater Invertebrates

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Received: 22 January 2003/Accepted: 23 August 2003

Abstract. The toxicity of the pyrethroid insecticide λ-cyhalothrin to freshwater invertebrates has been investigated using data from short-term laboratory toxicity tests and *in situ* bioassays and population-level effects in field microcosms. In laboratory tests, patterns of toxicity were consistent with previous data on pyrethroids. The midge *Chaoborus obscuripes* was most sensitive (48- and 96-h EC_{50} = 2.8 ng/L). Other insect larvae (Hemiptera, Ephemeroptera) and macrocrustacea (Amphipoda, Isopoda) were also relatively sensitive, with 48- and 96-h EC_{50} values between 10 and 100 ng/L. Generally, microcrustacea (Cladocera, Copepoda) and larvae of certain insect groups (Odonata and Chironomidae) were less sensitive, with 48-h EC_{50} values higher than 100 ng/L. Mollusca and Plathelminthes were insensitive and were unaffected at concentrations at and above the water solubility (5 μg/L). Generally, the EC_{50} values based on initial population responses in field enclosures were similar to values derived from laboratory tests with the same taxa. Also, the corresponding fifth and tenth percentile hazard concentrations (HC_5 and HC_{10}) were similar (laboratory HC_5 = 2.7 ng/L and field HC_5 = 4.1 ng/L; laboratory and field HC_{10} = 5.1 ng/L), at least when based on the same sensitive taxonomic groups (insects and crustaceans) and when a similar concentration range was taken into account. In the three field enclosure experiments and at a treatment level of 10 ng/L, consistent effects were observed for only one population (*Chaoborus obscuripes*), with recovery taking place within 3 to 6 weeks. The laboratory HC_5 (2.7 ng/L) and HC_{10} (5.1 ng/L) based on acute EC_{50} values of all aquatic arthropod taxa were both lower than this 10 ng/L, a concentration that might represent the "regulatory acceptable concentration." The HC_5 and HC_{10} values in this study in The Netherlands (based on static laboratory tests with freshwater arthropods) were very similar to those derived from a previous study in the United Kingdom (1.4 and 3.3 ng/L). This suggests that for pesticides like λ-cyhalothrin, HC_5 values based on static laboratory tests may provide a conservative estimate of the potential for community-level effects under field conditions. While these HC_5 values are conservative for initial effects, they do not provide information on recovery potential, which may be important for regulatory decision-making.

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λ-Cyhalothrin is a pyrethroid insecticide used to control insect pests in a wide range of crops. Like other pyrethroids, it is highly toxic to fish and aquatic arthropods in standard laboratory studies (Maund *et al.* 1998; Solomon *et al.* 2000). The mode of action of pyrethroids is through interference with various ion channels in the nerve axon, and in aquatic organisms disturbance of concentration gradients across membranes may also cause osmotic stress (Clark *et al.* 1982). Symptoms of toxicity occur within a few hours of exposure in aquatic arthropods. This results initially in hyperactivity and, in the longer term, disruption of the nervous system, which, if exposure is maintained long enough, can result in death. However, if exposure is reduced shortly after insecticide application, initially stressed organisms may recover, explaining why field-observed effects of pyrethroids are generally less severe than those suggested by standard laboratory tests, in which exposure concentrations usually are maintained more or less constant (Hill *et al.* 1994; Giddings *et al.* 2001). The very low water solubility (5 μg/L at 20°C), low volatility (vapor pressure = 2.0×10^{-4} mPa), and extremely high octanol–water partition coefficient ($\log K_{ow}$ = 7.0) mean that λ-cyhalothrin dissipates very rapidly, and generally exposure of aquatic organisms will be transient in surface waters (Hand *et al.* 2000). In ditch enclosure experiments, the time required for dissipation of 50% of the amount applied (DT_{50}) was less than 1 day (Leistra *et al.* 2003). Considering the mode of action and very rapid dissipation, acute laboratory toxicity data derived from static tests are therefore the most relevant for assessing potential effects of λ-cyhalothrin in aquatic ecosystems.

Higher-tier approaches for assessing potential risks of pesticides have been under discussion for a number of years (Campbell *et al.* 1999; Giddings *et al.* 2002). One option for refining the risk assessment is to test additional species to reduce the uncertainty (and hence safety factors needed) of assessments based on sensitive standard test species (e.g., *Daphnia* for invertebrate toxicity). One approach to using such data is to construct species sensitivity distributions (SSDs) (Campbell *et al.* 1999). The application of SSDs in risk assessment is currently under debate, particularly which species and toxicity data (acute or chronic) should be used and which

endpoint from the SSD is most appropriate. The HC_5 (hazardous concentration for 5% of the species) is one such SSD-derived "regulatory acceptable concentration" (Posthuma *et al.* 2002). Campbell *et al.* (1999) state that perhaps also HC_{10} values might be used to derive acceptable concentrations but that additional research is needed—with compounds that differ in agricultural use pattern (e.g., number and frequency of applications), environmental fate, and toxicity to freshwater organisms—to confirm this.

In this study, we compare the responses of freshwater invertebrates to λ-cyhalothrin in laboratory and field studies. Our general aims are to examine relationships between laboratory and field data, particularly to relate laboratory SSDs to field SSDs and to compare SSD-derived threshold levels to community threshold levels.

Specific aims of the work were as follows.

- To generate acute laboratory toxicity values for λ-cyhalothrin with a variety of lentic freshwater invertebrates typical of Dutch drainage ditches and shallow freshwater ecosystems
- To investigate the potential of laboratory toxicity tests with indigenous species in predicting treatment-related field responses
- To evaluate the SSD approach by comparing SSD curves derived from laboratory and field data to community-level endpoints

Materials and Methods

Laboratory Experiments

Test Species and Test Units. *Daphnia galeata, Simocephalus vetulus,* and *Proasellus coxalis* were obtained from temporary (2- to 4-month) laboratory cultures. These cultures were set up with individuals collected in shallow freshwater ecosystems in the vicinity of Wageningen. The other species used were collected from the field (Table 1). The life stage or the size of the organisms tested is given in Table 1. All species were acclimated to laboratory conditions for at least 2 days, during which suitable food material was provided (e.g., decomposing *Populus* leaves for detritivores and daphnids for the carnivorous midge *Chaoborus*).

Various glass test systems were used to perform the single species tests (Table 2). For *Notonecta glauca, Erythromma viridulum,* and *Sialis lutaria,* aquaria were divided with stainless-steel gauze into compartments to house the test organisms individually to avoid cannibalism. In test systems with other macroinvertebrates, stainless-steel gauze (approximately 90 cm^2) was provided as a substrate. All vessels and aquaria were covered with a glass lid.

Tests were performed with 10 organisms per test system, except those for *Daphnia galeata* and *Simocephalus vetulus,* where 25 organisms were used. For *Notonecta glauca, Erythromma viridulum,* and *Sialis lutaria* nine organisms were tested per test unit.

After dosing with λ-cyhalothrin and mixing the compound in the test solution with a glass rod, the organisms were allocated evenly to test units according to their body size.

Test Media and Exposure Concentration. In The Netherlands an important emission route of pesticides to surface water is spray drift. For this reason, all tests were performed with an emulsifiable concentrate formulation of Karate [a.i. λ-cyhalothrin, a 1:1 mixture of (*R*)-α-cyano-3-phenoxybenzyl (1*S*)-*cis*-3-(*Z*)-(2-chloro-3,3,3-trifluoroprop-1-enyl-2,2-dimethylcyclopropane-carboxylate and (*Z*)-α-cyano-3-phenoxybenzyl (1*S*)-*cis*-3-(*Z*)-(2-chloro-3,3,3-trifluoroprop-1-enyl-2,2-dimethylcyclopropanecarboxylate at a concentration of 50 g/L]. Stock solutions were made by diluting the test compound in distilled water and concentrations were checked analytically. Test media were prepared by diluting stock solutions in water collected from an uncontaminated pond (Sinderhoeve Experimental Station, The Netherlands). This water was first filtered using a plankton net with a mesh size of 55 μm. The 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^-, and 4.9–7.8 mg/L chloride and had an alkalinity of 0.60–0.88 meq/L.

All single species tests were set up as static tests with a single application of λ-cyhalothrin. Since λ-cyhalothrin shows fast dissipation under field conditions, the exposure regime in static tests is considered more appropriate than a more or less constant exposure. Tests were performed with six concentrations and an untreated control. For those species that were expected to be insensitive (*Lymnaea stagnalis, Bithynia tentaculata,* and *Polycelis nigra/tenuis*), only four concentrations were used. Test concentrations were chosen using a factor of 3. Test media without λ-cyhalothrin were used as controls (there was no blank formulation testing). All tests were done in duplicate.

Prior to application, samples of stock solutions were taken to measure concentrations of λ-cyhalothrin. The measured concentration in the stock solution was used to calculate the initial test concentration in the test vessels. In addition, samples of test media were taken from the vessels for λ-cyhalothrin analysis at 1 h after application to check the treatment concentration. Assessing the dynamics of the λ-cyhalothrin concentration through time was done measuring residues in the highest treatment level at 1, 4, 24, 48, and 96 h after application in the test units containing *Chaoborus obscuripes.* At 1, 24, and 96 h after dosing to test units containing *Lymnaea stagnalis, Bithynia tentaculata,* and *Polycelis nigra/tenuis,* water samples were taken to determine the influence of size of the test organism on the concentration decrease of the test compound.

Physicochemical Measurements. The tests were conducted in a temperature-controlled room (20°C) with a light/dark regime of 14 h light and 10 h darkness. During the tests, the temperature of the test media remained within the limits given in Table 2. No aeration of test media took place during tests.

Within 4 h of dosing, dissolved oxygen concentrations (YSI Model 58) and pH (WTW pH323, equipped with a Sentix pH electrode) were measured in all test units. At 24, 48, and 96 h after dosing, these parameters were measured at least in controls and treatments with highest concentrations.

Monitoring of Effects. Sublethal and/or lethal effects were monitored. Immobility was categorized in different ways for different species (Table 2). Since mortality is the ultimate phase of immobility and in ecosystems immobile organisms suffer a high risk of predation, scores for mortality were incorporated in those of immobility. For arthropods, effects were scored as mortality when no response of any kind was observed for about 10 s under a stereomicroscope after repeated stimulation of the organisms body with a dissection needle.

Except for zooplankton species, organisms were at least observed at 24, 48, and 96 h after application. Tests with zooplankton species were only observed at 24 and 48 h. For all toxicity data presented here, survival of organisms in controls was higher than 80%, except for the test with *Sigara striata.*

Field Experiments

Experimental Design of Enclosure Experiments. In 2000, three field experiments were performed with λ-cyhalothrin in enclosures placed

Table 1. Taxonomic group, origin, and stage of tested species

(Sub)class, order, genus, and species[e]	Source	Stage and length (mean ± SD; $n \geq 10$)
(Macro-)Crustacea		
Isopoda		
Asellus aquaticus Linnaeus	Ditches, Veenkampen, Wageningen	(Sub)adult, 8.8 ± 0.8 mm
Proasellus coxalis Dollfus	Laboratory culture[a]	(Sub)adult, 4.6 ± 0.5 mm
Amphipoda		
Gammarus pulex Linnaeus	Ditches, Veenkampen, Wageningen	(Sub)adult, 11.6 ± 1.4 mm
(Micro-)Crustacea		
Cladocera		
Daphnia galeata Richard	Laboratory culture[b]	(Sub)adult, 0.7 ± 0.08 mm
Simocephalus vetulus Müller	Laboratory culture[b]	(Sub)adult, 1.7 ± 0.3 mm
Insecta		
Ephemeroptera (mayflies)		
Cloeon dipterum Linnaeus	Ditches, Veenkampen, Wageningen	Larvae, 4.1 ± 0.9 mm
Caenis horaria Linnaeus	Experimental ditches, Alterra, Renkum	Larvae, 4.6 ± 0.7 mm
Hemiptera (true bugs)		
Sigara striata Linnaeus	Pond, Alterra, Wageningen	Adult, 7.4 ± 0.8 mm
Notonecta glauca Linnaeus	Experimental ditches, Alterra, Renkum	Adult, 14.4 ± 1.7 mm
Diptera (true flies)		
Chaobetus obscuripes Van der Wulp	Experimental ditches, Alterra, Renkum	Larvae,[c] 1.9 ± 0.1 mm[d]
Macropelopia sp. Thienemanns	Experimental ditches, Alterra, Renkum	Larvae, 7.6 ± 1.7 mm
Zygoptera (dragonflies)		
Erythromma viridulum Charp	Pond, Alterra, Wageningen	Larvae, 17.3 ± 2.0 mm
Megaloptera (alderflies)		
Sialis lutaria Linnaeus	Experimental ditches, Alterra, Renkum	Larvae, 17.8 ± 4.2 mm
Mollusca (mollusks)		
Gastropoda (snails)		
Lymnaea stagnalis Linnaeus	Ditches, Veenkampen, Wageningen	(Sub)adult, 24.5 ± 2.6 mm
Bithynia tentaculata Linnaeus	Ditches, Veenkampen, Wageningen	(Sub)adult, 9.7 ± 0.8 mm
Plathelminthes (flatworms)		
Turbellaria		
Polycelis nigra/tenuis	Ditches, Veenkampen, Wageningen	Adult (na)[f]

[a] Originally obtained from a ditch near Wageningen.
[b] Originally obtained from experimental ditches at the Sinderhoeve experimetal station (Alterra).
[c] Larvae stadia instar 3–4.
[d] Head length.
[e] Nomenclature according to Limnofauna Neerlandica (Mol 1984).
[f] na—data not available.

in experimental ditches at the Sinderhoeve experimental station, Renkum, The Netherlands. Two experiments were performed in spring and focused on the impact of λ-cyhalothrin on aquatic invertebrates in (1) a mesotrophic macrophyte-dominated ditch and (2) a eutrophic plankton-dominated ditch. The third experiment was performed in late summer in a macrophyte-dominated ditch in order to evaluate potential seasonal differences in effects of λ-cyhalothrin on aquatic invertebrates in macrophyte-dominated ditches.

In all three ditches, 14 enclosures (diameter, 1.05 m; height, 0.9 m) were installed. The depth of the water column was 0.5 m. Twelve enclosures were used for effect observations and two were used to study fate. In all three experiments, a regression design was performed: six concentrations of λ-cyhalothrin (0, 10, 25, 50, 100, 250 ng/L) were applied to duplicate enclosures. Concentrations of λ-cyhalothrin applied to the enclosures corresponded to 0.2, 0.5, 1, 2, and 5% spray drift emission at label-recommended rates for tulips (15 g a.i./ha) to a Dutch standard ditch of 30-cm water depth water. The compound was applied as a formulated product, Karate Zeon 10 CS (a.i. 100 g/L as capsule suspension). Three applications were made at weekly intervals.

The zooplankton was sampled each week by taking depth-integrated 5-L samples which were concentrated in a plankton net (mesh size, 55 μm) and preserved with formalin (4% vol/vol). Organisms in these samples were counted with an inverted and/or stereo microscope. Macroinvertebrates were sampled at 2-week intervals using artificial substrates. In each enclosure, two multiplate samplers and two pebble baskets served as artificial substrate. Macroinvertebrates present on the substrates were collected, identified, and counted alive. After sampling, organisms were returned to the enclosures. Full details of the enclosure experiments are presented by Roessink *et al.* (in press) and Van Wijngaarden *et al.* (in press).

In Situ *Bioassays.* During the enclosure experiments, in situ bioassays were performed with *Chaoborus obscuripes* and *Asellus aquaticus.* In the late summer experiment *Proasellus coxalis* was also tested. The bioassay cages were constructed of stainless-steel gauze (mesh size, 0.5 mm; length, 33 cm; diameter, 6 cm; and volume, approx 930 cm^3). In the spring experiments, one bioassay cage was placed in each enclosure for each organism. In the late summer experiment, three cages were used. The bioassay cages contained 30 *Chaoborus obscuripes* larvae, and 25 each of *Asellus aquaticus* and *Proasselus coxalis.* Survival was monitored for 48 h after λ-cyhalothrin application. Each day, the cages were carefully pulled up and down to improve exchange of water.

Table 2. Testing conditions for selected species in laboratory toxicity experiments with the insecticide λ-cyhalothrin

Species	Test unit volume (L)	Initial test conc. (ng/L), min–max (±mean CV)	Temperature (°C)	pH, min–max	O_2 (mg/L), min–max	Criterion (a) for effect endpoints
Asellus aquaticus	1.8	4.9–422 (±2.9%)	20 ± 0.6	7.0–7.3	6.4–7.6	TS + MB
Bithynia tentaculata	1.8	219–34,425[b] (±10%)	20 ± 0.4	7.0–7.9	6.8–11.0	MB + BA[a]
Caenis horaria	1.8	5.2–436 (±2.4%)	22 ± 1.4	6.9–7.5	6.4–7.9	TS + MB
Chaoborus obscuripes	1.8	0.3–27 (±0.3%)	20 ± 1.0	7.1–8.0	8.8–10.2	TS, MB
Cloeon dipterum	1.8	2.5–265 (±1.4%)	20 ± 0.8	6.5–7.8	8.0–9.4	TS + MB
Daphnia galeata	0.6	44.4–3,596 (±0.04%)	20 ± 0.8	7.3–8.0	7.9–9.3	MB
Erythromma viridulum	3.0	15.5–1,460 (±2.4%)	22 ± 1.0	7.1–7.7	5.6–7.8	TS + MB
Gammarus pulex	1.8	1.6–156 (±0.4%)	20 ± 0.5	6.1–7.3	4.1–7.9	TS + MB
Lymnaea stagnalis	1.8	207–29,129[b] (±5%)	20 ± 0.4	6.7–8.0	2.8–9.1	LB
Macropelopia sp.	0.65	26.5–2,202 (±0.8%)	20 ± 0.9	7.4–7.8	7.0–7.8	TS + MB
Notonecta glauca	3.0	2.5–265 (±0.4%)	20 ± 0.5	6.7–7.9	8.2–9.8	TS + MB
Polycelis nigra/tenuis	1.8	226–30,759[b] (±6.2%)	20 ± 0.2	7.4–7.9	9.5–11.2	LB
Proasellus coxalis	1.8	4.9–416 (±1%)	20 ± 0.4	7.1–7.4	6.3–7.6	TS + MB
Sialis lutaria	3.0	25.7–2,183 (±0.4%)	20 ± 0.5	6.6–7.8	6.7–7.6	TS + MB
Sigara striata	1.8	5–325 (±3.2%)	20 ± 0.7	6.8–7.7	8.2–9.4	TS, MB
Simocephalus vetulus	0.6	44.5–3,598 (±0.03%)	20 ± 0.8	7.2–8.0	7.9–9.0	MB

Note. Criteria for effect endpoints: TS, response to tactile stimuli; MB, mobility behavior; LB, locomotional behavior; BA, behavior of avoidance. ±mean CV, ±mean coefficient of variation in % of replicates within each treatment level.
[a] Closing operculum.
[b] Concentrations above the solubility of λ-cyhalothrin (5000 ng/L).

Chemical Analyses

Chemical analysis of the active substance was performed in stock solutions and in water samples from test systems. Depth-integrated water samples were taken out of the test units. The well-mixed subsamples were transferred into a 250-ml flask and the mass of each sample was recorded. A 30-ml volume of hexane was added to the flask and the water sample was shaken for at least 0.5 h. To measure the higher test concentrations, 1.5 ml of the hexane extract was added to a vial for analysis by gas–liquid chromatography (GLC). For the lower concentrations, a known volume of between 10 and 25 ml of the hexane extract was transferred to a tube, which was then placed in a water bath of 40°C. The solvent was evaporated by an air stream and the residue was taken up in 1.5 ml hexane for analysis by GLC.

The sample extractions were analyzed with a HP 5890 gas chromatograph equipped with an electron-capture detector and an HP 6890 autosampler. Samples of 3 μl were injected (splitless) with an HP6890 autosampler. The carrier gas was helium (2.3 ml/min). The flow rate of N_2 through the detector (as auxiliary gas) was 60 ml/min. The injection and detection temperatures were 250 and 325°C, respectively. A typical retention time of λ-cyhalothrin was 19.3 min. Known concentrations in the range of 0.2 to 25 μg/L were injected to construct the calibration curve.

The limit of determination in the water samples was 2 ng/L. Although recovery of the compound was not specifically determined in this study, results in concurrent studies in the same matrices showed typical recoveries of approximately 90% (Leistra *et al.* 2003).

Statistical Analyses

Statistical Analyses in Single Species Tests and Bioassays. The EC_{10} (or LC_{10}) and EC_{50} (or LC_{50}) values and their confidence intervals were calculated by a log concentration-logit effect regression model. A binomial distribution for the fraction of affected species was assumed. This model is used for single species and bioassay data because in both tests a fixed number of individuals was placed in the test units. Within the regression, calculated toxicity values were adapted for immobility or mortality in the controls. The concentration–effect model used was as follows:

$$\text{Expected affected fraction} = \frac{(1 - c)}{(1 + \exp -b[\text{In}(\text{concentration}) - a])}$$

where $a = \ln(EC_{50})$ or $\ln(LC_{50})$, b = slope of parameter, c = fraction of affected individuals in controls.

The possibility to calculate confidence limits depended on the number of concentrations with partial responses and their distribution over the 0 and 100% effect range (singularity in regression model). Results from duplicates per treatment were combined in one regression analysis but based on both sets of data.

Statistical Analyses in Field Enclosure Studies. Short-term response data of the enclosure experiments were the most appropriate to compare with acute laboratory toxicity data. Data from artificial substrate samples, collected 9 ± 1 days after the first application of λ-cyhalothrin, were used to calculate EC_{10} and EC_{50} values for field populations of macroinvertebrates. Similarly, densities of zooplankton in the water column of the enclosures as sampled 13 days post first application were used. Application of λ-cyhalothrin to enclosures occurred two times in this period (days 0 and 7). In contrast to single species tests and bioassays, the densities of individuals were not known a *priori* and could have been different between replicates. Therefore, the concentration–response model used for the field enclosure studies was different from the one based on a fixed number of exposed organisms (e.g., fraction affected species). Figure 1 shows the differences between the models for an example species. The model gives a sigmoid concentration–response curve for ln(concentration), and *numbers* were assumed to be quasi-Poisson distributed. The EC_{50} and the EC_{10} values of the field enclosures were defined as the concentrations at which numbers were reduced to the abundance in the controls by 50

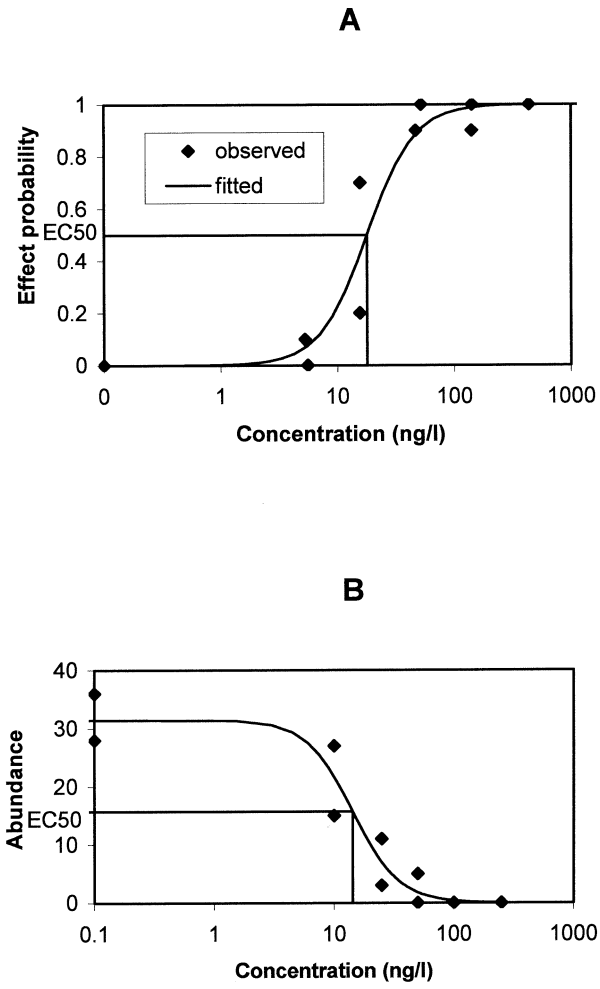

Fig. 1. Concentration–response curves as assessed in the laboratory single species test and the field enclosure study for the macroinvertebrate *Caenis horaria.* Curve **A** shows the logistic relation between probability of scored effect (e.g., immobility) and ln(concentration) of λ-cyhalothrin. The hypothetical model describing the expected decrease in numbers of individuals (abundance) of field populations in relation to increased exposure concentrations of λ-cyhalothrin is shown in curve **B.** The calculated laboratory EC_{50} and field EC_{50} values (ng/L) with their 95% confidence limits are, respectively, 14.4 (8.1–25.0) and 17.3 (12.8–25.1)

and 10%, respectively. The regression models were programmed in Genstat (Payne *et al.* 1987).

The concentration–effect model for enclosures was as follows:

$$\text{Expected number} = \frac{(d)}{(1 + \exp -\text{b}[\ln(\text{concentration}) - a])}$$

where $a = \ln(EC_{50})$, b = slope parameter, d = expected *number* in the control enclosures.

Responses at the community level were analyzed by the principal response curve (PRC) method (Van den Brink and Ter Braak 1999). No observed effect concentrations at the community level ($NOEC_{community}$) calculations were based on sample scores of the first principal component of each sampling date by applying the Williams (1972) test (Van den Brink *et al.* 1996). In this paper, only a summary of these data is used to compare with results of the laboratory-based toxicity data. Full discussion of the multivariate analyses is presented by Roessink *et al.* (in press) and Van Wijngaarden *et al.* (in press).

Species Sensitivity Distribution (SSDs). SSD analyses were conducted according to Aldenberg and Jaworska (2000) and the computer program ETX—version 1.403 (Van Vlaardingen and Traas 2002). The spreadsheet calculates the HC_5 and HC_{50} and their 95% confidence limits of toxicity data. In addition, HC_{10} values and confidence limits were calculated using k_s values provided by Aldenberg and Jaworska (2000).

The model used assumes a log-normal distribution of toxicity data; thus,

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

where $x = \ln(EC_{50})$, μ = median, $EC_{50} = \ln(HC_{50})$, σ = standard deviation of $\ln(EC_{50})$.

The SSD was defined as the cumulative frequency distribution of toxicity data as follows:

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

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 at $p \geq 0.05$ (Postuma *et al.* 2002). 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.

Results and Discussion

Exposure Concentrations of λ-Cyhalothrin

In the laboratory single species tests, calculated initial concentrations in the test units (based on measurements of the active substance in the stock solutions) were used to estimate EC_x or LC_x values. Initial concentrations were used because of the expected short-term effect and high dissipation rate of λ-cyhalothrin. Concentrations of λ-cyhalothrin measured 1 h post-treatment in water from test vessels indicated that the intended initial concentrations were reached (Table 3). The ranges of initial test concentrations for the different laboratory experiments are presented in Table 2. The data presented in Table 2 also show that, on average, the variation in concentration of the active substance between treatment replicates was small.

In laboratory studies, generally less than 50% of the initial concentration remained 1 day after application (Table 3). Test units with relatively small species (e.g., *Polycelis nigra/tenuis*) tended to show slower dissipation rates than those with larger species (e.g., *Lymnaea stagnalis*), suggesting that uptake and/or metabolism by the organisms may have also influenced dissipation.

For the *in situ* bioassays and free-living populations in the enclosures, initial nominal concentrations were used for the estimations of EC_x or LC_x values. Analyzed concentrations in the

Table 3. Percentages of remaining λ-cyhalothrin in water, relative to the initial test concentration, during laboratory single species tests with four aquatic invertebrates

Species	Fraction (%) of compound after			
	1 h	24 h	48 h	96 h
Lymnaea stagnalis	97	31	—	2
Bithynia tentaculata	88	47	—	9
Chaoborus obscuripes	100	49	34	10
Polycelis nigra/tenuis	98	51	—	15

solutions applied to the test systems varied on average between 93 and 112% of the intended nominal concentration between the three enclosure experiments and the first two applications per experiment (Table 4). The first two applications are of particular importance when interpreting the toxicity values based on abundance data of free-living populations of arthropods in the enclosures (sampled between the second and the third application). On average, the initial exposure concentrations based on measured levels in the application solutions were somewhat higher than the intended nominal concentrations. Consequently, the calculated field toxicity values based on initial nominal concentrations can be considered as more or less conservative.

Concentrations of λ-cyhalothrin in the water column of the field enclosures decreased more rapidly than in laboratory test systems. One day after application, only 24–40% of applied λ-cyhalothrin could be detected in the water column of the enclosures, while after 3 days this was 1.8–6.5% (Leistra *et al.* 2003) (Table 3).

Sensitivity of Indigenous Species to λ-Cyhalothrin in Laboratory Tests

Toxicity data for the 16 aquatic invertebrates tested by us are presented in Table 5. These data and those published by Maund *et al.* (1998) allow a comparison of the sensitivity profile of freshwater arthropods from Dutch shallow freshwater ecosystems (our dataset) with a similar (but independent) dataset from the United Kingdom. In Table 6, the taxa tested by us and by Maund *et al.* (1998) are ranked in order of decreasing short-term toxicity (48-h EC_{50}).

Of all species tested, *Chaoborus obscuripes* was the most sensitive. The EC_{50} value found for this species was similar to that of *Chaoborus* sp. reported in the study by Maund *et al.* (1998). A more or less equally sensitive species was the amphipod *Hyallella azteca* (48-h EC_{50} = 2.3 ng/L) (Table 6).

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*). *Daphnia galeata* was the most sensitive microcrustacean tested (48-h EC_{50} = 116 ng/L). 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 (Table 6).

The flatworm (*Polycelis nigra/tenuis*) and snails (*Lymnaea stagnalis* and *Bithynia tentaculata*) tested—all nonarthropods—can be characterized as nonsensitive species, since their EC_{50} values are above the solubility of λ-cyhalothrin. *Polycelis nigra/tenuis* and *Lymnaea stagnalis* showed no visible treatment-related response. *Bithynia tentaculata,* however, responded by means of closing the operculum—avoidance behavior. 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 effects.

Validity of Single Species Tests Performed

Internal Validity. For most of the tested species, the confidence intervals of the 48/96-h toxicity values are relative small (Table 5). Only in the case of extrapolation, e.g., when the $E(L)C_{50}$ value was greater than the highest test concentration, was a wide confidence interval determined (e.g., *Sialis lutaria*). For most of the 48/96-h toxicity data presented in Table 5, however, the lower and upper confidence limit differed by no more than a factor of 2–3.

External Validity. Of all tested species in the present study, four species can be directly compared with other data (Maund *et al.* 1998). Toxicity values reported for these taxa (*Chaoborus* sp., *Gammarus pulex, Asellus aquaticus,* and *Cloeon dipterum*) appear to be very similar (Table 6). It is known that under standardized laboratory conditions, the EC_{50} values of a toxicant can vary by approximately a factor of 3 within single species (Baird *et al.* 1989). Considering the relatively small confidence limits of the calculated EC_{50} values, and the similar results obtained by us and Maund *et al.* (1998), it is concluded that our laboratory data are consistent and can be used with confidence for the ecotoxicological risk assessment of λ-cyhalothrin.

Comparison of 48- and 96-h Toxicity Data and EC_{50} and LC_{50} Values

SSDs were used to compare the distributions of toxicity values and different time points. All log-transformed toxicity data (EC_{50} and LC_{50} for 48 and 96 h) were derived from a normal distribution ($p \geq 0.10$ for the Anderson–Darling test).

When considering all species tested, the overall trend was that the species sensitivity distributions were very similar between the 48-h and the 96-h EC_{50} values (Fig. 2A). No significant differences were determined ($p > 0.10$). Nevertheless, some individual taxa (e.g., *Sialis lutaria, Macropelopia* sp.) clearly showed a lower 96-h EC_{50} (Table 5). For these species the incipient effect, when scoring immobility, apparently was not yet reached after 48 h. When considering mortality, lower 96-h LC_{50} values were recorded for all species tested when compared with corresponding 48-h LC_{50} values. A higher overall mortality with increasing exposure time was also reflected in the SSD curves presented in Figure 2B, but significant differences in both curves could not be demonstrated ($p > 0.10$).

As might be expected when considering the mode of action of λ-cyhalothrin, EC_{50} values were lower than the corresponding LC_{50} values. As also might have been expected, such differences

Table 4. Percentages of calculated initial concentrations of λ-cyhalothrin in the overlying water of enclosures relative to intended nominal concentrations

	% of nominal concentration		
Experiment	Treatment 1	Treatment 2	Treatment 3
Macrophyte-dominated			
Spring	104 (100–115)	112 (105–126)	94 (75–113)
	$n = 5$	$n = 5$	$n = 5$
Summer	111 (105–117)	93 (85–104)	100 (81–120)
	$n = 5$	$n = 5$	$n = 5$
Plankton-dominated			
Spring	102 (97–105)	106 (100–114)	92 (74–116)
	$n = 5$	$n = 5$	$n = 5$

Note. Average values (and range) between treatment levels are presented for each application and the three different enclosure experiments. Calculated initial concentrations in the enclosures are based on measured concentrations in the application solutions.

Table 5. Results of short-term static laboratory toxicity tests with the insecticide λ-cyhalothrin

		EC_x (ng/L) (95% confidence limits)		LC_x (ng/L) (95% confidence limits)	
	x	48 h	96 h	48 h	96 h
Chaoborus obscuripes[a]	10	0.6 (0.3–1.3)	1.2 (0.6–2.2)	>27.4	5.4 (1.1–26.2)
	50	2.8 (1.8–4.4)	2.8 (2.0–3.9)	>27.4	75.7 (9.8–588)
Notonecta glauca	10	7.2 (3.5–14.6)	9.2 (1.7–48.3)	4.7 (1.5–14.5)	9.2 (1.7–48.3)
	50	14.8 (10.0–21.9)	16.4 (7.5–36.1)	22.6 (12.6–40.4)	16.4 (7.5–36.1)
Proasellus coxalis	10	13.0 (8.7–19.6)	14.8 (9.2–23.8)	9.7 (2.9–33.0)	8.9 (3.4–23.5)
	50	17.7 (13.1–23.9)	27.4 (20.5–36.7)	78.8 (45.1–138)	44.6 (27.4–72.4)
Caenis horaria	10	6.4 (3.6–11.4)	3.6 (1.2–11.1)	20.0 (6.7–59.6)	4.6 (1.0–21.9)
	50	17.9 (12.8–25.1)	13.6 (7.7–24.0)	257 (124–533)	34.6 (16.0–75.1)
Sigara striata[b]	10	7.5 (1.9–29.7)	—	17.8 (3.6–87.5)	—
	50	18.2 (9.2–36.1)	—	49.2 (21.7–112)	—
Gammarus pulex	10	14.2 (7.4–27.0)	13.1 (7.0–24.7)	17.9 (7.2–44.7)	13.1 (7.0–24.7)
	50	23.6 (16.0–34.9)	24.2 (15.9–36.7)	31.4 (19.8–49.8)	24.2 (15.9–36.7)
Asellus aquaticus	10	10.7 (6.6–17.6)	9.7 (5.7–16.7)	18.7 (7.8–44.9)	9.1 (3.4–24.8)
	50	24.8 (18.4–33.4)	24.8 (18.1–33.9)	140 (82.3–240)	75.2 (45.7–124)
Cloeon dipterum	10	7.2 (3.7–14.0)	74.6 (*)	18.8 (8.4–42.5)	25.3 (13.1–48.6)
	50	24.8 (17.2–35.8)	88.3 (*)	122 (71.9–207)	105 (68.4–162)
Sialis lutaria[c]	10	12.2 (4.0–37.1)	22.1 (8.3–58.8)	>2179	14.0 (0.0–>2179)
	50	51.5 (30.3–87.7)	28.0 (17.4–45.2)	>2179	>2179 (55.5–>2179)
Daphnia galeata	10	44.0 (24.8–78)	—	63.9 (28.1–146)	—
	50	117 (86.6–157)	—	397 (267–590)	—
Macropelopia sp.	10	125 (67.9–231)	16.2 (*)[e]	165 (71.0–384)	76.9 (23.0–257)
	50	244 (183–326)	64.3 (*)	1019 (608–1707)	698 (383–1274)
Erythromma viridulum	10	377 (223–635)	381 (190–765)	1104 (*)	381 (190–765)
	50	689 (479–992)	493 (280–869)	1583 (*)	493 (280–869)
Simocephalus vetulus	10	334 (176–631)	—	558 (338–921)	—
	50	957 (707–1295)	—	1340 (1042–1724)	—
Bithynia tentaculata	10	At conc. ≥8900 behavior of avoidance (closing of operculum) determined[d]			
	50				
Lymnaea stagnalis	10	No concentration–response relationship		No concentration–response relationship	
	50				
Polycelis nigra/tenuis	10	No concentration–response relationship		No concentration–response relationship	
	50				

[a] EC and LC values based on nominal concentration.
[b] Mortality in controls >20% (40%).
[c] Indicative because of cannibalism in controls. Cannibalism is not indicated as a negative response.
[d] LOEC.
[e] (*)Standard error of parameters not available due to singularity in regression model.

decreased when exposure time increased (Table 5). For example, the [LC_{50}/EC_{50}] ratios for *Proasellus coxalis* and exposure periods of 48 and 96 h are 4.5 and 1.6, respectively. This general trend is also reflected in species sensitivity distributions based on paired toxicity data (Figs. 2C and D). Only the differences presented in Figure 2C were significant ($p < 0.05$).

Table 6. The 48-h EC_{50} values for λ-cyhalothrin and indigenous species tested in this study and the study by Maund *et al.* (1998)

	48-h EC_{50} (ng/L) (95% confidence limits)	
Tested species	This study	Maund *et al.* (1998)[a]
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	
Bithynia tentaculata	>5000	
Polycelis nigra/tenuis	>5000	

[a] The confidence limits were kindly provided by Syngenta (personal communications, Maund 2002).
[d] Based on zooplankton sampling.
[e] *Gammarus pulex* juvenile.
[f] No test performed.

Comparison of Short-Term Toxicity Between Laboratory and Field

Of the various populations of aquatic invertebrates sampled in the three enclosure experiments, 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 (Table 7). In the case of *Chaoborus obscuripes* where three field EC_{50}/EC_{10} values could be calculated from different enclosure experiments, toxicity values were very similar. Furthermore, when comparing the field EC_{50} and EC_{10} values (free-living populations) and corresponding laboratory EC_x values for the same species, it also appears that these values are remarkably similar for all taxa except one (Tables 7 and 8). Only *Gammarus pulex* seemed more sensitive in the field, with a field population-level EC_{50} value of 9.0 ng/L, compared with our laboratory values of 23.6 ng/L (95% confidence limits, however, overlapped). The apparent increase in sensitivity for *Gammarus pulex* 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. Maund *et al.* (1998) report a laboratory EC_{50} of 14.0 ng/L for *Gammarus pulex,* a value closer to our field EC_{50} of 9.0 ng/L.

It could be argued that the overall similarity in EC_x values for the same taxa under field and laboratory conditions is not in line with expectations because dissipation of λ-cyhalothrin from water in the enclosures was more rapid than in the static laboratory tests. However, the field data on treatment-related responses of macroinvertebrates were obtained 8–10 days post first application. In this time frame two applications had occurred. In addition, the calculated initial concentrations in the enclosures directly after the first two applications were somewhat higher than the intended nominal concentrations (Table 4). Consequently, the application regime (two applications), the actual peak concentrations, and the time frame of the effects (8–10 days) might have been somewhat more worst-case in the field than in the laboratory experiments (one application, 48-h tests), compensating for the mitigating effects of a faster dissipation of the active substance under field conditions.

We also compared the field and laboratory EC_{50} and EC_{10} values with the corresponding 48-h EC_x values calculated for the same species in the in situ bioassays (Table 7). During spring, the most sensitive species, *Chaoborus obscuripes,* showed a similar sensitivity for the free-living population and the caged individuals in the enclosures of the macrophyte-dominated ditch. This was in contrast with the plankton-dominated system, where the *in situ* bioassay showed a relatively high EC_x value compared with that of the free-living population. EC_{50} and EC_{10} values of free-living *Chaoborus* and those of the *in situ* bioassays in the macrophyte-dominated enclosures were somewhat higher than the corresponding laboratory EC_x values. The 95% confidence limits for these field and

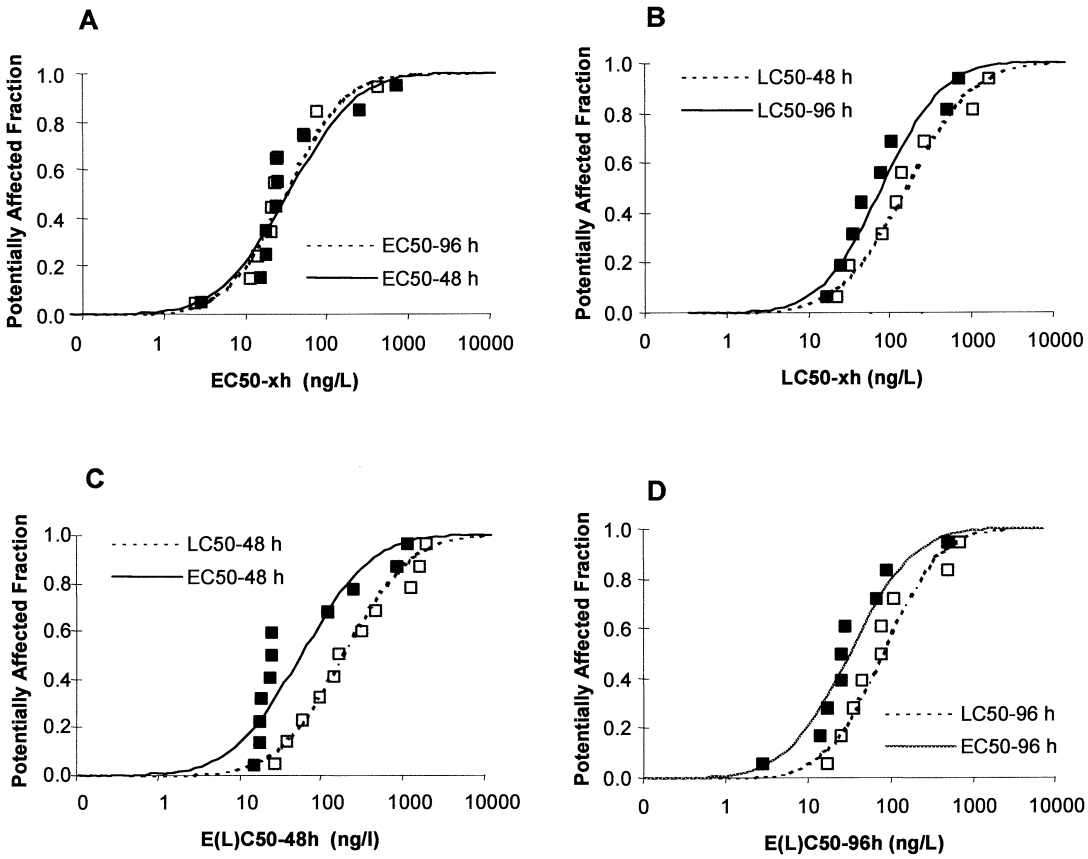

Fig. 2. Comparison of laboratory species sensitivity distribution (SSD) curves for λ-cyhalothrin and short-term toxicity data of aquatic arthropods. **A** and **B** show differences in SSD curves based on exposure time (48 versus 96 h). Differences in SSD curves based on effect endpoint (LC_{50} versus EC_{50}) are shown in **C** and **D.** The SSD curves in the same figure are based on paired toxicity data (Table 5)

laboratory EC_x values, however, overlapped except for the *in situ* bioassay of the plankton dominated system.

More pronounced differences in EC_x values between *in situ* bioassays and the laboratory were observed for the isopods *Asellus aquaticus* and *Proasellus coxalis* (Table 7). The responses observed in the *in situ* bioassays were less severe than those in single species laboratory tests, also when considering the 95% confidence limits of the EC_x values. In free-living populations of *Asellus* and *Proasellus,* no consistent concentration–response relationship could be demonstrated, due in part to the low abundance of these taxa in the enclosures in spring. During summer, *Asellus aquaticus* could be observed at somewhat higher densities in the macrophyte-dominated ditch, and a NOEC of 50 ng/L could be calculated for this species (Van Wijngaarden *et al.* in press). The fact that higher toxicity values for the isopods were observed in the enclosures (both the free-living population and the individuals incubated in the *in situ* bioassays) might be explained by a lower bioavailability of λ-cyhalothrin in the field than in the laboratory. In the *in situ* bioassays *Asellus* and *Proasellus* individuals could shelter between the *Populus* leaves that were placed in the cages as food for these detritivores. In the enclosures the free-living populations of isopods predominantly dwelled in the detritus layer on top of the sediment. Exposure to λ-cyhalothrin in these microhabitats might have been lower compared with the laboratory conditions of the single species tests, where only water was present as medium.

Verification of the SSD Concept

It is assumed that species sensitivity distributions derived on a limited number of species in the laboratory can be used to describe the range of sensitivities of all other "related" species (depending on taxonomy and the mode of toxicity). In addition, it is assumed that under comparable exposure conditions, the laboratory SSD represents the effects that would be observed on natural assemblages. To test these assumptions, we compared laboratory and field SSDs (Fig. 3).

Comparison of SSDs should take into account the concentration ranges that were tested and the likely response of organisms at those concentrations. Since the highest concentration tested in the field was 250 ng/L, the inclusion of organisms whose acute EC_{50} values were higher than approximately 100 ng/L might bias the comparison (because it would not be possible to adequately calculate EC_{50} values for these organisms).

Six EC_{50} values for macroinvertebrate arthropods were available from the enclosure experiments (Table 7). Two different laboratory-based SSDs were constructed to compare to the field SSD. One laboratory SSD was based on all 13 arthro-

Table 7. Comparison of short-term EC_{10} and EC_{50} values (ng/L) of arthropods between laboratory single species tests and outdoor field enclosure studies

		(Semi-)field experiments		EC_x, laboratory tests	
Species	x	EC_x, enclosure	EC_x bioassays	This study	Maund *et al*. (1998)
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)	nt[f]
	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,e]		14.2 (7.4–27.0)	
	50	9.0 (3.5–22.7)[b,e]	nt	23.6 (16.0–34.9)	14.0 (9.1–19)
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)	26.0 (18–36)
	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)	nt
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)	38.0 (23–93)
Ceanis luctuosa	10	5.0 (0.5–45.8)[c]			
	50	22.1 (7.2–67.4)[c]	nt	nt	nt
Ceanis 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)	nt
Corixidae/Corixa	10	3.7 (0–361)[c]			
	50	27.2 (2.6–288)[c]	nt	nt	30.0 (21–42)

Note. The bioassays (cage experiments) were performed in the same enclosures. In the three enclosure experiments, not always an EC_{10} or EC_{50} for the same taxon.
[a] Macrophyte-dominated system, spring.
[b] Macrophyte-dominated system, summer.
[c] Plankton-dominated system, spring.
[d] Based on zooplankton sampling.
[e] *Gammarus pulex* juvenile.
[f] No test performed.

Table 8. Comparison of threshold levels (NOECs) for λ-cyhalothrin at the community level and population level (top) with HC_5 and HC_{10} values (bottom) based on acute laboratory 48-h EC_{50} values (Table 6) and field-EC_{50} values (Table 7) of aquatic arthropods

	$NOEC_{field}$ (ng/L)	
Field experiments	Community (multivariate)	Most sensitive endpoint
Our enclosure experiments		
(Roessink *et al*. in press; Van Wijngaarden *et al*. in press)	10	<10[a]
Farmer *et al*. (1995)	—	17[b]
Hill *et al*. (1994)	—	16[c]
SSD-based threshold values		
Toxicity data	HC_5 (ng/L) (95% conf. limits)	HC_{10} (ng/L) (95% conf. limits)
Laboratory		
This study:		
All species (n = 13)	2.7 (0.5–7.5)	5.1 (1.3–12.5)
Species with an EC_{50} <100 ng/L (n = 9)	4.7 (1.7–8.2)	6.3 (2.6–10.4)
Maund et al. (1998)		
All species (n = 12)	1.4 (0.1–5.9)	3.3 (0.4–12.2)
Field (n = 6)	4.1 (1.0–7.7)	5.1 (1.5–9.1)

[a] *Chaoborus obscuripes.*
[b] Affected group of species: Hydracarina, Ephemeroptera, Hemiptera, Trichoptera, Diptera.
[c] Affected group of species: Gammaridae.
[d] Geometric mean was used when more data of one taxon were available.

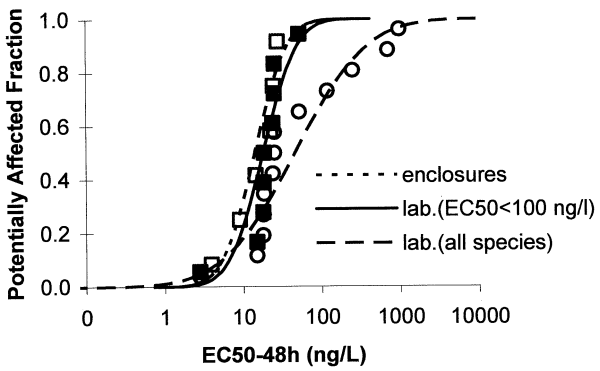

Fig. 3. Comparison of laboratory- and field-based species sensitivity distribution (SSD) curves for aquatic arthropods and the insecticide λ-cyhalothrin. For the analyses, data in Table 5 (48-h EC_{50} values) and Table 7 (enclosure EC_{50}) were used. For the enclosure curve EC_{50} values of macroinvertebrates *only* were available (□···). Besides a laboratory-based curve for all 13 arthropods species (○ - - -), a curve based on the 9 species of macroinvertebrates with an $EC_{50} \geq 100$ ng/L is given (■ ——)

pod species tested in the present study (Table 6). This included insects, macrocrustaceans, and microcrustaceans. In the second laboratory SSD, only toxicity data with an EC_{50} lower than 100 ng/L were used (in this case, these were all arthropod macroinvertebrates). The latter SSD curve was considered to be more appropriate for comparison to the field values for the reasons described above.

From the SSD curves presented in Figure 3, it appears that the overall laboratory SSD curve based on 13 taxa did not resemble that of the field SSD. Significant differences ($p < 0.05$) in variance (steepness of curves) and mean (positions of curves) occurred between these curves. Despite these differences the left tail of both curves (i.e., the region where the HC_5 and HC_{10} is located) were more similar. The position of the left tail of both curves is determined by sensitive species which were tested in the laboratory and of which representatives were also present in the field enclosures.

The second laboratory SSD, based on nine macroinvertebrate arthropod taxa much better resembled the field SSD. When applying the *F*-test and *t*-test to compare both data sets, significant differences could not be demonstrated ($p > 0.10$) for, respectively, variance (steepness of curve) and mean (position of curve). It was concluded that laboratory and field SSDs for λ-cyhalothrin were very similar when based on the same sensitive taxonomic groups (insects and crustaceans) and when a similar range of exposure concentrations was taken into account.

The toxicity data used for the laboratory-SSD curve are based on the endpoint immobility (EC_{50}), while the field SSD is based on abundance data of species. The field EC_{50} reflects the overall effect of λ-cyhalothrin at the population level (net results of death, birth, and ecological interactions over a ca. 10-day period in which two applications occurred). Despite these differences, SSDs based on field and laboratory toxicity data of aquatic arthropods were very similar. These results suggest that use of the SSD approach for this compound is relevant for field communities. Such a conclusion has been demonstrated previously by Van den Brink *et al.* (2002), who performed a similar assessment for chlorpyrifos on the basis of laboratory and field EC_{50}'s of freshwater arthropods. Chlorpyrifos is an organophosphorus insecticide with several physicochemical characteristics more or less comparable to λ-cyhalothrin (low water solubility, high octanol–water partition coefficient, and high dissipation rate from water). In addition, the specific toxic mode of action of both compounds is comparable, in that arthropods and fish are sensitive taxonomic groups in particular. For chlorpyrifos the laboratory-based and the field-based SSDs also showed a high similarity, at least when based on toxicity data of aquatic arthropods tested in a similar concentration range.

Laboratory- and Field-Derived Threshold Levels

Multivariate analysis of the invertebrate data resulted in more or less similar ecological threshold levels among the three enclosure experiments and in an overall community NOEC of approximately 10 ng/L (Table 8). The NOEC of the most sensitive population (*Chaoborus obscuripes*) in these enclosure experiments, however, was always lower than 10 ng/L (the lowest concentration tested), but recovery of this species took place within 3 to 6 weeks. For other free-living populations present in the enclosures treated with 10 ng/L, consistent treatment-related effects could not be demonstrated by means of the Williams test (Roessink *et al.* in press; Van Wijngaarden *et al.* in press). In other field experiments performed with λ-cyhalothrin (Farmer *et al.* 1995; Hill *et al.* 1994), a consistent NOEC of the most sensitive endpoint was 16–17 ng/L (upper part of Table 8). In the latter studies *Chaoborus* populations did not occur at high densities at the moment of insecticide application.

The field threshold levels for the total community or the most sensitive population presented in Table 8 can be compared to the HC_5 and HC_{10} values derived from laboratory single species tests (lower part of Table 8). Based on all our laboratory 48-h EC_{50} values of aquatic arthropods, a HC_5 and HC_{10} of 2.7 and 5.1 ng/L, respectively, was calculated for λ-cyhalothrin. The HC_5 and HC_{10} based on laboratory EC_{50} values <100 ng/L is 4.7 and 6.3 ng/L, respectively. Particularly the latter HC_x values are very similar to our field HC_5 (=4.1 ng/L) and field HC_{10} (5.1 ng/L). All these HC_5 values are below the overall community NOEC of 10 ng/L derived from our enclosure studies by means of multivariate techniques (Table 8). In addition, the HC_5 and HC_{10} value derived from our laboratory 48-h EC_{50} values of the 13 aquatic arthropods (2.7 and 5.1 ng/L, respectively) appears to be very similar to the HC_5 of 1.4 ng/L and the HC_{10} of 3.3 ng/L which we calculated from the acute toxicity data for aquatic arthropods published by Maund *et al.* (1998). Apparently, in the case of λ-cyhalothrin the species sensitivity distribution of aquatic arthropods is similar for taxa sampled in British ponds and Dutch drainage ditches. No significant differences ($p > 0.10$) for variances and means were determined between the two curves.

Our study shows that for the rapidly dissipating compound λ-cyhalothrin, the HC_5 based on 48-h EC_{50} values of static laboratory tests with indigenous species provide a conservative estimate of potential effects on aquatic communities in the field, even in the case of a repeated application at weekly intervals. Apparently, the sensitive populations present in the field enclosures were already affected after the first application, and the second appli-

cation hardly increased the magnitude of the effect. Even the upper confidence limits of the laboratory HC_5 values were lower than the overall community concentrations with no observed effect (10 ng/L) (Table 8). The results of our study with λ-cyhalothrin are in accordance with other studies that compared results of SSDs with responses in aquatic microcosm/mesocosm experiments (e.g., Emans *et al.* 1993; Solomon *et al.* 1996; Versteeg *et al.* 1999; Van den Brink *et al.* 2002).

The derived HC_5 or HC_{10} values may provide a cost-effective risk evaluation to provide "acceptable concentrations" to set targets for λ-cyhalotrin in the field. The SSD concept and, consequently, the derived HC_5 values do not take into account aspects like indirect effects and recovery of affected endpoints. Insight into these aspects, however, may be provided by microcosm and mesocosm tests. Consequently, the SSD approach cannot be seen as a complete alternative of semifield experiments.

Acknowledgment. We thank all direct colleagues of Alterra for advice and practical assistance, in particular, René van Wijngaarden for his advice when setting up the laboratory toxicity tests with indigenous species.

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