The Aquatic Ecotoxicology of the Synthetic Pyrethroids: From Laboratory to Landscape

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Thesis

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Dedicated to all my dear family…

… and in loving memory of my grandparents.

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Summary

The synthetic pyrethroids (SPs) provided a break-through in insect pest control by allowing broad-spectrum pest activity at low application rates, whilst having a relatively benign mammalian toxicological profile compared to earlier insecticidal chemical classes like organochlorines, organophosphates and carbamates. One area where concerns for SPs have been raised is their high toxicity to aquatic organisms in laboratory studies, particularly to fish and aquatic arthropods. However, due to their highly lipophilic properties, pyrethroids rapidly and extensively partition from the water phase to organic matter and sediment, thereby reducing exposure of water-column organisms. This behaviour has previously been demonstrated to provide mitigation of their effects on aquatic organisms under field conditions by reducing exposure.

In this thesis, the aquatic ecotoxicology of the synthetic pyrethroids is investigated further. The publications presented in the thesis set out to address three specific research questions concerning the aquatic ecotoxicology of the synthetic pyrethoids, namely:

- What is the toxicity and bioavailability of the pyrethroid cypermethrin in sediments, and how might approaches be developed to assess the risks of pyrethroids in sediment?
- How do environmental and ecological factors influence the impacts of SPs on aquatic organisms under field conditions, how can those factors be investigated in semi-field experiments, and what are the implications for higher-tier risk assessment?
- How might landscape factors like the co-occurrence of crops and surface water influence exposure to pyrethroids at a landscape level, and how could these be used to develop a higher-tier risk assessment methodology?

A summary of the experimental approaches to address these research questions and the resulting key findings are provided below.

What is the toxicity and bioavailability of the pyrethroid cypermethrin in sediments, and how might approaches be developed to assess the risks of pyrethroids in sediment?

Since pyrethroids partition rapidly and extensively to sediment, concerns have been raised about their potential bioavailability and toxicity in this compartment. To this end, the toxicity and bioavailability of the SP cypermethrin were investigated in water-sediment systems. Cypermethrin adsorbed extensively and rapidly to sediments, with an overall mean organic carbon (OC) adsorption partition coefficient (K_{OC}) of 350,000, and adsorption

was rapid with approximately 99% occurring within 24 hours. Bioavailability from sediments was measured via body burdens of the cladoceran crustacean *Daphnia magna* and the dipteran insect *Chironomus tentans*. Mean biota–sediment accumulation factors (BSAFs), i.e., the concentration in the organism as a proportion of the concentration in the sediment, decreased with increasing OC content. The BSAF values were 0.31, 0.14, and 0.08 for D. magna and 0.63, 0.19, and 0.08 for *C. tentans*, in 1, 3, and 13% OC sediments, respectively. Since the BSAF values were less than one (i.e. no evidence of bioaccumulation), these data indicated that the bioavailabilty of cypermethrin adsorbed to sediment was low.

In toxicity tests, the 10-d median lethal sediment concentrations (LC_{50}s) of cypermethrin were 3.6, 18, and 32 µg/kg for amphipod crustacean *Hyalella azteca* and 13, 67, and 62 µg/kg for *C. tentans* in 1, 3, and 13% OC sediments, respectively. Predictions of aqueous concentrations at the LC_{50} in sediments (based on K_{oc}) compared well to each other and to effect concentrations from studies in water alone, suggesting that equilibrium partitioning theory could be used reasonably to predict and normalize the toxicity of cypermethrin across sediments of differing OC content. The data indicated that using water-column toxicity data and sediment partitioning would allow adequate risk assessment of pyrethroids in sediments.

How do environmental and ecological factors influence the impacts of SPs on aquatic organisms under field conditions, how can those factors be investigated in semi-field experiments, and what are the implications for higher-tier risk assessment?

Under field conditions, a range of environmental and ecological factors will influence the effects of the SPs on aquatic organisms, and these should be considered in refining the risk assessment of this class of chemistry. Risk assessment of pesticides for aquatic ecosystems is typically based on comparisons of exposure and effect concentrations at a variety of levels (tiers) of increasing sophistication and complexity. At the higher tiers, effects assessment can involve generating data under field conditions, typically in micro- and mesocosm experiments. However, interpreting the ecological significance of effects measured in these studies can be difficult because ecological factors can influence the outcome of perturbations in the real world. These factors include interpreting the relevance of recovery (or lack thereof) seen under experimental conditions to those that occur in the real world. Such factors include rates of immigration and reproduction, interactions with other stressors not tested in the experimental system, differences in size, geographical location and climate. A clear example of the challenge of interpretation is demonstrated with data for the SP cypermethrin, where there were clear effects on

Gammaridae and Asellidae in a mesocosm study with only little evidence of recovery by the end of the study. However, in field studies in natural water bodies, recovery of these taxa was relatively rapid.

In order to review how such factors might influence the aquatic risk assessment of pyrethroids, three chapters of the thesis then investigate a number of these considerations. In one study, the SP *lambda*-cyhalothrin was applied as part of a 'crop' programme of treatments, evaluating the impact of several pesticides (as would be used in normal agricultural practice) as opposed to treatment with one active ingredient, as is usually done in mesocosm studies. Secondly, in order to evaluate the influence of experimental design, geographical location and trophic status, the available microcosm and mesocosm data for *lambda*-cyhalothrin from eight 'cosm studies were reviewed. Thirdly, the influences of immigration of organisms into treated systems and the of persistence of the test substance was experimentally investigated in a microcosm study with the SP cypermethrin and the persistent herbicide degradate 3,4-dichloroanaline.

Ecological Impact in Ditch Mesocosms of Simulated Spray Drift from a Crop Protection Program for Potatoes

In order to investigate how combinations of pesticides might affect the conclusions of aquatic risk assessments compared to those derived from individual compounds, outdoor ditch mesocosms were treated with a range of pesticides to simulate various spray drift rates resulting from a typical crop protection program used in the cultivation of potatoes in The Netherlands. Applications of prosulfocarb, metribuzin (both herbicides), *lambda*-cyhalothrin (insecticide), chlorothalonil, and fluazinam (both fungicides) were made in the sequence typical of the spray calendar for potatoes. A total of 15 treatments with the various compounds were made by spray application (to simulate spray drift) to the water surface at 0.2%, 1%, and 5% of the recommended label rates. Chemical fate and effects on ecosystem function and structure (phytoplankton, zooplankton, chlorophyll-α, macroinvertebrates, macrophytes, breakdown of plant litter) were investigated. To interpret the observed effects, treatment concentrations were also expressed in toxic units (TU), which describe the relative toxicity of the compounds with standard toxicity test organisms (*Daphnia* and algae).

After treatment, each compound disappeared from the water phase within 2 d, with the exception of prosulfocarb, for which 50% dissipation time (DT_{50}) values ranged between 6 and 7 d. At the 5% treatment level, an exposure peak of 0.9 TU_{algae} was observed, which resulted in short-term responses of pH, oxygen, and phytoplankton. Exposure concentrations also exceeded 0.1 $TU_{Daphnia}$, and this resulted in long-term effects on

zooplankton and macroinvertebrates, some of which did not fully recover by the end of the study. At the 1% treatment level, only slight transient effects were observed on a limited number of zooplankton and macroinvertebrate species and on pH. At the 0.2% level, no consistent treatment-related effects were observed. Most of the observed effects were consistent with the results from higher-tier and mesocosm studies with the individual compounds. Based on the combination of pesticides applied in the study, multiple and repeated stress appeared to play only a small role due to the rapid dissipation of most substances and the absence of many simultaneous applications. The data indicated that risk assessments based on the individual compounds would in this case have been sufficiently protective for their uses in a crop protection program.

Aquatic fate and effects of lambda-*cyhalothrin in model ecosystem experiments*

The available data on the fate and effects of the synthetic pyrethroid *lambda*-cyhalothrin in eight aquatic model ecosystem experiments were reviewed. In laboratory studies, *lambda*-cyhalothrin is highly toxic to fish and invertebrates. Its physico-chemical and laboratory fate properties indicate that it will dissipate rapidly from the water phase, reducing exposure for organisms in the water-column. For European aquatic risk assessments, where exposure models predict that spray drift is the main entry route from agricultural uses, this has been a key factor in refining higher-tier risk assessments for water-column organisms. Modified exposure studies in the laboratory confirmed that rapidly reduced exposure mitigates effects on fish and invertebrates.

Eight aquatic model ecosystem experiments have been published on *lambda*-cyhalothrin (six papers of which were co-authored by the thesis author) in a variety of indoor and outdoor test systems. These were of differing trophic status, and ranged in size from 0.43 to 450 m^3. The timing of application of test substance also varied between studies. The fate of the compound in the various experiments was consistent, typified by rapid dissipation (and degradation) in the water phase with median dissipation times (DT_{50}) of typically a day or less. Only 5-22% (where quantifiable) of the chemical applied to the water column reached the sediment, and it was not possible to calculate sediment DT_{50} values. Effects in the studies were driven by population responses of macrocrustacea and certain insects, along with zooplanktonic microcrustacea. Considering the range of test systems, the variety of locations, different trophic status of the test systems, differences in season of application, and differences in numbers of applications, the effects thresholds observed in the studies were remarkably consistent, with no to slight effects occurring consistently at initial nominal treatment concentrations up to 10 ng/L. The effects threshold values for clear effects with recovery were more variable than the no to slight

effects, but still reasonably consistent, with thresholds between initial nominal treatment concentrations of 16 and 50 ng/L. This gives considerable confidence in the potential to extrapolate the effects observed in one study to a different situation, at least in this case where effects tend to be of an acute nature, and the dissipation and degradation of the compound is rapid.

Influence of simulated immigration and chemical persistence on recovery of macroinvertebrates

Chemical dissipation and organism immigration are considered important factors that influence recovery potential from perturbation of aquatic macroinvertebrates. The effect of simulated immigration was investigated on the recovery of aquatic macroinvertebrates exposed in outdoor microcosms to ecotoxicologically similar concentrations of the rapidly-dissipating pyrethroid insecticide cypermethrin (70 ng/L) or the more persistent herbicide intermediate and degradate 3,4-dichloroaniline (10 mg/L). Microcosms were covered with light-permeable mesh to prevent recolonisation. Immigration was simulated by the regular addition of organisms after treatment. Microcosms exposed to 3,4-dichloroaniline treatment suffered substantial loss of taxon-richness and by ten months after treatment had only recovered where invertebrates had been added. Those treated with cypermethrin underwent an initial decline in certain crustacean and insect populations. These populations showed some signs of recovery over a period of five months through internal processes alone. However, rate of recovery was further enhanced where immigration was simulated, and in this case recovery had occurred around 100 days after treatment. Although not the only factors involved, simulated immigration and chemical fate clearly influence the ability of communities to recover from chemical exposure. Consideration of immigration processes and development of ecological recovery models will help to increase the realism of aquatic risk assessments.

How might landscape factors like the co-occurrence of crops and surface water influence exposure to pyrethroids at a landscape level, and how could these be used to develop a higher-tier risk assessment methodology?

Standard aquatic risk assessment methodologies for pesticides typically use a 'realistic worst-case' approach to evaluate aquatic exposures. As part of this, it is generally assumed that every application of the compound will occur in close proximity to surface water. However, in reality, many water bodies in the landscape are distant from agricultural areas, and so the standard approach has the potential to overestimate the extent to which surface water is exposed. Furthermore, lower tier risk assessments generally consider a worst-case predicted no effect concentration, based on the effect

endpoint of the most sensitive species tested in the laboratory with the application of a 'safety factor'. This approach can be refined by examing the distribution of sensitivity of organisms from laboratory studies, or by using data generated in semi-field experiments to refine the effects endpoint.

In order to investigate these considerations for the uses of SPs in the cotton crop in the USA, a landscape-level exposure characterization and risk assessment was conducted in Yazoo County, Mississippi, USA, and the results were published in a series of five papers (four of which were co-authored by the thesis author, and the synthesis of which is presented in Chapter 7). Yazoo County is considered by the US Environmental Protection Agency to constitute a worst-case scenario for cotton uses because of the extent of co-occurrence of the crop and surface water, and its climatic and soil conditions. The aim of the study was to provide a more realistic exposure characterization using landscape-level data, and to compare these exposures to laboratory and semi-field derived effects endpoints. Cypermethrin was used in the assessment as a representative cotton pyrethroid, and analysis of laboratory toxicity data supported the use of cypermethrin as a reasonable worst-case surrogate for the other pyrethroids for the purposes of risk assessment of pyrethroids as a class.

As a next step, data from aquatic semi-field studies with cypermethrin and esfenvalerate were analyzed and interpreted. A core group of seven mesocosm studies conducted on two continents over the course of a decade were examined, and additional observations from mesocosm and field studies with these and other cotton pyrethroids were also brought to bear. The results for cypermethrin and esfenvalerate were remarkably consistent. They revealed a trend in sensitivity from amphipods, isopods, midges, mayflies, copepods, and cladocerans (most sensitive) to fish, snails, oligochaetes, and rotifers (least sensitive). With few exceptions, populations affected by pyrethroids in the mesocosms recovered to normal levels before the end of the year of exposure; most populations recovered within weeks. Factors presumed responsible for population recovery included internal refuges (areas of low exposure), resistant life stages, rapid generation times, and egg deposition by adults from outside the treated systems. Indirect effects on fish (which have been hypothesized to occur when invertebrate food sources are reduced) were not observed. The lowest-observed-adverse-effect concentrations for the overall ecosystems for cypermethrin and esfenvalerate corresponded to the 54th and 41st centiles of acute toxicity endpoints (LC_{50}s) for arthropods measured in laboratory studies with these compounds, implying that a risk characterization based on 10th centiles would be highly conservative.

In order to refine the standard tier 1 and tier 2 exposure estimates, a landscape-level exposure analysis was carried out for Yazoo County. Remotely-sensed land use and land cover data were collected, and an image processing technique and geographic information system were developed to investigate the number and size of the water bodies in the county and their proximity to cotton. Variables critical to aquatic exposure modelling were measured for approximately 600 static water bodies in the study area. Quantitative information on the relative spatial orientation of cotton and water, regional soil texture and slope, and the detailed nature of the composition of physical buffers between agricultural fields and water bodies was also obtained. Results showed that remote sensing and geographic information systems could be used cost effectively to characterize the agricultural landscape and provide verifiable data to refine conservative model assumptions. For example, 68% of all ponds in the region have no cotton within 360 m and 92% of the ponds have no cotton within 60 m. Only 2% of ponds have cotton present in all directions around the ponds and within 120 m.

Using the landscape-level data on the proximity of the 600 static water bodies in Yazoo County to cotton fields, a higher-tier exposure analysis was conducted. This was based on the standard U.S. Environmental Protection Agency tier II regulatory scenario for cotton, which assumes a 1-ha pond surrounded by 10 ha of treated crop, with high levels of runoff, erosion, and drift entering the pond. The regulatory scenario was modified to include simplified landscape-level information on the proximity of cotton to ponds derived from the remote sensing study. No-spray buffers between pyrethroid applications and surface waters mandated on all cotton pyrethroid labels (which differentiate applications made by air and by ground-based equipment), and the percentage of cotton area that is treated with pyrethroids were also included. Incorporation of these landscape-level factors into the analysis reduced the predicted aquatic exposure concentrations approximately 50- to 100-fold. Because many other conservative assumptions in the original tier II exposure analysis were not revised, the modified exposure predictions were still overestimates of true field exposure concentrations.

The higher-tier exposure and effects data were then combined to develop an evaluation of potential risks at the landscape level. Standard tier I and II approaches (U.S. Environmental Protection Agency) indicated, as expected, potential concerns for fish and aquatic invertebrates. The assessment was then refined by comparing landscape-level exposure calculations for ponds and lakes in Yazoo County (modified tier II analysis) with distributions of laboratory effect concentrations and with data from field studies. The modified tier II analysis showed that exposure concentrations are unlikely to exceed concentrations that might cause ecologically significant effects. Indeed, in the vast

majority of cases, concentrations in the modified tier II analysis were several orders of magnitude lower than those at which effects would be predicted on the basis of laboratory and field data. The conclusion of minimal potential for adverse ecological effects was also supported by semi-field studies, which showed that impacts on aquatic systems were negligible, even at concentrations many times higher than the modified tier II exposure concentrations.

Conclusions

The experiments presented in this thesis address a number of key factors concerning the development of higher-tier aquatic risk assessments for pyrethroids. Overall, the studies indicate that it is possible to accrue the agronomic benefits of SP uses (efficacious, broad-spectrum insect pest control) whilst appropriately managing the potential risks to aquatic organisms.

Due to their highly lipophilic nature, concerns have been expressed regarding the potential for effects of SPs on sediment-dwelling organisms. The data from laboratory studies on cypermethrin presented in Chapter 2 demonstrate that bioavailability in sediment is low. Furthermore, effects on aquatic macroinvertebrates could be adequately predicted by taking into account partitioning behavior and effect concentrations from tests conducted in the water phase alone. Hence it should be possible to assess the potential risks of SPs in sediments largely with the existing regulatory data (provided appropriate estimates of sediment exposure are available).

In Chapter 3, the range of ecological factors that influence the effects of SPs under field conditions was discussed, and in the subsequent Chapters 4, 5, and 6, a variety of these factors have been explored experimentally. The results presented in Chapter 4, where several pesticides were applied to simulate uses in a potato crop, showed that effects could be adequately predicted on the basis of the effects of individual compounds (at least in this case, where dissipation was reasonably rapid). A review of eight micro- and mesocosm studies with *lambda*-cyhalothrin (Chapter 5) demonstrated that due to their rapid dissipation, effects observed in micro- and mesocosm studies with pyrethroids are remarkably consistent, irrespective of the size, composition, trophic status or location of the test system. In Chapter 6, the importance of immigration as a factor in the recovery of macroinvertebrates following effects from cypermethrin treatment was demonstrated. Under field conditions, the immigration of animals from unaffected regions within the water body or from neighbouring surface waters is likely in many cases to lead to faster recovery than might necessarily be seen in standard ‘cosm studies. Taken together, these

findings provide substantial reassurance that the application of semi-field study endpoints in a higher-tier risk assessment for SPs will provide a reasonably conservative evaluation.

In Chapter 7, laboratory and field effects data were combined with a landscape-level exposure characterization (developed using geographical information systems) for the use of the SP cypermethrin in cotton in a worst-case county in the USA. By refining the exposure assessment with data on the proximity of static surface water bodies to the cotton crop, it was possible to show that adverse effects on aquatic organisms (based on laboratory and field data) were unlikely in the vast majority of cases. The ever-increasing availability of landscape-level data and the further development of methodologies in this area will allow the broader application of such approaches in the future.

Chapter 1: General Introduction

Discovery and Introduction of Synthetic Pyrethroids

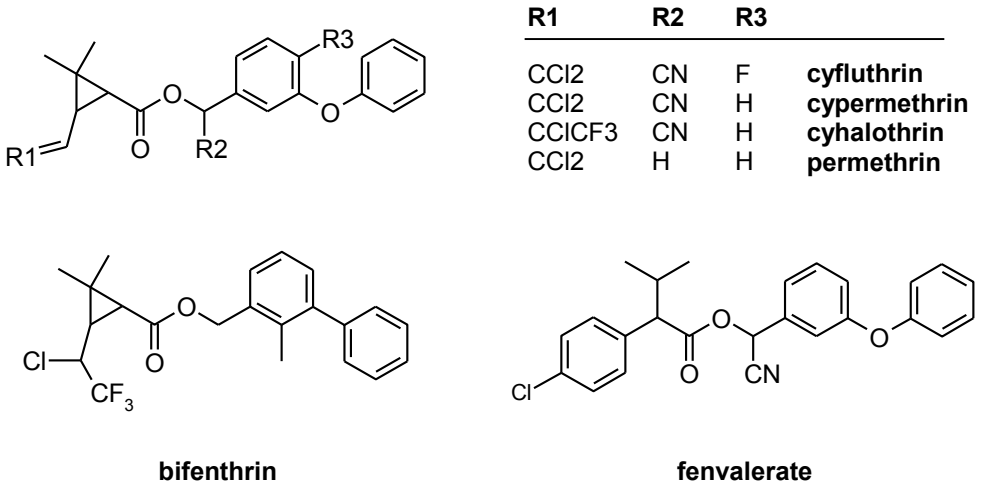
Although the insecticidal properties of naturally-occuring pyrethrins (extracted from the flower-heads of *Chrysanthemum cinerariaefolium* as seen in Figure 1) have been known since the 1950s, until the 1970s their agronomic use was limited to indoor applications because the compounds are photo-labile. At that time, work began to develop new analogues that would be sufficiently stable for efficacious outdoor use as insecticides for pest control, culminating in the mid-1970s with the commercial introduction of the first synthetic pyrethroid (SP), permethrin. Since that time, a wide range of SP analogues have been introduced (e.g., cypermethrin, fenvalerate, cyfluthrin, cyhalothrin, deltamethrin, bifenthrin, fenpropathrin, tralomethrin, fluvalinate, flucythrinate).

Figure 1: *Chrysanthemum cinerariaefolium* - the source of the natural pyrethrums

Chemically, the SP structure is characterised by an acid moiety and alcohol moiety, joined centrally by an ester bridge (Figure 2). The acid moiety has two chiral carbons which means that pyrethroids typically exist as stereo-isomers. Some compounds also contain a chiral carbon on the alcohol moiety with the potential for three chiral carbons and a total of eight different stereo-enantiomers. The optical isomeric nature of many of the molecules has led to further refinements in their agrochemical uses, with the more recent products containing the resolved stereo-isomers that are responsible for insecticidal activity (e.g. esfenvalerate, *beta*-cyfluthrin, *lambda*-cyhalothrin, *alpha*- and *zeta*-cypermethrin, *tau*-fluvalinate).

Based on their toxicological properties in vertebrates and invertebrates, pyrethroids are sometimes categorised as type I or type II. Type I compounds (e.g. permethrin, bifenthrin) induce tremors in rats and insects whereas Type II compounds (e.g. cypermethrin,

cyfluthrin, cyhalothrin) induce seizures (1). Structurally, type I compounds have no cyano group in the alcohol moiety, whereas type II compounds do. The primary mode of action of pyrethroids in vertebrates and invertebrates is the disruption of voltage sensitive sodium channels in the neurons (2). By delaying the closing of the ion channel, multiple action potentials result, leading to multiple firing of the neurons, and neurological disruption. Unlike the earlier organophosphate or carbamate insecticide chemistries, pyrethroids do not inhibit acetyl cholinesterase.



R1	R2	R3	
CCl2	CN	F	**cyfluthrin**
CCl2	CN	H	**cypermethrin**
CClCF3	CN	H	**cyhalothrin**
CCl2	H	H	**permethrin**

Figure 2 Chemical structures of example pyrethroids. The left hand side of the molecule is the acid moiety, and the right hand side the alcohol, with the ester bridge in the centre of the molecule.

The introduction of the SPs represented a step-change in insect pest control compared to earlier insecticide chemistries (e.g. organochlorines, carbamates and organophosphates). They combined broad-spectrum control of a wide range of insect groups with low use rates (for earlier analogues typically around 100-200 g/ha, and as the chemistry was refined with rates down to less than 10 g/ha for some pests), coupled with relatively low toxicity to vertebrate wildlife compared to many of their predecessors. Due to their cost-effectiveness, they gained rapid and widespread adoption by the agricultural community, and soon were used globally on a broad range of crops. In addition, since the mode of action of the SPs (interference with ion channels in the nerve axon) was different from previous insecticide chemistry, the compounds offered new tools for insecticide resistance management. They soon began to find widespread use in non-agricultural pest control sectors, for example forestry, animal health, and vector control. Overall, synthetic

pyrethroids have provided enormous benefits to mankind by enabling effective control of damage and disease caused by arthropod pests. However, use in agriculture also implies that the compounds are released into the environment, and may potentially expose non-target organisms. Consequently, environmental risk assessments are needed for non-target organism groups (e.g. birds and mammalian wildlife, aquatic organisms, soil organisms, honeybees).

Fate in the environment

The physical and chemical properties of the pyrethroids have been reviewed extensively by Laskowski (3). All pyrethroids are highly non-polar molecules with logarithmic octanol:water partition (log P coefficients) in the region of 6 to 7. This in turn leads to low water solubility, typically in the low microgram per litre range, and potential for bioaccumulation, with fish bioconcentration factors (BCF) of several hundred to several thousand (although metabolism limits to some degree the amount of bioaccumulation), and depuration is rapid (3). Pyrethroids are not particularly volatile from either soil or water.

Their high lipophilicity also means that SPs adsorb rapidly and extensively to soils and sediments (3). Soil organic carbon adsorption constants (K_{oc}) are in the range from 100, 000 to 700, 000. Once in soil or sediment, pyrethroids degrade readily. Aerobic soil half lives are in the range of 20 to 100 days, and aerobic aquatic half lives are similar. Pyrethroids are generally hydrolytically stable at circumneutral or acidic pH values, but under alkaline conditions, the ester bridge is cleaved, and for certain pyrethroids (e.g. cyfluthrin, cypermethrin, *lambda*-cyhalothrin) this results in rapid degradation, with half-lives of a matter of days at pH 9 (3). For aquatic ecosystems in agricultural areas, where alkaline conditions tend to predominate, this may be an important factor for consideration. SPs tend to be photolytically stable (3).

Effects on Non-target Organisms

Based on extensive mammalian toxicology data generated for registration, it can be concluded that SPs are relatively safe for humans. Although the mode of action is potentially relevant to man, systemic neurotoxicity is considered unlikely to occur from use (1). Some pyrethroids generate a local skin effect which causes tingling of the skin (termed parasthesia), but it is however completely and quickly reversible. The reasons for the difference in sensitivity between mammals and insects is because insects have increased sodium channel sensitivity, smaller body size and lower body temperature

(resulting in less metabolism). Despite their extensive use around the world in many different applications, there are very few reported cases of human pyrethroid poisoning (4). Similarly, low vertebrate toxicity means that pyrethroids pose negligible risks to avian and mammalian wildlife. Due to their highly efficacious and broad-spectrum insecticidal efficacy, pyrethroids are highly toxic to bees and non-target arthropods.

Identification and Development of Regulatory Aquatic Concerns

Due undoubtedly to the close taxonomic relationship to the target terrestrial species, high levels of toxicity were observed in laboratory acute and chronic studies to a range of aquatic Arthropoda, particularly crustaceans and certain aquatic insects (5). Although of relatively low toxicity to terrestrial vertebrates for insecticides (due to their rapid metabolism by birds and mammals), fish were also found to be relatively sensitive most probably because of a less pronounced ability to metabolise the compounds (6). Based on their inherent toxicity in laboratory studies, pyrethroids are still among the most aquatic ecotoxicologically active of all pesticides, with effect concentrations in standard acute and chronic studies with fish and arthropods ranging from the microgram to nanogram per litre level (7).

Such inherent levels of activity triggered regulatory concerns about the potential for impact on non-target aquatic ecosystems during agricultural use. However, due to the extremely lipophilic nature of the molecules, it was anticipated that when pyrethroids entered the aquatic environment, exposures in the water phase would be rapidly reduced through processes of adsorption and degradation. Hence the predicted levels of effects based on standard laboratory toxicity data (where exposure concentrations are maintained throughout the course of the experiment) would probably overestimate the effects observed in the field due to the mitigating impact of reduced exposure.

In order to investigate this phenomenon, during the 1980s, manufacturers of pyrethroids and other researchers performed many field studies to investigate the impact of SPs under natural conditions. These included farm pond monitoring studies (where pyrethroids were treated in a pond catchment, and residues and impacts were monitored) and replicated, large-scale pond mesocosm studies to compare pyrethroid-treated ponds with untreated controls. By the late 1980s, there was a substantial database of studies which had investigated the potential effects of SPs under laboratory and field conditions (5, 8).

Recent Developments in SP Regulation

The early 1990s brought a sea-change in the way that pesticides were to be regulated in the USA and Europe. In 1992, the United States Environmental Protection Agency (US-EPA) issued its 'New Paradigm', which de-emphasised aquatic field studies, instead focusing on exposure modelling, mitigation, and monitoring as the regulatory instruments for managing compounds like SPs that were presumed to present unacceptable risks to non-target aquatic organisms. At this time, there was also a review of the many regulatory aquatic field studies with SPs that had been submitted to the Agency. In most cases, the US-EPA review determined that the data submitted did not fully negate the presumption of risk (although this was a point of much debate with the study submitters). Principal among the concerns was the difficulty of interpreting the submitted farm pond and mesocosm studies (Figure 3). This was due *i.a.* to: the difficulty of attributing cause and effect in farm pond studies; difficulties in interpreting effects in mesocosm studies due to the presence of fish which obscured invertebrate endpoints; inherent variability in the mesocosms, meaning that using analysis of variance (this was before the advent of more refined statistical tools for evaluating mesocosm data) the experimental power to detect statistical differences between treatments and controls was much lower than that in laboratory studies.

Figure 3: A 450 m^3 US pond mesocosm facility at Rocky Mount, North Carolina, USA in 1992.

The US-EPA also raised an additional concern; if the effects of pyrethroids were mitigated by adsorption to particulates and sediments, what was the potential bioavailability and impact of those adsorbed residues? Concerns regarding sediment toxicity were also raised in Europe. New requirements were therefore put in place to assess sediment toxicity and bioavailability, a topic area that was attracting increased scientific and regulatory interest at the time (9, 10)

Similarly, in the European Union (EU), pesticide registration procedures were under-going major revisions in the early 1990s. This culminated in the issuing of the Directive Concerning the Placing of Plant Protection Products on the Market (91/414/EEC) which required that all existing and new pesticides were approved at the European level before products could be sold in EU Member States. The SPs were identified as one class of pesticide that should be prioritised on the review lists of existing substances, and most of the pyrethroids were included on the first and second EU review lists.

These changes in regulatory requirements stimulated new reviews of the existing data on SPs and spurred new research to develop better understanding of the aquatic ecotoxicology of the SPs. In the US, the EPA implemented a Pyrethroid Working Group (PWG), consisting of all the major manufacturers of pyrethroids who were tasked to work together to address regulatory concerns. In the aquatic area, to begin with this principally concerned the generation of data on sediment toxicity and bioavailability. The PWG also initiated a set of further studies on the potential exposure and effects of pyrethroids under realistic use conditions in the agricultural landscape were also started, using cypermethrin as a representative compound. In the late 1990s, as the organophosphorus compounds were gradually removed from the market, pyrethroids began to be used extensively in home and garden uses for pest control. Surveys of sediments have now established significant pyrethroid residues in urban areas, and the potential effects of these on aquatic ecosystems is currently being investigated (11).

In Europe, under the new scheme outlined in 91/414 manufacturers now had to reassess the ecological risks of their compounds. In the preliminary aquatic risk assessment scheme, predicted environmental concentrations (PECs) were generated by calculating the concentrations that would occur from worst-case spray drift rates into a static water body of 30 cm depth. Not surprisingly, SPs generally failed this preliminary assessment for both acute and chronic toxicity to aquatic invertebrates and fish, requiring that higher-tier assessments would be needed to assess the potential for effects under field conditions. Critical in these debates was the interpretation of the existing microcosm (Figure 4) and mesocosm data, particularly considering the potential of aquatic ecosystems to recovery

from the short-term exposures typical of pyrethroids. Such considerations began to be included in a more ecological approach to SP risk assessment, including the use of ecological models and landscape level data.

Figure 4: A 1 m^3 pond microcosm test system used for European regulatory purposes at Jealott's Hill Research Station, Bracknell, Berkshire, UK in 1994.

Impact of SP research on the development of aquatic ecotoxicological regulatory science

Over the last two decades, research into the aquatic effects of pyrethroids has contributed substantially to the development of higher-tier tools and techniques for assessing the risks of pesticides to aquatic organisms. Such developments have included: sediment toxicity and bioavailability assessments; the use of species sensitivity distributions to summarize laboratory effects data; the application of ecological models to predict impact and recovery from pesticide exposures, the inclusion of the aquatic macrophytes to more realistically assess aquatic fate; the development of landscape-level probabilistic assessments to better predict levels of exposure under realistic agronomic conditions.

Indeed, much of the research generated during that time with SPs has provided precedents for the way that higher-tier risk assessments would be performed in the future. Research with SPs (along with studies on other pesticides) has contributed substantially to new initiatives to develop consensus on suitable higher-tier approaches to aquatic risk assessment of pesticides that began to appear throughout the mid- to late 1990s and at the start of this decade. These included the reports of the Aquatic Risk Assessment and Mitigation Dialog Group (12) and ECOFRAM (13) in the USA, and the outputs of the HARAP (14), CLASSIC (15), EUPRA (16) and LEMTOX (17) workshops in the EU. From this perspective, not only have SPs contributed substantially in agronomy, they have also provided considerable material for the development of better tools for aquatic risk assessment, which ultimately contributes to the development of sustainable solutions for pest, disease and weed control.

Aims of the Thesis

The publications presented in the thesis set out to address three specific objectives concerning the aquatic ecotoxicology of the synthetic pyrethoids, namely:

To investigate the toxicity and bioavailability of the pyrethroid cypermethrin in sediments.

To consider the range of environmental and ecological factors that may influence the impacts of SPs on aquatic organisms under field conditions, and investigate factors that influence effects and recovery in semi-field experiments.

To develop a landscape-level probabilistic risk assessment for an example pyrethroid, cypermethrin for uses in US cotton, considering the available laboratory and field ecotoxicity data, and assessing the likelihood of exposure under realistic agronomic use conditions.

Outline of the Thesis

In the thesis, publications are presented which describe research into the aquatic ecotoxicology of the pyrethroids building on laboratory data, through semi-field microcosm and mesocosm studies, and arriving at a landscape level risk assessment for US cotton – hence the title of the thesis ‘The Aquatic Ecotoxicology of the Synthetic Pyrethroids: From Laboratory to Landscape’.

In Chapter 2, sediment toxicity and bioavailability are considered. A detailed assessment of the behaviour of cypermethrin in sediment is presented along with data on its bioavailability and toxicity in sediments to water-column and benthic organisms.

Moving from laboratory to the field, Chapter 3 discusses how ecological factors should be considered in the interpretation of pyrethroid mesocosms. In Chapters 4, 5 and 6, these factors are evaluated through the use of semi-field microcosm and mesocosm experiments. In Chapter 4, an experiment to describe the effects of the pyrethroid *lambda*-cyhalothrin as part of a multi-chemical simulated crop protection regime in potatoes is described, evaluating whether combinations of stressors can be predicted on the basis of individual compound effects. I then review the available microcosm and mesocosm data for the SP *lambda*-cyhalothrin in Chapter 5 (including several publications on which I was co-author), and evaluate the influence of various environmental factors on effects and recovery. In Chapter 6, I describe a microcosm experiment that measured the effects of organism immigration and chemical persistence on the rate of recovery. This study compared the fast-degrading pyrethroid cypermethrin with a more persistent chemical, 3,4-dichloroaniline.

Chapter 7 describes a landscape-level probabilistic risk assessment for the use of cypermethrin in cotton in Mississippi, USA, using cypermethrin as a representative SP. This assessment brings together all of the available laboratory and field effects data on cypermethrin, conducts a landscape-level exposure analysis, and describes the potential risks of use in cotton in a probabilistic manner.

In the summary and concluding remarks chapter 8, the current state-of-the science for the aquatic ecotoxicology of the SPs is discussed with reference to the objectives of the thesis. Finally concluding remarks and suggestions for future research needs are also presented.

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Chapter 2: Partitioning, Bioavailability, and Toxicity of the Pyrethroid Insecticide Cypermethrin in Sediments[1]

Summary

The partitioning, bioavailability, and toxicity of cypermethrin in water–sediment systems was investigated. Cypermethrin adsorbed extensively and rapidly, with an overall mean organic carbon (OC) adsorption partition coefficient (K_{oc}) of 350,000, and approximately 99% adsorption occurred within 24 h. Bioavailability was measured via body burdens of *Daphnia magna* and *Chironomus tentans*. Mean biota–sediment accumulation factors (BSAFs), that is, the concentration in the organism as a proportion of the concentration in the sediment, decreased with increasing OC content. The BSAF values were 0.31, 0.14, and 0.08 for *D. magna* and 0.63, 0.19, and 0.08 for *C. tentans*, in 1, 3, and 13% OC sediments, respectively. The 10-d median lethal sediment concentrations (LC_{50}s) of cypermethrin were 3.6, 18, and 32 µg/kg for *Hyalella azteca* and 13, 67, and 62 µg/kg for *C. tentans* in 1, 3, and 13% OC sediments, respectively. Predictions of aqueous concentrations at the LC_{50} in sediments (based on K_{oc}) compared well to each other and to effect concentrations from studies in water alone, suggesting that equilibrium partitioning theory could be used reasonably to predict and normalize the toxicity of cypermethrin across sediments of differing OC content.

Introduction

Synthetic pyrethroid insecticides have been used for more than 20 years to control insect pests in a variety of crops. Historically, concerns have existed regarding toxicity to aquatic organisms, particularly fish and arthropod invertebrates, because of the high degree of toxicity observed in standard laboratory studies (where exposures are maintained for periods of days to weeks). However, it has also been widely recognized that aquatic organisms are less likely to be affected under field conditions. The amelioration of effects results from a reduction in exposure because of the tendency of this group of insecticides to bind rapidly and extensively to suspended particulate matter, sediments, and aquatic plants (1,2). Although this adsorption provides significant mitigation of possible effects for

[1] Published in Environmental Toxicology and Chemistry, 21, 9-15. Maund SJ, Hamer MJ, Lane MGC, Goggin UM, Gentle WE.

water-column organisms, it raises the question of the potential influence of those chemical residues adsorbed to sediment on benthic and infaunal organisms.

Studies on pyrethroids and other chemicals of similar lipophilicity indicate that a number of factors may affect toxicity and bioavailability in sediment. Pyrethroids readily adsorb to sediments, which greatly reduces bioavailability to water-column organisms (3). However, once associated with sediments, the potential exists for exposure of benthic organisms via sediment particles (by ingestion or contact) or from interstitial water (4). The extent to which lipophilic compounds such as pyrethroids are bioavailable in sediments has been the focus of recent research. To date, indications are that bioavailability for nonionic, organic chemicals is determined by a chemical equilibrium between water, sediment, and organism phases, with bioavailability best predicted from the concentration of chemical in the water phase (so-called equilibrium partitioning theory). It has been demonstrated (5) that reasonable predictions of toxicity (within a factor of two to three) can be made from predicted water-phase concentrations calculated with the sediment organic carbon (OC) partition coefficient (K_{oc}). Furthermore, for chemicals of similar lipophilicity to pyrethroids, it has been demonstrated that OC content is often the most significant sediment component in determining the partitioning (6,7). In this paper we describe a range of studies in which we investigated the extent of adsorption, bioavailability when adsorbed, and the resulting toxicity in sediment of the synthetic pyrethroid cypermethrin (IUPAC name (RS)-α-cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate).

Materials and Methods

Test sediments

Sediments were selected for use in the studies to cover a range of OC contents. A number of natural sediments were obtained from researchers in the United States from which three sediments that contained approximately 1, 3, and 13% OC were selected for use in the studies (Table 1). The 1% OC sediment (Mississippi 2) that was used in the first study to assess bioavailability to Daphnia (see below) was substituted by an alternative 1% OC sediment (Florissant) in subsequent studies.

Table 1. Properties of sediments used in the studies. Mississippi 2 and 3 are from the University of Mississippi Biological Field Station (Oxford, MS, USA); Florissant is from the Midwest Science Center (Columbia, MO, USA); and Duluth is from the University of Wisconsin–Superior (Superior, WI, USA)

	Mississippi 2[a]	Florissant[b]	Mississippi 3[ab]	Duluth[ab]
% Organic carbon	1	1	3	13
Textural properties				
% Clay	10	24	25	25
% Sand	61	6	10	30
% Silt	29	70	65	45
CEC[c] (meq/100 g)	4.0	14.5	13.2	43.6
pH	4.9	6.0	5.1	7.2
Classification	Sandy loam	Silt loam	Silt loam	Loam

[a] Used for *Daphnia* bioaccumulation study

[b] Used for adsorption-desorption studies, *Chironomus* bioaccumulation studies, and *Chironomus* and *Chironomus* and *Hyalella* toxicity studies

[c] CEC = cation exchange capacity

Methods for the analysis of the sediment physicochemical properties were as follows. The sediment pH was determined by slurrying a suspension of one part sediment to two parts water and measuring the pH with a Phillips CE13 sleeve junction combined electrode (Radiometer, Crawley, UK). Particle size analysis was performed by wet sieving for sand content (50- to 2,000-µm fraction), sequential sedimentation, and analysis of supernatant for silt (2 to 50-µm fraction) and clay (<2-µm fraction). Organic matter was determined by the method of Walkley and Black (8), which involves oxidation with K2Cr2O7 followed by titration of the excess dichromate with FeSO4 with barium diphenylamine sulfonate as an indicator. Organic matter content was then divided by 1.724 to estimate OC content. Cation exchange capacity was measured by sodium saturation at pH 7 and flame photometry.

Test chemical

In all of the studies, ^{14}C-phenoxy-labeled cypermethrin (Figure 1) with a specific activity of 2.1 G Bq/mmol and a purity of >99% was used. Cypermethrin is used on a variety of agricultural crops, but also has a number of public health and veterinary uses. The compound has also been extensively studied in laboratory and field ecotoxicological studies (2,9). The water solubility of cypermethrin at 20°C and pH 7 is 4 µg/L (10).

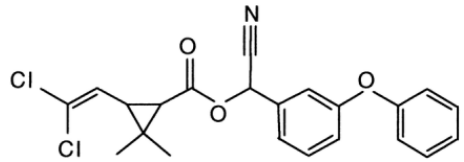

Figure 1. Chemical structure of cypermethrin

Rate and extent of partitioning of cypermethrin in sediment

Measurement of adsorption dynamics and equilibria are important factors for understanding the bioavailability and toxicity of chemicals in sediments. Previous studies have shown that adsorption of pyrethroids is extensive and rapid, occurring in the most part within several hours (11). To confirm these data for cypermethrin, adsorption and desorption properties were determined in the three sediments. Measurements were made with methodologies that broadly complied with U.S. Environmental Protection Agency test guideline 163-1 (12). In addition, to determine the rate at which cypermethrin adsorbed, measurements of adsorption were made at various time intervals after addition of the chemical to the sediment–water system.

In all studies, an air-dried mass of 1 g of sediment (gamma irradiated with 35 kilograys, a standard dose for medical sterilization, to prevent microbial degradation of the test chemical) together with 25 ml of 0.01 M CaCl2 solution was placed in a 50-ml centrifuge tube with a ground-glass stopper. The test system was mixed before the addition of test chemical by shaking on an end-over-end shaker at approximately 1,300 revolutions per hour.

To estimate the time to equilibrium in the test system, cypermethrin was added to the test system in 100 µl of acetone to achieve a nominal concentration in the water phase of 0.045 µg/ml. Ten replicate test systems were prepared for each sediment. The test systems were then placed on an end-over-end shaker at approximately 1,300 revolutions per hour. Two replicate test systems were then analyzed after 2, 6, 24, 48, and 72 h. Test systems without sediment were analyzed after 72 h.

Before analysis, the water–sediment slurries were centrifuged at 1,200 g for 15 to 30 min until the supernatant appeared clear. A 15-ml aliquot of supernatant was then removed and extracted with 5 ml of hexane. The hexane was subsampled for liquid scintillation counting (LSC) on an LKB 1217 Rackbeta counter (Perkin-Elmer, Cambridge, UK), with Optiphase Safe (Fisher Scientific, Loughborough, UK) as the scintillation cocktail. The

remainder of the supernatant aqueous phase was transferred to a glass vial and weighed. The residual sediment pellet was air-dried in the tube, and transferred as completely as possible to a plastic centrifuge tube for extraction with acetonitrile (30 ml, with end-over-end shaking for 2 h). The extract was centrifuged at 1,600 g for 15 min, and the supernatant was removed. This extraction process was repeated two further times for each sample. Supernatants were then combined and made up to 100 ml with acetonitrile, and extracts were analyzed by LSC to quantify the amount of radiochemical recovered. The remaining sediment pellet was then air-dried and combusted to determine the total amount of radioactivity present with a Harvey OX300 Biological Oxidizer (Lab Impex, Teddington, UK). Evolved 14CO2 was trapped in 2-methoxyethylamine and analyzed by LSC. Pyrethroids readily adsorb to glassware, and, therefore, all centrifuge tubes were extracted twice by shaking in acetonitrile and the combined extracts were subsequently analyzed by LSC.

Because LSC only determines the total amount of radioactivity present, further analyses were conducted on all aqueous and sediment extracts to determine what proportion of the extracted radioactivity was parent cypermethrin (as opposed to other breakdown products). Aliquots from each extract were adjusted in volume to approximately 100 µl, which was applied in a 1.5-cm band to a thin-layer chromatography (TLC) plate (CAM-LAB precoated, Fisher Scientific). The plate was immediately placed in a 60:20:4:1 (v/v/v/v) toluene: hexane: chloroform: acetonitrile solvent system. Extracts were cochromatographed with an analytical standard of cypermethrin. Radioactivity on the plates was quantified with either a Rita 68000 or 3200 Automatic TLC Analyzer (Raytex, Sheffield, UK). Autoradiograms were made with a Fuji BAS Phosphorimager (Raytex).

As a result of the time to equilibrium studies (see below), an equilibration time of 24 h was selected for subsequent adsorption and desorption studies. The same test systems as described above were treated with radiolabeled cypermethrin to obtain nominal concentrations in the aqueous phase of 0.015, 0.045, 0.135, and 0.405 µg/ml. Four replicates of each of the three test sediments were prepared for each concentration. After 24 h, two replicate tubes were analyzed to determine the concentration in the water and sediment phase, with the same methods as described above. Two further replicate tubes were used for the desorption step. The tubes were centrifuged, and an aliquot was removed and extracted into hexane for LSC. The aqueous phase was removed and replaced with an equivalent volume of 0.01 M CaCl2 to permit desorption from the sediment. The test system was then shaken for 24 h at 1,300 revolutions per hour, after which the water and sediment phases were analyzed as described above.

Adsorption and desorption were expressed as the sediment adsorption/desorption coefficient (K_d), normalized to the OC content of the sediment (K_{oc}), where:

$$K_d = \frac{\mu g \text{ chemical per g sediment dry weight}}{\mu g \text{ chemical per cm}^3 \text{ of the aqueous phase}}$$

$$K_{oc} = \frac{K_d}{\% \text{ OC}} \times 100$$

Bioavailability of cypermethrin to Daphnia magna *and* Chironomus tentans

Estimating bioavailability of pyrethroids simply from measurements of the chemical in the aqueous phase of water–sediment systems can be difficult because small amounts of particulates, dissolved organic carbon (DOC), or colloids in the water can adsorb the chemical, reducing bioavailability (13,14). Therefore, ascertaining what is truly in the water phase in such small test systems can be difficult. Determination of the relative influence of the various routes of uptake in a multiphase system without affecting the chemical equilibria also is difficult. Therefore, the approach to estimating bioavailability that was considered most likely to succeed was by measuring organism body burdens. Bioaccumulation studies were performed with radiolabeled cypermethrin in three water–sediment systems. One study utilized *D. magna* to estimate bioavailability to organisms exposed principally through the overlying water phase. The second used *C. tentans* to measure bioavailability to a benthic organism additionally exposed through interstitial water and solid phases (by contact or ingestion).

Late (third to fourth)-instar *C. tentans* and adult female *D. magna* were obtained from laboratory cultures. Organisms of this size were required to allow sufficient tissue to be present for subsequent analyses. Test systems consisted of 500-ml glass jars containing 10 g dry weight of sediment and 250 ml of water. In the test systems containing *C. tentans*, 8 mg of ground TetraMin® (Tetra Sales, Blackburg, VA, USA) also was added as a food source. Given the small amount of food relative to the mass of the sediment, it was considered that this would have minimal influence on the partitioning and bioavailability of cypermethrin. *Daphnia magna* were fed with algal and yeast 24 h after their addition to the test systems.

For the *C. tentans* studies, nominal application rates were 40, 100, and 150 µg/kg sediment dry weight for the Florissant, Mississippi 3, and Duluth sediments, respectively. For the *D. magna* studies, nominal application rates were 423, 1,260, and 5,320 µg/kg

sediment dry weight for the Mississippi 2, Mississippi 3, and Duluth sediments, respectively. Application rates were selected to provide concentrations that were high enough to permit test organisms to accumulate sufficient chemical to quantify, while being low enough to avoid acute toxic effects, based on the water-only toxicity data and the expected partitioning of the compound (2). After application of the test chemical in a 100-µl aliquot of acetone, the jars were placed on a rolling mill overnight to ensure even mixing of the chemical in the test system. The test systems were then allowed to settle for 2 d before the introduction of 10 organisms and were then incubated in a water bath at 20°C and 23°C for the *D. magna* and *C. tentans*, respectively. After 24, 48, 72, and 96 h, test systems were analyzed for concentrations of the test chemical in the sediment, sediment pore water, overlying water, and test organisms. For the *D. magna* studies, two replicates were analyzed at each time point. For the *C. tentans* study, a further replicate was analyzed at each time point because an additional test system was required for analysis of the sediment pore water (this was not possible after removal of the test organisms because of disruption of the test system).

To analyze the overlying water, a 100-ml aliquot was removed and extracted into 5 ml of hexane and subequently analyzed by LSC. The remaining overlying water was then removed, and in the case of the *D. magna* studies, the organisms were also removed. Sediment pore water was then extracted from the sediment of one replicate. The sediment was placed in a centrifuge tube and centrifuged at 2,100 g for 15 min to separate the pore water from the sediment. The pore water was then removed and extracted with hexane for subsequent analysis by LSC (as described above). For the *C. tentans* studies, the organisms were removed from the sediments in the remaining two replicates. The sediments from all replicates were extracted with acetonitrile in an end-over-end shaker for 1 h, and were centrifuged at 2,100 g for 15 min. The extracts were then quantified by LSC. Any remaining sediment was dried and combusted (as described above) to determine the amount of radioactivity remaining in the sediment. The organisms were counted, rinsed with water, blotted dry, then wet-weighed in a combustion cone before analysis by LSC.

Aliquots of water and sediment extracts were also analyzed by TLC (as above) to determine what proportion of the extracted radioactivity was cypermethrin. Extraction and characterization of the radioactivity in the organisms was not possible because too little radioactivity was present. However, because the breakdown products of cypermethrin are polar and readily excreted, the majority of radioactivity measured in the organisms was assumed to be parent cypermethrin. Some 14C may have been

incorporated into tissues, and assuming that this was cypermethrin would have overestimated the amount bioconcentrated, and hence the bioavailability.

Toxicity of cypermethrin in sediment to Hyalella azteca *and* Chironomus tentans

Sediment toxicity tests were conducted with *H. azteca* and *C. tentans*, to evaluate effects on mortality and growth over 10 d. The test method was based on the methods of the U.S. Environmental Protection Agency (15).

Test systems were prepared in the same way as those described in the bioavailability studies above. For *C. tentans*, nominal sediment test concentrations ranged from 2.2 to 180, 3.4 to 300, and 5.6 to 450 µg/kg, for the Florissant, Mississippi 3, and Duluth sediments, respectively. For *H. azteca*, nominal sediment test concentrations ranged from 2.5 to 40, 0.74 to 60, and 1.9 to 150 µg/kg for the Florissant, Mississippi 3, and Duluth sediments, respectively. Six replicates were prepared for each test concentration. In addition, dilution water and solvent controls were prepared for each sediment and organism. Test organisms were obtained from laboratory cultures and third-instar *C. tentans* (confirmed by head capsule measurements) and 7- to 14-d-old *H. azteca* (selected by sieving through a 500-µm mesh and retaining on a 250-µm mesh) were used to initiate the tests. Ten organisms were added to each test system, after the test system had equilibrated. Test systems were covered to reduce evaporation and were incubated in a water bath at 23°C on a 16:8 h light:dark cycle at approximately 800 lux (lumen per square meter). *Chironomus tentans* were fed at discretion (i.e., if no food was visible at the sediment surface) with ground TetraMin. In practice, larvae were provided with 20 mg of TetraMin on two to three occasions during the study. Given the small amount of food relative to the mass of the sediment, it was considered that this would have minimal influence on the relative toxicity of cypermethrin in sediment. After 10 d, a 100-ml aliquot of water was removed from the overlying water of all of the test systems. This was extracted into 5 ml of hexane and analyzed by LSC (as above).

Organisms were carefully removed from four of the six replicate test systems. The number surviving was recorded and remaining organisms then were preserved for length (*H. azteca* only) and dry weight determinations. The overlying water from the two remaining test systems was then removed and the sediment pore water was extracted as described in the bioavailability studies above. The remaining sediment from all replicates was analyzed for radioactivity as described above. Some of the sediment extracts were also analyzed by TLC (as described above) to characterize the radioactivity.

Survival data were analyzed by the technique of iteratively reweighted linear regression of logit response on log 10 concentration. Length and weight data were analyzed by analysis of variance with the Statistical Analysis System (SAS®, Cary, NC, USA).

Results and Discussion

Adsorption dynamics

The average recovery of radioactivity applied was 109%, indicating that the extraction procedures had been effective. The TLC analysis of sediment extracts confirmed that >97% of the radioactivity in the extracts was cypermethrin. The TLC analyses of aqueous extracts were very difficult to quantify because of the low amounts of radioactivity present. Cypermethrin typically constituted >60% of the radioactivity, but small amounts of more polar compounds were sometimes apparent, suggesting that some degradation had occurred. However, for all calculations of partition coefficients, all of the radioactivity in the water phase was assumed to be parent cypermethrin. Because this would increase the estimate of water concentration, this approach would underestimate the degree of partitioning.

As expected, cypermethrin adsorbed rapidly to sediments, reaching equilibrium in the sediment in all cases in less than 24 h (Figure 2). Indeed, the vast majority (>98%) of total adsorption had already occurred within 2 h of application of the chemical. For all three sediments, approximately 99% or more of the total amount of chemical applied to the test system was adsorbed to the sediment at equilibrium.

A direct relationship was found between adsorption and increasing OC content, with mean sediment partition coefficients (K_ds) of 2,360, 15,700, and 23,600 for 1, 3, and 13% OC sediments, respectively. In theory, if partitioning was only determined by OC content, a direct relationship would exist between OC and K_d, which would result in an expected ratio of 1:3:13 between these K_d values. Here, the average ratio was 1:6:10, which seems to support the hypothesis that adsorption is closely related to OC content, within a factor of two (5).

Normalizing adsorption to sediment OC content, mean adsorption K_{oc} values were 238,000 (standard deviation (SD) = 38,000; coefficient of variation (CV) = 16%), 502,000 (SD = 27,000; CV = 5%), and 177,000 (SD = 40,000; CV = 23%) for the 1, 3, and 13% OC sediments, respectively. The overall mean adsorption K_{oc} of cypermethrin was 350,000. Theoretically, K_{oc} should be a constant for a particular chemical, that is, an increase in OC content should lead to a direct increase in adsorption. However, adsorption probably also

is affected by the physical nature of the OC present in the sediment and the surface area available for adsorption (the latter being a function of particle size distribution within the soils).

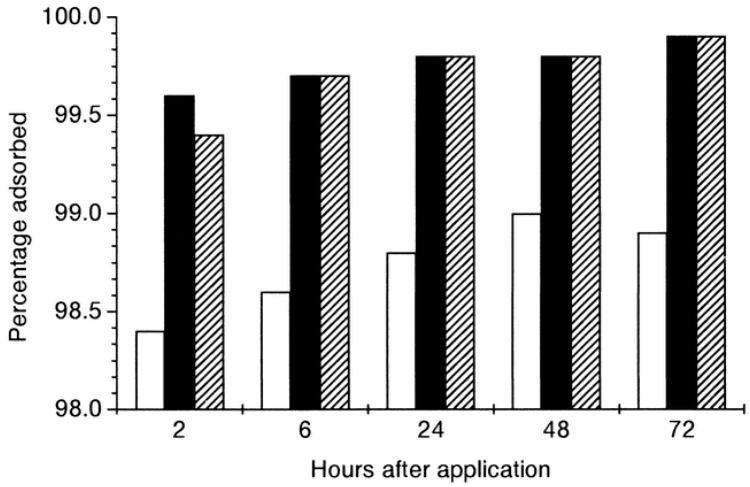

Figure 2. Rate of adsorption of cypermethrin to three aquatic sediments (column legend: white = Florissant; black = Mississippi 3; cross-hatched = Duluth)

Organic carbon that is present in small, often cominuted particles or coating the surface of mineral particles is likely to present a greater potential for adsorption than larger intact particles of OC, because of increased surface area. However, the method of Walkley and Black of analyzing for OC content (as used here) does not take surface area into account, because OC is digested from the sediment in its entirety (8). Therefore, two sediments that have the same measured OC content, but different surface areas of OC, possibly could adsorb the chemical slightly differently. This factor probably accounts for the variation in K_{oc} values observed in this study. Furthermore, aggregated OC would be expected to cause more variability in adsorption measurements because of the more heterogeneous distribution of larger particles. This was observed for the 13% OC sediment in this study, whose adsorption characteristics were more variable than the lower OC sediments (see coefficients of variation for adsorption K_{oc} values described above and desorption K_{oc} values described below).

Measured K_{oc} values after the desorption step were similar to, although somewhat higher, than those measured after the adsorption step, averaging 281,000 (SD = 28,000; CV

=10%), 582,000 (SD = 75,000; CV = 13%), and 182,000 (SD = 73,000; CV = 40%) for the 1, 3, and 13% OC sediments, respectively. This suggests that the adsorption of cypermethrin may not be entirely reversible, indicating that adsorption K_d values might be somewhat conservative in estimating potential bioavailability.

Bioavailability

Measured concentrations (based on total radioactivity) in the sediment ranged from 80 to 85% of nominal in the studies with *D. magna*, and from 74 to 97% in studies with *C. tentans* (Table 2). Extractable radioactivity (which was determined to be only 14C-cypermethrin by TLC) accounted for the vast majority (88–98%) of the total radioactivity, and the vast majority of total radioactivity was cypermethrin, with no other major products present. Relatively little change occurred in the concentration of cypermethrin in the water, sediment, or organism phases through time, indicating that the organisms rapidly reached equilibrium with the test system. Similar rapid equilibration has been found in previous studies with pyrethroids (11). In addition to organisms and sediment, concentrations of cypermethrin in the interstitial and overlying water were analyzed (Table 2). Cypermethrin was extracted from whole water samples (no centrifugation) and hence the amount of cypermethrin truly in solution and that associated with DOC and suspended and colloidal material would not be distinguished. The analytical methods used will not readily allow the concentration truly in solution and that associated with DOC and suspended material to be distinguished at such low concentrations.

In the majority of cases, concentrations of cypermethrin in the interstitial water were up to an order of magnitude higher than those in the overlying water. This may have resulted from the centrifugation extraction process for pore water, causing additional amounts of DOC and colloids with their associated cypermethrin to be released from the sediment, thereby increasing the apparent concentration of cypermethrin in the interstitial water. Similar results have been observed with another lipophilic pesticide, chlorpyrifos (14). Measurements of cypermethrin in the overlying water may also have reflected a similar influence of increasing DOC and colloids with increasing OC content in the sediment, because average K_{oc} values decreased with increasing OC content in both studies (Table 3).

To compare the relative bioavailability of cypermethrin to the organisms in the various studies, bioavailability was expressed as biota–sediment accumulation factor (BSAF), that is, the concentration in the organism divided by the concentration in the sediment. This ratio indicates the proportion of the chemical adsorbed to the sediment that has accumulated in the organism. For example, if the concentration in the sediment is 100

mg/kg and the concentration in the organism is 50 mg/kg, the BSAF would be 0.5; if the sediment concentration is 100 mg/kg and that in the organism is 10 mg/kg, the BSAF would be 0.1. Hence, BSAF decreases as bioavailability decreases.

Table 2. Measured concentrations in the water, sediment, and organism phases in bioavailability studies with *Daphnia magna* and *Chironomus tentans*

Sediment	Time (h)	Body burden (µg/kg)	Overlying water (ng/L)	Sediment (µg/kg)	Pore water (ng/L)
***D. magna*[a]**					
Mississippi 2	24	90	34	307	170
	48	110	41	377	220
	72	123	27	378	185
	96	107	21	383	145
Mississippi 3	24	109	54	1061	480
	48	130	66	989	535
	72	130	44	1093	285
	96	171	28	1111	270
Duluth	24	424	535	3959	1850
	48	282	645	4287	980
	72	292	390	4410	2000
	96	133	260	4345	1350
***C. tentans*[b]**					
Mississippi 2	24	13	8	31	70
	48	21	9	30	60
	72	15	9	29	40
	96	18	9	29	40
Mississippi 3	24	16	18	95	40
	48	18	16	98	40
	72	19	13	100	50
	96	21	11	96	70
Duluth	24	11	25	133	20
	48	12	35	129	40
	72	10	22	137	40
	96	9	4	140	20

[a] Number of replicates for each phase was two.
[b] Number of samples for each phase was: organism 2; overlying water 3; sediment 3, pore water 1

Table 3. Mean sediment organic carbon partition coefficients for 1, 3, and 13% organic carbon sediments measured in bioavailability studies with *Daphnia magna* (*n* = 2) and *Chironomus tentans* (*n* = 3). Standard deviations of means for each time interval are shown in parentheses below the mean values

Time (h)	1% organic carbon		3% organic carbon		13% organic carbon	
	D. magna	*C. tentans*	*D. magna*	*C. tentans*	*D. magna*	*C. tentans*
24	850 000	363 000	650 000	193 000	50 000	38 000
	(99 000)	(25 000)	(190 000)	(60 000)	(4 200)	(7 700)
48	920 000	382 000	470 000	228 000	47 000	34 000
	(99 000)	(128 000)	(64 000)	(68 000)	(3 500)	(12 000)
72	1 390 000	307 000	780 000	292 000	84 000	58 000
	(190 000)	(61 000)	(42 000)	(98 000)	(6 400)	(22 000)
96	1 820 000	370 000	1 250 000	438 000	118 000	192 000
	(280 000)	(136 000)	(78 000)	(249 000)	(7 100)	(98 000)
Average	1 245 000	356 000	788 000	288 000	75 000	81 000

For the 3 and 13% OC sediments, the mean sediment BSAFs for *Daphnia* and *Chironomus* were very similar (Table 4). For the 1% OC sediments, small differences of a factor of two occurred in bioavailability. This may have been due to the use of different sediments in the two studies (Mississippi 2 for Daphnia and Florissant for *Chironomus*; see Table 1). For these 1% OC sediments, cypermethrin seemed to be more bioavailable in the Mississippi 2 sediment than in the Florissant sediment, as perhaps might be expected with the higher sand content of Mississippi 2. Where the same sediments were used, analysis of the data clearly demonstrated that no difference occurred in the relative bioavailability to water column or benthic organisms of cypermethrin adsorbed to sediments in the test systems used.

Comparison of the BSAFs for the different sediment types (Table 4) demonstrated decreases in bioavailability with increasing OC content. If bioavailability is inversely related to OC content, a linear decrease in bioavailability would be expected as OC content increases. Relative differences between the sediments broadly followed this pattern. The average ratio between bioavailabilities for 1 and 3% OC sediments was 1:3, exactly as expected for this difference in OC content. However, the ratio between the 3 and 13% OC sediments was 1:2, compared to an expected ratio of 1:4. Once again, this difference may be due to smaller surface area of OC in the 13% OC sediment (see above), providing slightly less adsorption as a function of OC than expected. However, a discrepancy of a factor of approximately two to three is consistent with the expected precision of

bioavailability estimates based on equilibrium partitioning (5). The bioavailability results observed for cypermethrin were consistent with those observed for another pyrethroid, λ-cyhalothrin (16).

Table 4. Mean biota–sediment accumulation factors (BSAFs) for cypermethrin in sediment of varying organic carbon content. Values in parentheses are 95% confidence limits

Sediment organic carbon content (%)	BSAF *D. magna*	BSAF *C. tentans*	Mean BSAF
1[a]	0.31 (0.28 – 0.34)	0.63 (0.50 – 0.76)	0.47
3	0.14 (0.12 – 0.16)	0.19 (0.17 – 0.21)	0.17
13	0.08 (0.06 – 0.10)	0.08 (0.06 – 0.10)	0.08

[a] Studies were performed using two different 1% organic carbon sediments – Mississippi 2 and Florissant for *Daphnia* and *Chironomus* respectively (see Table 1 for sediment characteristics)

The observed differences in the amount of chemical in the water phase (overlying and interstitial) were clearly not reflected in the bioavailability data, where bioaccumulation decreased with increasing sediment OC (Table 4). However, the relative bioavailability would have been adequately described by differences in K_d values from the adsorption–desorption study described above. The difference between the studies can be explained by differences in methodologies between the two study types. Adsorption–desorption studies overcome the problem of chemical associated with DOC and colloids in the water phase by removing these materials by addition of a flocculating agent, calcium chloride. This coagulates organic material, promoting settling and thereby reducing apparent water-phase concentrations, but giving a more realistic measurement of actual dissolved concentrations. Such additions are impractical when organisms are present because of the potentially toxic effects of concentrated calcium chloride. However, indications of a similar process occurring through settling of DOC and colloids with time were also apparent in the bioavailability studies, where K_{oc} values tended to increase throughout the study (Table 3).

In combination, the potential errors that may result from water-phase measurements (overlying or interstitial) due to the presence of DOC and colloids suggest that whole water-phase concentrations may not be a very good predictor of bioavailability and hence toxicity for pyrethroids or other highly lipophilic chemicals because of the difficulty of establishing what is truly in the aqueous phase. Concentrations of the chemical in the sediment together with sediment OC content seem to provide a more robust predictor of the bioavailability.

Sediment toxicity studies

Measured concentrations in the sediment on day 0 were generally similar to, albeit slightly lower than, the nominal concentrations. For *H. azteca* studies, extractable sediment residues on day 10 were approximately 65, 75, and 80% of day 0 values for the Florissant, Mississippi 3, and Duluth sediments, respectively. For *C. tentans* studies, extractable sediment residues on day 10 were approximately 70, 90, and 90% of day 0 values for the Florissant, Mississippi 3, and Duluth sediments, respectively. Effects on survival and growth of *H. azteca* and *C. tentans* after 10-d exposures in sediment are shown in Table 5. Effect concentrations were calculated on the basis of initial (day 0) measured concentrations in the sediment.

Data from the toxicity studies (Table 5) were not as precise as those developed in bioavailability studies (note the confidence limits for mortality data). Two factors are likely to have influenced this. First, a number of experimental variables other than the toxicant can affect the expression of whole-organism toxic responses (e.g., energetic, growth, or reproductive status of test organism; interaction with test sediment; and so on). Second, designing tests that contain concentrations that are wholly suitable for measuring endpoints for both mortality and growth is difficult. Some compromise in the test concentrations is necessary. Nevertheless, for the 1 and 3% OC sediments, toxicity followed the same pattern that was seen in the adsorption and bioavailability studies, with decreases in toxicity observed with increasing OC content, as would be expected. Reductions in toxicity were similar to what would have been expected in the 3% OC sediment, with median lethal concentration (LC_{50}) values approximately five times greater than the 1% OC sediment. However, toxicity of cypermethrin in the 13% OC sediment was similar to that observed in the 3% OC sediment.

As with the bioavailability studies described above, although differences in OC content (as measured by soil characterization methods) would be predicted to cause decreases in bioavailability and hence toxicity, the possibility exists that not all of the OC measured in the 13% OC sediment is available to adsorb the chemical. This would lead to differences in the apparent toxicity because of sediment sorption. Differences in adsorption are also reflected in the relatively low K_{oc} for the 13% OC sediment measured in this study (average K_{oc} was 78,000), which suggests that a smaller proportion of cypermethrin was adsorbing than would be predicted from the OC content (average K_{oc} from adsorption–desorption studies was ~350,000).

Table 5. Effects of cypermethrin in three sediments on survival and growth of *Hyalella azteca* and *Chironomus tentans.* Confidence limits for survival measurements are given in parentheses[a]

Sediment organic carbon content (%)	*H. azteca*		*C. tentans*	
	10 d LC_{50} (µg/kg)	NOEC growth (µg/kg)	10 d LC_{50} (µg/kg)	NOEC growth (µg/kg)
1	3.6 (3.1 – 4.2)	< 1.8	13 (4.5 – 42)	3.8
3	18 (15 – 23)	2.3	67 (24 – 215)	25
13	23 (19 – 28)	1.8	62 (23 – 176)	14

[a] LC_{50} = median lethal concentration; NOEC = no observed effect concentration

Predictions of the water-phase concentrations at the LC_{50} and no-observed-effect concentration in the three sediments can be made by using the K_{oc} measured for each sediment in the adsorption and bioavailability studies (see formulae in methods). Hence, when the K_{oc} is 350,000, the sediment has an OC content of 3%, and the concentration on the sediment in the test system (10 g) is 10 µg/kg, then the concentration in the water phase (250 ml) would be predicted to be 0.001 µg/L. Table 6 shows predicted water-phase concentrations derived from the sediment toxicity values based on the K_{oc} measured for each sediment and from the overall mean K_{oc} (350,000).

Effect concentrations for both Hyalella and Chironomus were highly consistent when the data were normalized according to the concentration that was predicted to be in the water phase (Table 6). This demonstrates that K_{oc} (both measured for a specific sediment or as an overall constant for a particular chemical) is a reasonable approach for obtaining a preliminary indication of potential exposure concentrations for sediment organisms.

Previous studies with *H. azteca* (7–14 d old) and *Chironomus riparius* (first instar) generated 48-h LC_{50}s in water alone of 5.3 and 6.9 ng/L, respectively (Zeneca Agrochemicals, unpublished data). When normalized to concentrations predicted in the water phase, the effect concentrations in the sediment studies were of the same order of the toxicity measured in water alone, albeit slightly lower probably because of the longer duration of the study (10 d as opposed to 2 d). The predicted water-phase values also were similar to other acute toxicity data for cypermethrin with sensitive insects and amphipods (2). This suggests that reasonable predictions of cypermethrin toxicity in sediment could be made by estimating the concentration of cypermethrin in the aqueous phase and comparing that to toxicity data from water-only studies.

Table 6. Effect concentrations (mortality and growth) normalized according to predicted concentrations in the water phase (based on organic carbon partition coefficients (K_{oc}s) measurements from adsorption–desorption and bioavailability studies) from sediment toxicity studies on *Hyalella azteca* and *Chironomus tentans* with cypermethrin[a]

Sediment organic carbon (%)	Predicted water phase concentration (ng/L)				
		H. azteca		*C. tentans*	
	K_{oc}	LC_{50}[a]	NOEC[a]	LC_{50}	NOEC
1	239 000[b]	1.5	< 0.76	5.5	1.60
	350 000[c]	1.0	< 0.52	3.8	1.10
3	503 000[b]	1.6	0.22	4.3	1.58
	350 000[c]	1.6	0.22	6.2	2.27
13	178 000[b]	1.0	0.08	2.6	0.59
	350 000[c]	0.5	0.05	1.3	0.30

[a] LC_{50} = median lethal concentration; NOEC = no observed effect concentration
[b] Mean K_{oc} for sediment in adsorption-desorption studies.
[c] Overall mean adsorption K_{oc}

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Chapter 3: Ecological Considerations in Pesticide Risk Assessment for Aquatic Ecosystems[2]

Summary

Risk assessment of pesticides for aquatic ecosystems is typically based on comparisons of exposure and effect concentrations at a variety of levels (tiers). At the highest tier, effects assessment can involve generating data under field conditions, typically in mesocosm experiments. However, interpreting the ecological significance of effects measured in these studies can be difficult because ecological factors can influence the outcome of perturbations in the real world. The influence of ecological factors is not readily addressed experimentally and so a strategic modelling approach is proposed which may aid in defining acceptability of effects.

Introduction

In this chapter, the interpretation of mesocosm data in pesticide risk assessment with regard to determining acceptable levels of effect will be discussed. An acceptable effect can be defined as a measurable difference between untreated and pesticide-treated mesocosms which is of little ecological significance. Modelling approaches which may provide guidance on the acceptability of effects under different environmental conditions will be described.

Procedures for assessing potential risk from the use or release of pesticides and other organic chemicals to aquatic environments are reasonably well-established with various national and international regulatory authorities. Although differing in certain details, these are mostly based on a similar general concept i.e. that potential risk can be described by comparison of concentrations which elicit effects on organisms with concentrations which are predicted to occur in the environment for a specific use pattern.

For pesticides, products are considered to present no risk if the ratio of these two figures is less than a pre-determined safety or application factor, usually between 10 and 100 for acute assessments and between 1 and 10 for chronic. This approach is conservative;

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effect concentrations are generated under worst-case exposure conditions in the laboratory and environmental concentrations are predicted under extreme use and exposure scenarios. Therefore, if a compound is determined to present no potential risk from this preliminary assessment, adverse impacts are unlikely to occur during use. But the converse is not necessarily true; if a compound is identified as presenting a potential risk in preliminary assessments, it does not inevitably mean there will be a risk in reality because the process is conservative. This is because other factors (changes in bioavailability in the environment, interspecies differences in sensitivity, etc.) not included in the initial evaluation could significantly mitigate potential risks.

If a potential risk is identified from preliminary assessments, two courses of action can be taken: either management measures can be used to reduce exposure by limiting use patterns (e.g. by lowering label use rates and numbers of applications, or by imposing buffer zones between the use area and water courses) so that the potential for the chemical entering the environment is reduced or by generating additional laboratory data on potential effects and/or exposure to provide a more realistic quantification of risks, usually to account for a mitigating factor not included in the preliminary assessment. At the extreme of experimental complexity, this latter option also includes generating data under field conditions, usually through the use of outdoor microcosms or mesocosms - experimental systems which attempt more realistically to simulate ecological and chemical fate processes and therefore produce a better representation of the likely environmental effects of the chemical.

Interpreting Effects in Mesocosm Studies

There has been a great deal of debate concerning the use of microcosm and mesocosm studies in pesticide risk assessment, particularly with regard to the experimental design and interpretation of such studies (1,2). Mesocosms are designed to measure differences (often by analysis of variance) between control and treated mesocosms for a variety of different variables. Interpreting the ecological importance of such differences can be difficult and requires consideration of a number of important factors.

Recovery from perturbation

Although impacts may be observed on certain organisms during a study, they may subsequently recover during the course of the experiment. Some consideration should be given to the duration of a difference before it is considered ecologically significant. This will also vary for different types of organisms. Even what may appear as relatively easily interpretable endpoints, such as differences in abundance (i.e. population reductions), may not be so obvious depending on the life history of organism. The mortality of an individual may be important for an organism with low reproductive rates and a long life-cycle, but may be of little significance to a fecund species with a short generation time.

Relevance to other aquatic ecosystems

Measuring effects in one type of experimental system may provide useful information for determining what happens in that specific system. However, it may be difficult to interpret those effects in relation to other types of ecosystem. It has been suggested (3) that differences in climate, size, location, etc., may mean that results from one study may not always be readily extrapolated to all other ecosystems. In many cases, mesocosms may provide an ecological worst-case because of their relatively small size and isolation.

Influence of experimental design

For analysis of variance, whether a significant difference is detected between treatment and control depends on the sensitivity of the experiment; two experiments identical but for differing amounts of experimental noise (error) could give apparently very different results. A “noisy” experiment is less likely to detect differences than a less variable study. Consequently, if the conclusions of the studies (effect or no effect) were based on number of significant differences alone, then the conclusions reached may be erroneous. At present, there are no guidelines for what constitutes acceptable levels of variation within a mesocosm study and this decision is often delegated to “expert judgment”. Furthermore, noise can vary enormously between different sampling events. For every statistical comparison, there is always a defined probability that a difference will be designated as significant, when in fact it is not (Type II error or false positive when there is a null hypothesis of no effect). For example, at the 95% significance level, there is a 5% probability of measuring a false positive. Mesocosm studies typically evaluate a very large number of variables over a relatively long time period, which has implications for interpretation, since the number of false positives will increase proportionally with the

number of measurements. It is apparent, therefore, that a measured statistical difference may not always be ecologically important. Uncontrollable chance events which Hurlbert (4) defines as "demonic intrusions" can also lead to differences between control and treatments which may not necessarily be related to treatment concentration, often resulting in an apparent lack of dose response. The results of such experimental "catastrophes" are usually reasonably apparent. However, more subtle intrusions such as minor environmental gradients may go undetected.

An Example with the Pyrethroid Insecticide Cypermethrin

In order to illustrate some of the difficulties that can arise from interpreting mesocosm studies, data on the effects of cypermethrin on two peracarid crustacean families, the Gammaridae and Asellidae, will be described. Cypermethrin is a pyrethroid insecticide which is used in a wide variety of crops. There is a substantial amount of information on the compound, including a number of mesocosm and large-scale field studies which have been carried out under realistic agricultural conditions (5). Cypermethrin is of high inherent toxicity to Peracarida, with acute toxicity values in the region of 0.01 µg/L (Zeneca, unpublished, and Ref. 6). Depending on the crop and use rate, instantaneous worst-case predicted environmental concentrations from agricultural uses are estimated in the region of 0.01 – 0.1 µg/L. Preliminary risk assessment of cypermethrin would therefore suggest that there is potential for effects on Peracarida. Additional laboratory studies have shown (7) however, that, due to the rapid adsorption of cypermethrin to sediment and other organic matter (organic carbon partition coefficient (K_{OC} is approximately 200 000), exposure of crustacea in the field is likely to be reduced substantially, by around 300 times. This information indicates that the preliminary risk assessment overestimates the potential risk of cypermethrin, such that investigation of effects under more realistic conditions is likely to indicate that potential risk will be greatly mitigated. A mesocosm study performed at exposure rates similar to those that might be expected from agricultural spray drift (1%), investigated impacts on crustacea in 25 m^3 mesocosms (8). In this study there were significant reductions in both Asellidae and Gammaridae. There were indications that the Asellidae may have been beginning to recover by the end of the study, but Gammaridae were eliminated from the treated mesocosms. The results may seem fairly unequivocal - statistically significant effects were observed on both taxa and the apparent elimination of Gammaridae from the mesocosm could certainly be regarded as of potential ecological significance. But how representative are these data of natural ecosystems and how relevant are they to other types of ecosystem? Ecologically important processes such as immigration from other water bodies and recolonization

from unaffected sites within the water body may not have occurred in these experimental systems at rates that would occur in nature. Further doubt is shed on the interpretation of these effects by examining data from larger-scale field studies. In a series of experiments which aimed to simulate realistic agricultural exposure to cypermethrin (9, 10) several natural water courses containing Peracarida have been studied. These studies indicated that additional processes are occurring which may not have been effectively simulated in the mesocosm study. In a study in a farm ditch neighbouring a cypermethrin application (9), effects were apparent on Gammaridae close to the application area, but recovery occurred very rapidly (in contrast to the mesocosm study), probably from recolonisation from unaffected sites. In another study on several farm ponds to which cypermethrin was applied (10) interpretation became even more difficult when comparing several ponds, since each appeared to respond in a different way. It is clear from these data that very different "effects" can be observed on Asellidae depending on the type, size and location of the ecosystem in question and its condition at the time of perturbation.

What Constitutes an Unacceptable Effect?

The data described above demonstrate that, to be able to interpret results from mesocosm and field studies, some definition of what constitutes an effect of ecological importance is required. Aquatic ecosystems are diverse and contain organisms which are related to target organisms of crop protection compounds (e.g. arthropods for insecticides, algae and macrophytes for herbicides). Thus, depending on the specificity of the compound to a particular taxon, if there is potential for exposure, then under certain conditions there may be potential for some sort of effect. But having reached the higher tiers of risk assessment associated with mesocosm studies, we have already identified that there may be potential for some effects under certain conditions. In order to be able to design an effective study, it is necessary to specify what degree of effect is acceptable for various endpoints. This clearly cannot be simply prescriptive and depends on the objective of the risk assessment. One could consider several different potential objectives for the risk assessment:

- Maintaining biodiversity: ensuring that there are no species extinctions (although it should be recognised that local extinctions are a natural, albeit usually slow, process, associated with species ranges and environmental change);
- Maintaining a certain degree of functionality: e.g. production of a certain biomass of invertebrate food species for fisheries ;

- Protecting a certain type of habitat from degradation (wetland, areas of outstanding natural beauty, sites of special scientific interest) ;
- Protecting rare, threatened or endangered species.

These are clearly not trivial considerations and involve not only scientific considerations, but aesthetic, economic (efficient food production) and political (environmental quality objectives) concerns. It should also be remembered that pesticide risk assessment should not be viewed in isolation but should also include a benefits evaluation against which any potential environmental cost can be balanced. To help overcome some of this complexity, it would clearly be helpful if ecotoxicologists could provide a scientific framework for guiding such decisions. With an objective of maintaining biodiversity, for example, this would involve developing approaches that could identify scenarios of perturbation which would describe conditions under which all species would persist and also identify those cases where perturbation may lead to the extinction of a species within a specific region. The development of such a management tool would enable risk assessors and managers to identify which uses of pesticides would be acceptable, even given the broad range of objectives described above.

Modelling Approaches

Developing such guidance on the ecological acceptability of perturbation is a considerable undertaking. A large number of factors will determine the persistence of populations of aquatic organisms after exposure to pesticides. Although estimates of exposure and effect concentrations are useful, they are often insufficient alone to predict adequately the consequences of pesticides on a given species. In order to make accurate predictions of population persistence, other factors should be considered, including:

- The spatio-temporal dynamics of the organisms (e.g. intrinsic rate of increase, carrying capacity, immigration, mobility, position in the habitat) ;
- The spatio-temporal dynamics of the chemical perturbation (e.g. timing, magnitude, duration, frequency, diffusion, decay) ;
- The overall susceptibility of other species in the ecological community (e.g. competitors, predators, parasites, symboints).

Accounting for all of these interacting factors simultaneously is a huge challenge. However, by extrapolating the results of investigations of short-term dynamics in small-scale experimental systems, we can nevertheless make some coarse predictions. The process of extrapolation in arriving at these predictions is of fundamental importance, not

least because we can never hope to evaluate directly the consequences of chemical perturbation for every species in every form of aquatic habitat. Formal mathematical and computer modelling is perhaps the most objective and reliable tool for extrapolation, although even this approach is not without its drawbacks. For instance, ecological models are notoriously poor at accurately predicting quantities such as population size, and the behaviour of some of the more complex ecological models can be as difficult to understand as the real world. Nevertheless, while modelling is not a substitute for laboratory research, it is the only affordable, ethical and objective way of predicting the large-scale ecological impacts of certain chemicals. How can such modelling approaches be developed?

Top-down or "holistic" models are generally capable of more accurate forecasts than bottom-up, reductionist models. For instance, by analyses of current experimental databases, it may be possible to identify those combinations of ecological life-history and habitat attributes which make aquatic organisms susceptible to certain types of pesticide. Thus, for example, with enough experimental data it might be possible to make a probabilistic estimate of the likelihood of a given species becoming extinct under a particular set of conditions, based on what is already known. Besides predictive power, holistic models are popular because they can be easily applied and are not based on unreliable theorising. The problems with holistic models are that they usually require extensive data (which increase dramatically with the number of factors considered), they give no indication of cause *per se*, and they are not easily extended to ranges of factors outside the range of data included (they are better at interpolation than extrapolation in the strict sense of the word).

A complementary method to holistic modelling is the reductionist approach, which aims to develop predictions based on more general ecological principles. Reductionist models can be conveniently classified into tactical (detailed models aimed at accurate forecasts) and strategic (general formulations aimed at generating broad properties) approaches (11). In many respects the reductionist approach to bridging the gap between experiment and prediction is preferable. For instance, the formalisation of general principles helps us to identify hidden assumptions that we may hold, it serves as a framework for testing causal relationships and it does not rely as heavily on interpolation. Most reductionist ecotoxicological models have concentrated on a limited number of well-studied organisms and tried to relate physiological changes in individuals to changes in the population - a tactical approach. However, without large amounts of physiological data on a very wide range of species, the applications of this approach are

limited. Since we wish to maintain a general approach (identify types of organisms of risk under certain pesticide uses), then strategic models are more appropriate.

Recently, there has been some progress in formulating strategic reductionist models of the impact of xenobiotics on terrestrial invertebrates (12, 13) and freshwater species (for example see Figure 1). Figure 1 shows that by the use of relatively simple ecological relationships, such as that between the rates of immigration and reproduction (e.g. the Euler-Lotka equation), general principles of likely recovery of organisms of different life history traits can be defined. For organisms with high reproductive rates (increasing r on Figure 1), the rate of immigration (increasing a on Figure 1) is less important in determining the rate of recovery than for those with low reproductive rates. When reproductive rate is low, immigration will be critical to the rate at which a population recovers. This may seem an obvious conclusion, yet the implication at the landscape scale may be extremely important, when one considers that immigration rates will be greatly dependent on the degree of isolation of the ecosystem and the availability of recolonising organisms from neighbouring habitats.

The great benefit of the strategic approach is that it should allow us to make quantitative generalisations for organisms with similar life-history strategies in particular habitats. In common with most ecotoxicological theory, the complete set of models that we are developing will integrate three classes of information: chemical fate (predicted spatial and temporal profile of chemical), exposure (predicted spatial and temporal coincidence of population and chemical) and toxicity (predicted demographic changes in the population as a result of a range of chemical concentrations). Models are most trustworthy if it can be demonstrated that they are valid under specified conditions. An extensive validation programme will be required which will involve surveying existing databases as well as manipulations of outdoor experimental pond systems to mimic the influence of a chemical perturbation and different recovery scenarios. At this early stage of development, the models will be viewed as successful if they are simply better at ranking the overall susceptibility of aquatic species to a chemical than a rank based on exposure and effect concentrations alone.

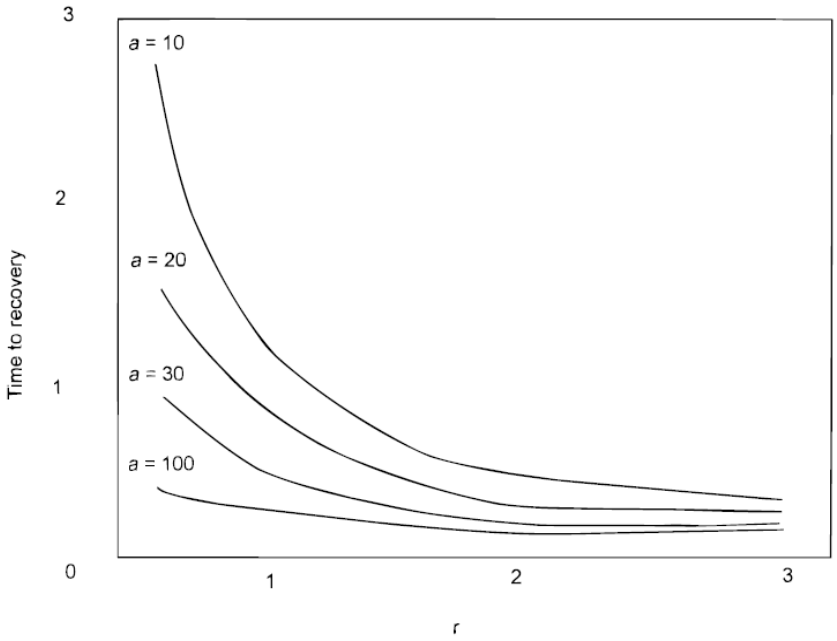

Figure 1. Relationship between time to recovery from perturbation with reproductive rate (r-number of offspring reaching reproductive status per unit time) and immigration rate (anumber arriving per unit time) derived from the Euler-Lotka equation (T. Stickland & T. Sherratt, unpublished). Values for r and a are hypothetical and are used for illustrative purposes.

Conclusions

Although mesocosm and field studies are regarded as the ultimate level of aquatic assessment for pesticides, there can be difficulties with interpreting the effects observed in these systems and extrapolating them to the wide range of scenarios that may occur in the real world. In order to be able to understand whether ecologically important effects may occur, it is necessary to understand more than the susceptibility of the organism and its exposure to the pesticide. Ecological factors can play a significant role in either mitigating or enhancing the potential impacts of a pesticide perturbation. Understanding the importance of these for a chemical under a wide variety of situations is clearly beyond

the scope of traditional experimental techniques. However, ecological models offer a technique which may allow us to formalise the principles by which these factors affect the overall risk of a pesticide to the aquatic environment. Of the available modelling approaches, reductionist strategic techniques seem to offer the greatest potential because they can be used to develop rules about the huge variety of organisms that exist in the real world, based on making generalisations about organisms with similar life-histories. Only by formalising these general principles will it be possible to understand fully whether a particular organism in a certain habitat is likely to be affected by a toxicant perturbation. Once such models are developed, extensive experimental validation will be required.

Acknowledgement

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Chapter 4: Ecological Impact in Ditch Mesocosms of Simulated Spray Drift from a Crop Protection Program for Potatoes[3]

Summary

Outdoor aquatic ditch mesocosms were treated with a range of pesticides to simulate various spray drift rates resulting from a typical crop protection program used in the cultivation of potatoes in The Netherlands. The main experimental aims of the present study were to provide information on the fate and ecological effects of drift of the pesticides into surface water and to evaluate the effectiveness of drift-reduction measures in mitigating risks. The pesticides selected and the dosage, frequency, and timing of application were based on normal agricultural practices in the potato crop. Applications of prosulfocarb, metribuzin (both herbicides), *lambda*-cyhalothrin (insecticide), chlorothalonil, and fluazinam (both fungicides) were made in the sequence typical of the spray calendar for potatoes. A total of 15 treatments with the various compounds were made by spray application to the water surface at 0.2%, 1%, and 5% of the recommended label rates. Chemical fate and effects on ecosystem function and structure (phytoplankton, zooplankton, chlorophyll-α, macroinvertebrates, macrophytes, breakdown of plant litter) were investigated. To interpret the observed effects, treatment concentrations were also expressed in toxic units (TU), which describe the relative toxicity of the compounds with standard toxicity test organisms (*Daphnia* and algae). After treatment, each compound disappeared from the water phase within 2 d, with the exception of prosulfocarb, for which 50% dissipation time (DT_{50}) values ranged between 6 and 7 d. At the 5% treatment level, an exposure peak of 0.9 TU_{algae} was observed, which resulted in short-term responses of pH, oxygen, and phytoplankton. At the 5% treatment level, exposure concentrations also exceeded 0.1 $TU_{Daphnia}$, and this resulted in long-term effects on zooplankton and macroinvertebrates, some of which did not fully recover by the end of the present study. At the 1% treatment level, only slight transient effects were observed on a limited number of zooplankton and macroinvertebrate species and on pH.

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At the 0.2% level, no consistent treatment-related effects were observed. Most of the observed effects were consistent with the results from higher-tier and mesocosm studies with the individual compounds. Multiple and repeated stress played a small role within the applied pesticide package, because of rapid dissipation of most substances and the absence of many simultaneous applications. This suggests that risk assessments based on the individual compounds would in this case have been sufficiently protective for their uses in a crop protection program.

Introduction

The use of pesticides in agriculture may lead to the entry of small amounts of these compounds into aquatic ecosystems through a variety of potential routes including spray drift, drainage, surface runoff, or accidental spills (1). In the flat, polder landscape of The Netherlands, spray drift into edge-of-field ditches is considered to be the main entry route of pesticides to surface water (2). Here, these pesticides can exhibit undesirable side effects on the survival and recruitment of non-target aquatic organisms. The entry rate of spray drift depends on the crop, application technique, implementation of risk mitigation measures, and environmental conditions on the day of application. Subsequent aquatic exposure patterns then depend on the number and frequency of applications and the dissipation rate of the compound (3).

Regulatory schemes for pesticides are currently based on assessments of individual compounds (4, 5). It is common practice, however, for several different pesticides to be applied to protect crops and for their uses to be simultaneous and/or repeated. Only relatively few studies have investigated the effects of combinations of pesticides in aquatic mesocosm experiments (6, 7), and such combinations of compounds are often not related to treatment programs used in specific crops. Only one previous study describes a crop-based approach to assess the combined and repeated effects of pesticides: The effects of a crop-protection program for flower bulb crops (8).

In this chapter, an experiment to evaluate the fate and effects in outdoor ditch mesocosms of a typical (with respect to compounds, treatment rate, frequency, and timing) crop-protection program for potatoes in The Netherlands is described. Potato crops cover a large area of The Netherlands (9), and because of the disease pressure they face (e.g., blights such as *Phytophtora infestans*), are characterized by an intensive use of pesticides (10). The spray drift treatment rates used in study followed Dutch regulatory scenarios. The mesocosms used are representative of the macrophyte-dominated

drainage ditches that are typical in the Dutch agricultural landscape and harbour populations of aquatic organisms representative for several trophic levels.

Materials and Methods

Experimental systems and study design

The experiment was performed in twelve ditch mesocosms (Figure 1), each with a length of 40 m, a width of 3.3 m at the water surface and 1.6 m at the sediment surface, a water depth of 0.5 m, a sediment depth of 0.25 m, and a total volume of approximately 55 m^3. Ditch sediment consisted of sandy loam with a moderate nutrient content. Construction and technical details of the mesocosms are described elsewhere (11). The test systems and their aquatic community structure resemble shallow, macrophyte-dominated drainage ditches on sandy loam and clay in the agricultural landscape. The mesocosms are not dominated by free-floating *Lemna* species, as is predominantly the case in the hypertrophic ditches in the agricultural Dutch landscape. Therefore, they may receive higher aquatic exposure of pesticides entering by spray drift compared with that of *Lemna*-dominated ditches.

Figure 1. Photo of ditch mesocosms.

To increase similarity between ditch mesocosms (and hence increase experimental power), two measures were taken at the start of the experiment. Firstly, ditches were reset to a pioneer stage by removal of the organic detritus layer in the autumn of 2000. After removal, recovery of macrophytes and macrofauna was stimulated by introduction of *Elodea nuttallii* shoots and macroinvertebrates originating from ditches that were situated in an area that was not in agricultural use ("Veenkampen"). *Gammarus pulex* was obtained from the groundwater basin at the experimental station. In 2001, fine detritus and macroinvertebrates from the same area were seeded into the ditches for a second time. In 2002, the year in which the experiment was performed, individuals of *Asellus aquaticus* and *Proasellus* juv. from ditches in the Veenkampen area were added to the ditches in the pre-treatment period. Secondly, water was circulated between the systems for about one month in the pre-treatment period. During the experiment, however, each ditch was isolated from the others, meaning that both duration of exposure (owing to an absence of dilution) and external recovery of organisms by water (due to lack of input of diaspores and organisms) were more or less the worst case.

The experimental set up followed a regression design. We applied spray drift rates that varied from 0.2% to 5% of label-recommended rates of the chosen pesticides. Four spray drift scenarios were applied: 0%, 0.2%, 1%, and 5%. Four ditches served as control ditches. Two ditches received a low spray drift emission of 0.2%, which is the percentage that can be achieved if several mitigation measures are applied simultaneously (12, 13). In three of the ditches, a spray drift emission of 1% was applied, which is the drift emission rate used for regulatory purposes for crops such as potatoes. In three other ditches, a spray drift emission of 5% was applied, which is the spray drift emission without mitigation measures (*ibid*.). The last scenario was intended as a realistic worst-case. The four treatments were randomly assigned to one of three rows of ditches at the field station (randomized block design).

The typical pesticide treatment regimes for ware and starch potatoes (9) were combined to develop a typical application scenario for potatoes based on active ingredients currently on the Dutch market. In potatoes, herbicides are applied at the beginning of the cultivation period, followed by various fungicide and insecticide treatments. Before harvest to aid tuber collection, above ground crop plants are removed either mechanically or with dessicant herbicides; in this case, we assumed mechanical removal and did not apply an herbicide at the end of the season.

Table 1. Application scheme, use rates, emission levels, calculated intended target concentrations and nominal concentrations of pesticides (μg/L active ingredients) in the ditches.

Active ingredient	Appli-cation week	Use rate (g ai/ha)	Concen-tration	Emission level (μg/L)		
				PEC 5%drift	PEC 1%drift	PEC 0.2%drift
Prosulfocarb (n = 1)	0	3200	Target	76	15	3.0
			Nominal	76.4	15.9	3.2
Metribuzin (n = 1)	2	350	Target	8.3	1.7	0.33
			Nominal	8.2	1.5	0.27
Lambda-cyhalothrin (n = 2)	5 and 9	5	Target	0.120	0.024	0.0048
			Nominal	0.085 (0.057-0.112)	0.016 (0.008-0.023)	0.0040 (0.0033-0.0047)
Chlorothalonil (n = 4)	6-9	1010	Target	24	4.8	0.96
			Nominal	22.5 (19.8-24.0)	5.1 (4.6-5.7)	0.95 (0.53-1.18)
Fluazinam (n = 8)	10-17	200	Target	4.8	0.95	0.19
			Nominal	4.6 (4.0-5.1)	0.95 (0.9-1.1)	0.18 (0.14-0.22)

Calculation method for PEC values presented in Table 1. The given example is a 5 % emission of prosulfocarb. The calculation follows the PEC calculation for a standard ditch as described in TOXSWA (2). Ditch width 1.0 m; Ditch depth 0.3 m; Ditch width at the sediment surface 0.4 m; Side-slope 1:1 (hor:vert); Ditch capacity per running metre: 0.4*0.3 + 2*0.3*0.5*0.3 = 0.21 m3/m = 210 L/m. Use rate of Prosulfocarb = 3200 g A.I./ha = 0.32 g A.I./m^2 = 320 mg A.I./m^2 sediment surface.5 % emission: 0,05 * 320 mg = 16 mg/m^2 sediment surface; 16 mg/m^2 sediment surface /210 L /m^2 = 0,076 mg/L = 76 μg/L.

The pesticides used and the sequence and frequency of their application in the spray program are presented in Table 1. The use rate of the compounds was based on the label recommendations for 2003. The treatment program included prosulfocarb, metribuzin, *lambda*-cyhalothrin, chlorothalonil, and fluazinam (Table 1). Prosulfocarb is a thiocarbamate herbicide that inhibits weed shoot growth. Metribuzin is a triazinone herbicide that inhibits photosynthetic electron transport and is widely used for the control of grasses and broadleaf weeds in numerous crops. *Lambda*-cyhalothrin is a synthetic

pyrethroid insecticide that disrupts ion channels in nerves. Fluazinam is a dinitroaniline fungicide that uncouples mitochondrial oxidative phosphorilation in fungi. Chlorothalonil is a widely-used, non-systemic chlorophenyl fungicide used to control various plant diseases in wetter regions in the United States and in The Netherlands. At least part of chlorothalonil toxicity is due to respiratory disruption.

Endpoints in the ditch mesocosms (Table 2) were investigated for a period of 30 weeks (18 March–14 October 2002), including a pre-treatment period of 5 weeks, a treatment period of 18 weeks, and a post-treatment period of 7 weeks. In the pre-treatment period, all endpoints were sampled once or twice to establish starting conditions and to check sampling methods. In the post-treatment period, all endpoints were sampled at least twice. Measurements and sampling activities (fate sampling, artificial substrates and litter bags, macrophyte bioassays, phytoplankton and zooplankton sampling and measurement of water quality endpoints) were non-randomly assigned to specific ditch segments in order to avoid mutual disturbance. On each sampling date, measurements and sampling activities were located in their assigned, specific ditch segments. Endpoints and sampling frequencies are presented in Table 2.

Pesticide application and sampling

To mimic spray drift deposition of pesticides on the water surface of the ditch mesocosms, pesticides were applied by means of a shielded spray boom (Design 1990 Technical Department of Wageningen University and Research Centre, formerly Institute of Agricultural and Environmental Engineering (14)). By this method, the desired spray volume is dosed accurately and evenly over the water surface of each ditch and the risks of cross-contamination are minimized. The spray boom is shielded from the wind to enable its use irrespective of wind conditions. To prevent prolonged stratification of pesticide concentrations in the top layer after application, the upper water layer was mixed by moving a metal plate, which was fixed to the platforms of the movable bridges and extended to a water depth of approximately 10 to 20 cm, from one end to the other end of the ditch.

Table 2. Ecological endpoints and sampling frequencies.

End-point	Frequency once in:
Phytoplankton	
Chl-a	3 weeks
Taxonomic analysis	3 weeks
Macrophytes	
Bioassays	6 weeks
Vegetation cover	6 / 7 weeks
Zooplankton sampling (combined with phytoplankton and Chl-a)	3 weeks
Macroinvertebrates	
Artificial substrates	4 weeks
Litter bags	4 weeks
Physico-chemistry	
O_2, pH, temp	2 to 3 days
Alkalinity and EC	4 weeks
Nutrient concentrations in water	3 weeks

Prosulfocarb was applied as the formulated product Boxer® (806 g/L prosulfocarb; density, 1.020 g/mL; Syngenta, UK). Metribuzin was applied as 70% metribuzin (powder; DIC 1468 70 WG, Bayer, Germany). *Lambda*-cyhalothrin was applied as Karate Zeon® (98 g/L *lambda*-cyhalothrin; density, 1.064 g/mL; Zeneca Crop Protection, UK). Chlorothalonil was applied as the formulated product Bravo® (40.4% w/w chlorothalonil; density, 1.254 g/mL; formulation YF 10934, Syngenta). Fluazinam was applied as the formulated product Shirlan® (39.5% w/w fluazinam; density, 1.273 g/mL; Syngenta). In the laboratory, a known amount of the compound was weighed into a brown glass bottle, and tap water was added. The glass bottles were transported to the experimental site, and just before application, the entire contents of a bottle were transferred to a spraying vessel that was filled with groundwater to a final volume of 12 L, weighed, and thoroughly mixed. The ditches were sprayed for about 60 to 70 s at a pressure of 3.5 bar, resulting in a spray volume of 7 L. After the application, the spraying vessel with remaining spray solution was weighed to calculate the amount that was sprayed. Two subsamples were transferred from the remaining spray solution directly into high-pressure liquid chromatography (HPLC) vials for measuring the concentration of the application solution.

The intended treatment concentrations were calculated from product use rates, emission percentages and predicted environmental concentration (PEC) calculations for the standard Dutch ditch (Table 1). Initial concentrations in the ditches were calculated from residue measurements in the spray solution, the amounts of solution sprayed, and the estimated amount of water (55 m^3) in the ditches (Table 1). To measure exposure concentrations, water samples were collected at intervals after application (2, 8, 24, 72, 168, 336, and 672 h) in the water compartment of each ditch mesocosm or over shorter periods if dissipation was anticipated to be rapid. Depth-integrated water samples were collected with a Perspex tube (length, 50 cm; internal diameter, 3.9 cm) at three fixed sampling locations in each ditch. The tube was inserted vertically into the water column and was sealed with rubber stoppers. Water samples were collected from all ditches in this way. The water samples were transferred into a 1 L glass beaker and were thoroughly mixed. A subsample was then transferred into a 500 mL flask, which was sealed and transferred to the laboratory for extraction within 2 h.

Chemical analysis

Prosulfocarb, metribuzin, and fluazinam were extracted from the water samples by solid-phase extraction with OASIS extraction columns. The bottle containing the water sample was weighed before extraction. The OASIS columns (Waters® OASIS HLB extraction columns 3 mL; 60 mg) were pre-conditioned with 1 mL acetonitrile (LAB-SCAN HPLC quality) and 5 mL distilled water for prosulfocarb and metribuzin and with 2 mL of LAB-SCAN methanol (HPLC quality) and 5 mL distilled water for fluazinam. The bottle with the water sample was connected to the extraction column with stainless steel tubing, and water was filtered through the column. After extraction, the bottle with water sample was weighed again to measure the amount of water extracted. Prosulfocarb, metribuzin, and fluazinam were eluted from the extraction columns with acetonitrile (2 × 0.5 mL) into volumetric tubes. For each compound, both eludates were collected in a 10 mL graduated tube, and the samples were diluted with distilled water to a known final volume. A subsample was transferred into an HPLC vial, and the samples were analyzed on an HPLC system equipped with an ultraviolet detector.

Table 3 summarizes the parameters used for the HPLC analysis. Concentration calculations were based on external standard samples. Recovery efficiencies from spiked water samples (control ditches) were 92.2% (n = 15; SD 7.9%), 96.6% (n = 11; SD 3.1%), and 83.9% (n = 59; SD 3.3%) for prosulfocarb, metribuzin, and fluazinam, respectively. Concentrations were corrected for recovery.

Table 3: Parameters for HPLC and GC/ECD analysis of the pesticides prosulfocarb, metribuzin, fluazinam, *lambda*-cyhalothrin and chlorothalonil.

HPLC analysis	**Prosulfocarb**	**Metribuzin**	**Fluazinam**
HPLC model	Waters M590 + Perkin Elmer ISS 100 autosampler + Waters LC 90 UV detector		
Injection volume	100 µl		
Flow	1 ml/min.		
Oven temperature	40 °C		
Column	Waters XTerra™ MSC 18, 3.5 µm, 4.6 × 150 mm		Waters Novapak® C-18, 4 µm, 4.6×150 mm
Guard column	Waters XTerra™ MSC 18, 3.8 µm, 3.9 × 20 mm		Waters Novapak® C-18, 4 µm, 3.9×20 mm
Mobile phase	Acetonitrile 65% HPLC-water 35%	Acetonitrile 30% HPLC water 70%	Acetonitrile 75% HPLC water 25% Acetic acid 0.1 %
Wavelength	220 nm	296 nm	260 nm
Retention time	8.4 min	8.2 min.	4.1 min.
Detection limit	< 0.5 µg/L	< 0.05 µg/L	< 0.05 µg/L

GC analysis	***Lambda*-cyhalothrin**	**Chlorothalonil**
GC-model	HP-5890 gas chromatograph + HP 6890 autosampler	
Mobile phase	Helium 15 psi.	
Column	WCOT 25 m×0.32 mm CP Sil 8 (filmthickness: 0.4 µm)	
Detection temperature	325 °C	
Injection volume	3 µl splitless	3 µl split
Injection temperature	240 °C	150 °C
Temperature program	Init. Temp: 80 °C Init. Time: 1 min. Rate: 30 °C/min. Final temp: 280 °C Final time: 4 min.	Init. Temp: 120 °C Init. Time: 1 min. Rate: 30 °C/min. Final temp: 280 °C Final time: 4 min.
Retention time	10.3 min.	5.8 min.
Detection limit	< 0.005 µg/L	< 0.1 µg/L

Lambda-cyhalothrin and chlorothalonil were extracted from water samples by liquid–liquid extraction with either hexane (own distillate; *lambda*-cyhalothrin) or toluene (Across Organics, Belgium, toluene pro analysis; chlorothalonil). A known volume (c. 300 mL) of water was transferred to a flask, and 35 mL of petroleum ether was added. The flasks were closed and shaken thoroughly for at least 15 min. The organic layer was concentrated by evaporation of the petroleum ether (own distillate) on a rotary evaporator, and the residue was dissolved in 1.5 mL hexane. Chlorothalonil samples (c.20 mL water) were extracted with 2 mL toluene by shaking thoroughly for 15 min in 35-mL tubes. Samples were analyzed without clean up by gas chromatography with an electron capture detector. Table 3 summarizes the parameters used for the gas chromatography and electron capture detector analysis. Concentration calculations were based on external standard samples. Recovery efficiencies from spiked water samples (control ditches) were 97.5% (n = 3; SD 3.7%) for *lambda*-cyhalothrin and 104.9% (n = 12; SD 10.6%) for chlorothalonil. Concentrations were corrected for recovery. The time required for dissipation of 50% of the mass originally present (DT_{50} values) were calculated based on linear least-squares regression of the natural logarithm of the concentration versus time, for mean concentrations at the 0.2%, 1%, and 5% treatment levels (only measurements above detection limit).

Water quality endpoints

Temperature, pH, and dissolved oxygen (DO) were measured three times a week between 09:00 AM and 11:00. The DO was measured with a Wissenschaftlich-Technische Werkstätte (WTW) Oxi 320 Set oxygen meter at 10 and 40 cm below water surface, and pH was measured with a WTW pH196 pH meter at a water depth of 25 cm. Alkalinity and conductivity were measured every 4 weeks. Conductivity was measured with a WTW LF 96 conductivity meter at a depth of −25 cm. Alkalinity was analyzed in 100 mL samples, taken from a depth of 25 cm, by titration with 0.02 M hydrochloride down to pH 4.2. Depth-integrated water samples filtered through a 40-µm mesh net (100 mL) were stored in labelled polyethylene vials in a deep freeze at a temperature below −20°C for nutrient analysis. After termination of the experiment, a Skalar 5100 Autoanalyser (Breda, The Netherlands) was used to colourimetrically analyze for nitrate/nitrite, ammonium, orthophosphate, and chloride.

Macroinvertebrates

Macroinvertebrates were sampled by means of artificial substrates and litter bags. The litter bag technique is described in the "decomposition" section below. Four weeks before the first sampling, 4 pebble baskets and 4 litter bags were placed in the ditches in order to allow colonization by microfauna and macrofauna. Pebble baskets and litter bags were assigned to four specific ditch segments, evenly distributed over each ditch. The pebble baskets were sampled every 4 weeks and, in total, 7 times during the experiment. Macroinvertebrates that were present on the substrates were identified, counted and then returned to the ditch from which they were sampled. The species composition of macroinvertebrates was determined to the lowest practical taxonomic level. For each ditch, the abundance of macroinvertebrates on pebble baskets and in litter bags was summed before analysis of the data.

Phytoplankton and zooplankton

Depth-integrated plankton samples were taken every three weeks. Phytoplankton and zooplankton were collected in a single sample. Plankton samples were taken with Perspex tubes (0.4 m long, 0.8 L in volume) from three sampling locations assigned to specific ditch segments (c. 5 samples taken at random per sampling location) and mixed to give a c. 13 L sample volume per experimental ditch. Five litres of each sample were filtered through a 55-µm mesh net to collect zooplankton and were preserved with formalin (4%). Eight litres were filtered through a 40-µm mesh net to concentrate phytoplankton and were preserved with formalin (4%). Phytoplankton samples were split on a weight basis. One set of samples was used for identification. Of the remaining unprocessed water sample, 1 L was collected for chlorophyll-α and nutrient analysis. For the chlorophyll-α determinations, 1 L of the water sample was concentrated over a Schleicher and Schuell glass fibre filter (GF52; diameter, 4.7 cm; mesh size, 1.2 µm), by use of a vacuum pump. The filter was stored in a labelled Petri dish, wrapped in aluminium foil, at a temperature below −20°C for a maximum period of 3 months. Extraction of chlorophyll-α was performed by use of the method described by Moed and Hallegraeff (15). Chlorophyll-α content was analyzed by spectrophotometric measurement (dichromatic following protocol NEN 6520).

Subsamples of approximately 2 to 3 mL were taken from one preserved sample. Subsamples were recalculated to the overall sample and the numbers in 1 L by weight. Cladocera were identified to species level. The remaining zooplankton taxa (e.g., Copepoda, Ostracoda, and Rotifera) were identified to the lowest practical level. For a

few samples, whether counting of 1 subsample was sufficiently representative was checked by taking an extra subsample.

Phytoplankton species composition was studied by counting the number of cells of a known volume. Taxa and number of cells were based on 40 counting fields of an object glass under a microscope (magnification, ×400). For colony-forming and filamentous algae, the number of colonies/filaments was counted. Identification took place to the lowest practical taxonomic level.

Macrophytes

Seasonal development of macrophyte species composition was investigated by monitoring macrophyte cover, abundance, and structure every 6 to 7 weeks (6 times during the experiment). Cover/abundance (percentage) of each macrophyte species was estimated per 5-m ditch length by direct measurement. To describe the macrophyte structure, the mean, minimum, and maximum height of the vegetation was measured per 5-m ditch length by direct measurement.

Macrophyte bioassays were performed to determine the effects of the pesticide application regime on growth of submerged water plants. The macrophyte used in the bioassays was *Myriophyllum spicatum*. Plant shoots (length, 10 cm) were obtained from plants growing in the ditches. Six to eight shoots (total weight of 5–7 g) were planted in a flowerpot. The flowerpots were filled with ditch sediment. In each ditch, six flowerpots were placed on the sediment surface at each of two locations in specific ditch segments. After an incubation period of 6 weeks, above- and below-sediment plant material was harvested, rinsed to remove sediment particles, dried in aluminium foil (105 °C, 24 h), and weighed. The material from two locations in each ditch was pooled. Sampling of macrophyte biomass was repeated three times at intervals of 6 weeks.

Leaf litter decomposition

Decomposition of coarse particulate organic material was studied using litter bags. The organic matter used in these litter bags were leaves of *Populus* × *canadensis* (2 g dry weight dried at 60 °C). These leaves had been soaked three times for 2 d to remove soluble humic compounds. After soaking, they were dried for 72 h at 60 °C and stored until use. Decomposition of these leaves by shredding macroinvertebrates was studied. To achieve this, two holes of 0.5 mm were placed in the stainless steel wire (mesh size, 0.7 × 0.7 mm) around the glass Petri dishes (diameter, 11.6 cm) in order to allow macroinvertebrates to enter. Two litter bags were introduced at the sediment surface of

a ditch at each of two locations. After an incubation period of 2 weeks, each litter bag was emptied in a white tray to separate coarse particulate organic material and macroinvertebrates. Macroinvertebrates on the outer surface of the litter bags (snails, leeches) were removed and not included in the sample. Macroinvertebrates were identified alive, counted, and released again into the ditch. Remaining organic plant material was dried in aluminium foil at a temperature of 105°C. After 24 h, dry weight was determined. The decomposition over a 2-week period was expressed as percentage of remaining organic material. New litter bags were incubated two weeks before the next sampling date. Decomposition measurements were repeated seven times.

Toxic units

To assist with the interpretation of the observed effects, we calculated toxic units (TU) and applied the concept of concentration addition (16). The TU were based on acute toxicity data of the most sensitive standard test species and measured exposure concentrations in the water compartment of the ditches. The toxicity for sensitive invertebrates (zooplankton and macroinvertebrates) was scaled to the toxicity of the five pesticides for *Daphnia magna*:

$$yTU = \Sigma (C_i/EC50_i) \qquad (1)$$

where C_i is the actual concentration of the compound i, $EC50_i$ is the geometric mean 48-h EC50 of compound i for *D. magna* (Table 4), and y is the resulting fraction of TU. The toxicity for phytoplankton was scaled to the toxicity of the 5 pesticides for algae by using Equation 1. EC50*i* is the geometric mean EC50 for *Selenastrum capricornutum*.

The toxicity of the pesticide package for macrophytes was calculated on the basis of the toxicity of the herbicides prosulfocarb, metribuzin, and chlorothalonil to *Lemna minor* or *L. gibba*. No data were available for either of the other compounds. Previous studies with pesticides in experimental ecosystems have demonstrated that effects on primary producers are likely to occur at $TU_{algae} > 0.1$, and effects on invertebrates are likely to occur at $TU_{Daphnia} > 0.01–0.1$ (17, 18).

Table 4: Geometric means of toxicity data of species representative of primary producers and invertebrates for the pesticides used in the experiment [a]

Pesticide	Taxon	E(L)C_{50} µg/L	N	Exposure time
Prosulfocarb	*Selenastrum capricornutum*	86.7	1	96 h
	Lemna gibba	690	1	14 d
	Daphnia magna	1531	2	48 h
Metribuzin[b]	*Selenastrum capricornutum*	39.7		72 – 96 h
	Lemna minor	36.5		96 h
	Daphnia magna	14,983		48 h
Lambda-cyhalothrin[c]	*Selenastrum capricornutum*	>1000		96 h
	Lemna sp.	--		--
	Daphnia magna	0.35		48 h
	Oncorhynchus mykiss	0.32		96 h
Chlorothalonil	*Selenastrum capricornutum*	190.4	3	72 – 120 h
	Lemna gibba	510	1	14 d
	Daphnia magna	105.2	7	48 h
Fluazinam	*Selenastrum capricornutum*	160	1	96 h
	Lemna gibba	--		--
	Daphnia magna	201.9	4	48 h

[a] Geometric mean EC_{50}s (µg/L) are based on toxicity data for standard laboratory test species (N = number of values or the reference for the values) commonly used in the first-tier risk assessment procedure for the administration of pesticides. Data were from the ECOTOX database (www.epa.gov./ecotox/), from the RIVM database (Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven, The Netherlands) and from data provided by industry (Syngenta, Jealotts Hill, UK).

[b] Data originate from Brock et al. (2004).

[c] Data originate from Van Wijngaarden et al. (2004).

Data analysis

In the data analysis, we used a combination of multivariate (principal response curves [PRC]) and univariate (Williams test (19)) techniques. The PRC technique serves to identify the treatment levels that affect the community and to indicate the taxa for which the responses affiliate most to the treatment regime. The Williams test is used to analyze the dynamics at the taxon level and helps to further identify the number of taxa affected. A graphical interpretation of the univariate analysis is often needed to decide whether the statistically significant deviations can be considered consistent effects and whether these deviations make any sense in relation to variations in abundance. At the taxon level, no observed effect concentrations (NOEC) resulted from the Williams test (ANOVA, p 0.05). Analyses were made with the Community Analysis computer program (20). Data of macrophyte bioassays and leaf litter composition were also analysed with the Williams test (ANOVA, p 0.05).

Before univariate and multivariate analysis of abundance values, the macroinvertebrate data were ln(2x + 1) transformed, the zooplankton data were ln(10x + 1) transformed, and the phytoplankton data were ln(0.2x + 1) transformed, where x equals the abundance value. This transformation was performed to down-weight high abundance values and approximate a normal distribution of the data (21).

The effects of the treatment on the community level of macroinvertebrates and zooplankton were analyzed by the PRC method, which is based on the redundancy analysis ordination technique, the constrained form of principal component analysis (22, 23). The PRC method is a multivariate technique specially designed for the analysis of data from microcosm and mesocosm experiments. PRC produces a diagram that shows the sampling weeks on the x-axis and the 1st principal component of the treatment effects on the y-axis. This generates a diagram that shows the deviations in time of the treatments compared with the control. The species weights (bk), shown on the right side of the diagram, can be interpreted as a correlation of each species with the response given in the diagram. Thus, the species that has the highest weight is indicated to have decreased most at the highest treatment level. The species with the lowest negative weight has increased most at the highest treatment level. A complete description and discussion of the PRC method is given by Van den Brink and Ter Braak (22, 23). PRC analysis was performed by means of Canoco for Windows software package, version 4 (24).

In the Canoco computer program, redundancy analysis is accompanied by Monte Carlo permutation tests to assess the statistical significance of the effects of the explanatory variables on species composition of the samples (25). The significance of the PRC diagram in terms of displayed treatment variance was tested by Monte Carlo permutation of entire time series in the redundancy analysis from which PRC is obtained, by using an F-type test statistic based on the Eigenvalue of the component (23).

Although the 1st principal component extracts the maximum amount of information from the multivariate treatment effects, it does not necessarily describe the effects of each treatment on all taxa in sufficient detail. Further components can be extracted from the residual variation. The significance of the 2nd and higher PRC diagrams was tested by means of Monte Carlo permutation tests. It showed that the 2nd and higher components were not significant and were therefore not considered. The results of the PRC analysis can be evaluated in terms of the fractions of the variance explained by the factors time and treatment. The fraction of variance explained by treatment is shown in the PRC diagram.

Monte Carlo permutation tests were also performed for each sampling date, by use of the ln-transformed treatment levels as the explanatory variable (25). This procedure served to test the significance of the treatment for each sampling date. In addition to the overall significance of the treatment regime, we tested which treatments differed significantly from the controls, so as to infer the NOEC at the community level ($NOEC_{community}$). The $NOEC_{community}$ calculations were done by applying the Williams test to the sample scores of the 1st principal component of the PCA of each sampling date in turn.

Results

Exposure concentrations

Overall, initial treatment concentrations (based on measurements in the spray solution and the amounts of solution sprayed) in the ditch mesocosms were close to the intended peak concentrations (Table 1). The only exception was that the second application of *lambda*-cyhalothrin was approximately 50% lower than intended, which was considered to be due to an incorrect 50% lower concentration in the application solution. Average treatment concentrations in the treatment regimes as a function of time are shown in Figure 2.

Prosulfocarb disappeared from the water layer most slowly. Mean calculated dissipation DT_{50} value from the water layer was about 7 d at the 5% treatment level (Table 5). Dissipation DT_{50} values for metribuzin and fluazinam in the water compartment were between 1 and 2 d (Table 5). For *lambda*-cyhalothrin, a DT_{50} value in the water layer of about 1 d was calculated. With water DT_{50} values of 0.3 to 0.5 d, dissipation of chlorothalonil was the most rapid (Table 5).

Table 5. Calculated dissipation DT_{50} values (days) for the compounds in the pesticide package in the water column of the ditches.[a]

Drift (%)	Prosulfocarb			Metribuzin			Chlorothalonil			*Lambda*-cyhalothrin			Fluazinam		
	Med	Low	High	Med	Low	High	Med	Low	High	Med	Low	High	Med	Low	High
0.2	6.1	5.6	6.7	1.3	1.1	1.6	0.3	0.1	1.0	n.c.	n.c.	n.c.	2.0	1.1	10.0
1	6.0	5.4	6.7	1.7	1.3	2.6	0.3	0.1	2.7	1.2	0.5	2.7	1.7	1.2	2.9
5	7.0	6.6	7.5	1.7	1.4	2.2	0.5	0.3	1.2	0.9	0.7	1.2	1.4	1.3	1.6

[a] The table presents median values over time for the three different treatments as well as 95 % lowest and highest values. N.c. = could not be calculated because of very fast dissipation of the compound. Med = median; Low = lowest value; High = highest value.

Peak exposure concentrations reached 0.88 TU for algae at the highest treatment level (5%) (Figure 3). The largest component of the toxicity was caused by prosulfocarb in the first few weeks of the experiment. Based on toxic units, metribuzin was expected to contribute to the toxicity for algae as well (0.1 TU; Figure 3). However, data from mesocosm studies indicate that effects of metribuzin at the ecosystem level are unlikely to occur at the applied concentrations. The contribution of chlorothalonil to the toxicity to algae was low (short exposures to 0.12–0.13 TU), whereas the contribution of fluazinam was negligible. At the 1% treatment level, only prosulfocarb reached concentrations at which toxicity for algae could be expected (Figure 3). For invertebrates, peak exposure concentrations reached 0.4 TU at the highest treatment level (5%) (Figure 3).

For these groups, *lambda*-cyhalothrin and chlorothalonil were expected to contribute most to the observed toxicity. Contributions of the other compounds at the 5% treatment level and of *lambda*-cyhalothrin and chlorothalonil at the 1% treatment level were in the range 0.01 to 0.1 TU. Within this range, low levels of effects cannot be ruled out. At the 0.2% level, TU values were less than 0.014 for invertebrates and less than 0.06 for algae, so no effects were anticipated

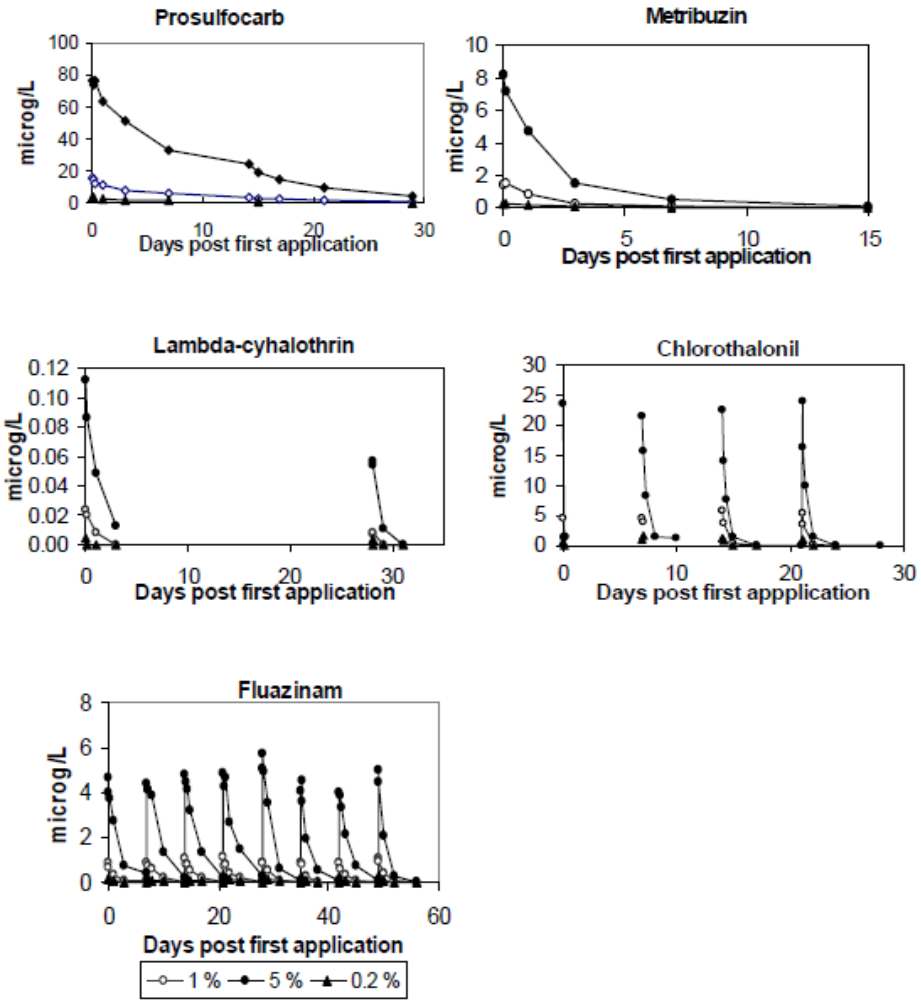

Figure 2. Trends in concentrations of pesticides applied in the potato spray program. Concentrations have been measured in the water compartment of the ditch mesocosms in the 0.2%, 1%, and 5% treatment regimes. Note that the different pesticides were applied on different dates (see Table 1).

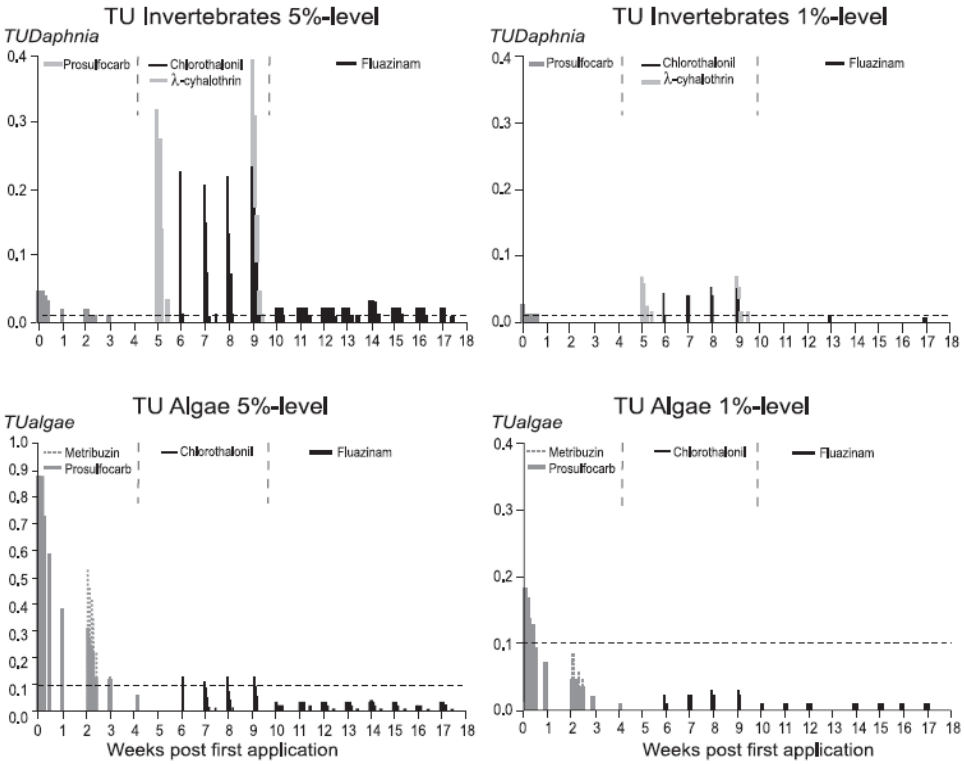

Figure 3. Toxicity of the pesticide package for invertebrates and algae at 1% and 5% drift emission of label-recommended rates of pesticides expressed as Toxic Units (TU) and the contribution of each pesticide to the toxicity (0.2% not shown). See text for rationale for these values. The lines of 0.01 and 0.1 TU are marked.

Water quality endpoints

Table 6. Concentrations of nutrients and chloride (mg/L) in surface water of ditch mesocosms.

	Sum NO_2^- NO_3^-	NH_4^+	o-P	Cl^-
Controls	0.079 ± 0.089	0.032 ± 0.012	0.006 ± 0.002	6.656 ± 0.740
0.2 %	0.081 ± 0.074	0.036 ± 0.011	0.006 ± 0.002	7.026 ± 0.890
1 %	0.094 ± 0.114	0.029 ± 0.010	0.007 ± 0.003	7.124 ± 0.819
5 %	0.082 ± 0.104	0.029 ± 0.014	0.006 ± 0.002	6.977 ± 0.737

Nutrient concentrations in the water layer were low (Table 6), and nutrients and chloride were not different between the treatments. Table 7 presents the results of NOEC calculations for pH and DO.

Table 7. Results of NOEC calculations of the physico-chemical parameters (drift percentage; Williams test, p < 0.05) [a]

Day	pH decrease	O_2 surface decrease	Day	pH increase	O_2 surface increase
-4		NM	88		
1	0.20%		91		
2	0.20%		92		
3	0.20%		94		
4		1%	98		
9	0.20%		99		
14	1%	1%	101		
15	0.20%		105		
16	1%	1%	106		
21			108		
23			112		1%
29			113		1%
36			115		
42			119		1%
43			120		
49	1%		123		1%
50	1%		126		
53	1%		127		
56			130		
57			133		
60			134		
63			137		
64			140		
67			141		
70		1%	144		
71			147		
74			148		
77			151		
78			154		
81			161		
84			169		
85			176		

[a] Only endpoints showing a consistent response (NOECs calculated for two consecutive sampling dates) are displayed. Blank cells indicate NOEC ≥ 5%. NM means not measured.

There was a significant decrease in pH of approximately 0.5 to 1 pH unit from day 1 to day 16 at the 5% treatment level, and from day 1 to day 9 at the 1% treatment level. In addition, there was a significant decrease of oxygen of approximately 0.75 mg/L on day 3 during this period at the 5% treatment level. The responses observed can most probably be attributed to prosulfocarb. A significant decrease in pH also occurred from day 49 to day 53 at the 5% treatment level, overlapping slightly with the application period of chlorothalonil. In the last week of the application period (days 112 through 123), DO in the water column significantly increased at the 5% treatment level (Table 7). Increased oxygen levels coincided with an increase in turbidity of the water at the 1% and 5% treatment levels (data not shown), and an increase in chlorophyll-α (NOEC week 14 = 0.2%), indicating an increase in algal densities.

Macroinvertebrates

Over the experimental period, a total of 81 different macroinvertebrate taxa were identified, belonging to eight classes, with almost half of the taxa (34) being Insecta. The mesocosms were characterized by Crustacea (dominant species: *G. pulex, Proasellus meridianus/coxalis*), Insecta (Ephemeroptera; dominant species: *Cloeon dipterum, Caenis horaria*; Odonata; Diptera: *Chaoborus* sp., Tanypodinae, Ceratopogonidae), Hirudinea (dominant species: *Erpobdella octoculata*), Gastropoda (dominant species: *Armiger cristata*) and Turbellaria (dominant species: *Polycelis nigra/tenuis*). Of the total variance, 24% could be attributed to the treatment regime by the PRC analysis (Figure 4). The PRC diagram displayed a significant amount of the treatment variance ($p = 0.003$). Clear treatment effects were observed at the 5% and 1% treatment levels compared with the controls from week 7 onward (Figure 4 and Table 8). At the 5% level, the PRC diagram shows long-lasting effects on the macroinvertebrate community without full recovery (Figure 4). These effects first became evident after the first application of *lambda*-cyhalothrin. Effects at the community level also persisted during the application periods of chlorothalonil and fluazinam, presumably because there was no recovery from the *lambda*-cyhalothrin effects. At the 1% level, effects at the community level were first observed in week 7 and seemed to be associated with the application of *lambda*-cyhalothrin in week 5. A similar effect was not observed after the second application of *lambda*-cyhalothrin, possibly because dosages were lower than the intended peak concentration and just below or above threshold levels (Figure 2 and Table 11). Statistical calculations resulted in an isolated community level $NOEC_{macroinvertebrates}$ at the 0.2% treatment level and an overall $NOEC_{community}$ of 1% (Table 8).

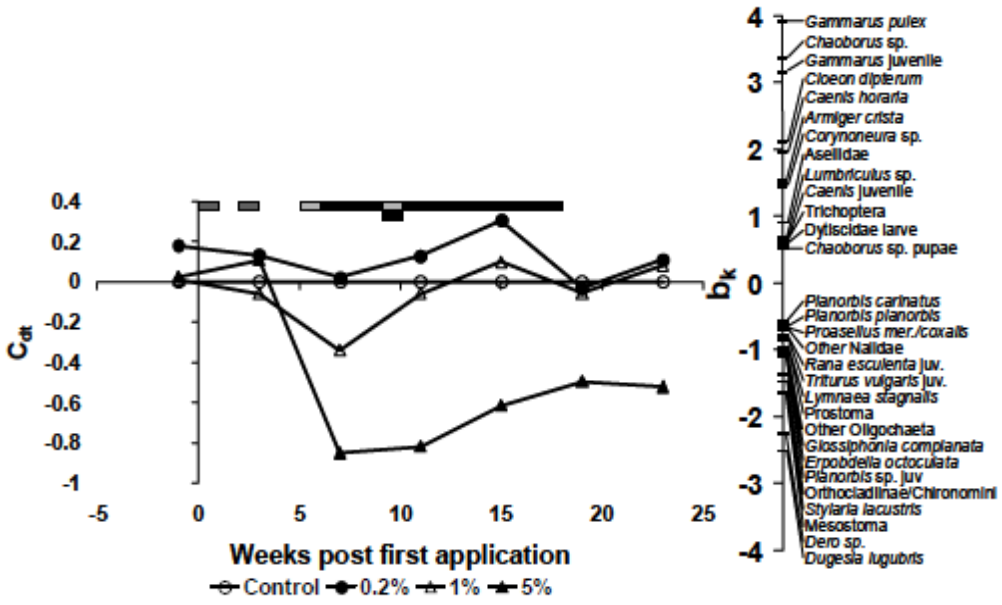

Figure 4. Principal response curves with species weights (bk) for the macroinvertebrate data set, indicating the effects of applications of compounds of the pesticide package. Of the variance, 38% could be attributed to sampling date and is displayed on the horizontal axis. Differences between replicates accounted for 38% of the variance. Twenty-four percent of the variance could be attributed to the treatment scenario. Of this percentage, 34% is displayed on the vertical axis. The species weight can be interpreted as the affinity of the taxon to the principal response curve. Taxa that have a species weight between –0.5 and 0.5 have a low correlation with the response curve and are therefore not displayed. The PRC diagram does display a significant amount of the treatment variance (p = 0.003). Application of herbicides, medium shading; application of insecticide, light shading; application of fungicides, solid.

Taxa with high positive weights (i.e., those taxa that decreased most strongly in association with changes in community structure) in the PRC analysis (Figure 4) were *G. pulex* (Figure 5A), *Chaoborus* sp. (Figure 5B), *C. horaria* (Figure 5C), and *C. dipterum* (Figure 5D). The populations of these taxa declined significantly at the highest treatment level. Recovery was observed for the affected insect taxa but was not observed for *G. pulex*. Taxa with high negative weights shown in the PRC diagram (Figure 4) comprise *Dero* sp. (Figure 5F), *Stylaria lacustris* (Figure 5I), and Orthocladiinae/Chironomini (Figure 5H). The population abundance of these taxa all increased, at least temporarily.

Table 8. Results of the Monte Carlo permutation test (p-value) and NOECs on the zooplankton, phytoplankton and macro-invertebrate community level (drift percentage; Williams test, p < 0.05) for the different treatment levels of a pesticide package containing prosulfocarb, metribuzin, lambda-cyhalothrin, chlorothalonil and fluazinam.

Week	Zooplankton		Phytoplankton		Macroinvertebrates	
	p-value	NOEC	*p*-value	NOEC	*p*-value	NOEC
-1	> 0.10	> 5 %	> 0.10	> 5 %	> 0.10	> 5 %
2	> 0.10	> 5 %	0.08	> 5 %	> 0.10	> 5 %
3	-	-	-	-	-	-
5	0.08	> 5 %	0.04	1%	≤ 0.001	0.20%
7	-	-	-	-	-	-
8	0.009	1%	>0.10	> 5 %	-	-
11	0.02	1%	>0.10	> 5 %	0.005	1%
14	0.015	1%	>0.10	> 5 %	-	-
15	-	-	-	-	0.017	1%
17	> 0.10	> 5 %	>0.10	> 5 %	-	-
19	-	-	-	-	-	1%
20	> 0.10	> 5 %	>0.10	> 5 %	-	-
23	-	-	-	-	0.001	1%
24	> 0.10	> 5 %	>0.10	> 5 %	-	-

Statistical analysis of treatment-related responses for individual macroinvertebrate populations resulted in NOECs for 8 taxa (Table 9). Adverse effects of treatments were apparent from week 7 onward. After week 15, recovery of most insect populations had occurred. Consistent responses, defined as statistically significant deviations pointing in the same direction on at least two consecutive sampling dates, were observed for all the presented taxa in Table 9. Longer-lasting reductions were observed at the 5% treatment levels and occurred with *G. pulex* (Figure 5A). Recovery of the populations of this species was hindered by isolation of the ditches. At the 1% treatment level, long-lasting reductions were observed within *Chaoborus* sp. (Figure 5B) and *C. horaria* (Figure 5C). The lowest consistent NOECs were calculated for *Chaoborus* sp. at the 0.2% treatment level. For *C. horaria* and Orthocladiinae/Chironomini, an isolated NOEC was calculated of 0.2%. NOECs for other species and sampling dates were at the 1% treatment level.

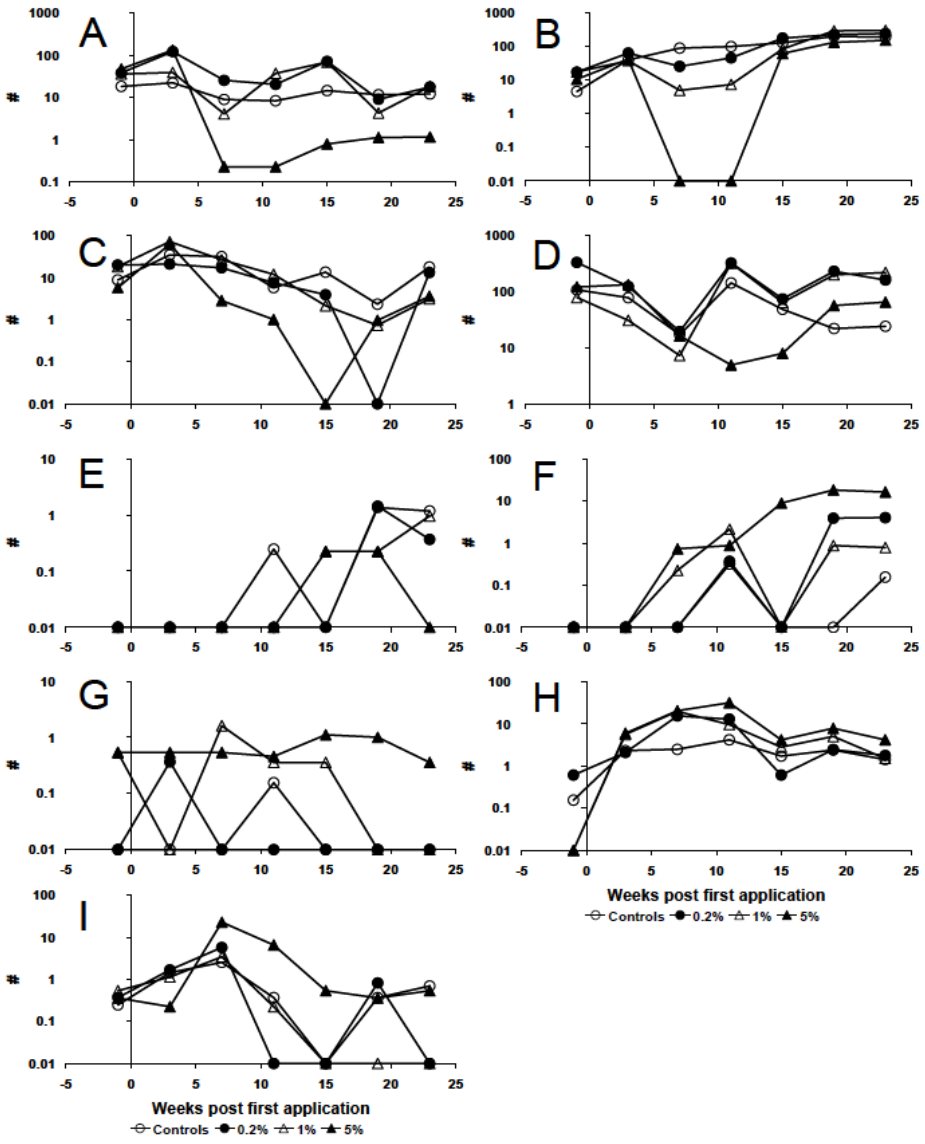

Figure 5. Dynamics of macroinvertebrate populations showing consistent treatment-related responses (Table 9) after applications of compounds of the pesticide package. Numbers per artificial substrata are geometric mean abundance numbers of *Gammarus pulex* (A), *Chaoborus* sp. (B), *Caenis horaria* (C), *Cloeon dipterum* (D), Haliplidae (E), *Dero* sp. (F), *Lymnaea stagnalis* (G), Orthocladiinae/Chironomini (H), and *Stylaria lacustris* (I)

Table 9. Results of NOEC calculations of zooplankton, phytoplankton, macroinvertebrate taxa and percentage cover of floating filamentous algae (drift percentage; Williams test, p < 0.05) for the different treatment levels of a pesticide package containing prosulfocarb, metribuzin, *lambda*-cyhalothrin, chlorothalonil and fluazinam. [a]

	Zooplankton			Phyto-plankton	Macrophytes
Week	*Hex-arthra* sp.	*Polyarthra remata*	*Daphnia* group *galeata*	Flagellatae sp. (ca. 5-6 micron, 2 flag.)	Filamentous algae
-1					
2				1 % ↑	
5				1 % ↑	
8		1 %↓	0.2 % ↑		
11		1 %↓	1 % ↑		
14	1 % ↓	1 %↓			
17	0.2 %↓				
19					0.2 %↓
20	1 % ↓				
24					1 % ↓

	Macroinvertebrates								
Week	*Gammarus pulex*	*Chaoborus* sp.	*Caenis horaria*	*Cloeon dipterum*	Halip-lidae	*Dero* sp.	*Lymnaea stagnalis*	Ortho-cladiinae/ Chirono-mini	Stylaria lacustris
-1							0.2 % ↑		
3									
7	1 %↓	0.2 %↓	1 %↓			1 % ↑	0.2 % ↑	0.2 % ↑	
11	1 %↓	0.2 %↓	1 %↓	1 %↓				1 % ↑	1 % ↑
15		1 %↓	0.2 %↓	1 %↓		1 % ↑	1 % ↑		1 % ↑
19	1 %↓				0.2%↓	1 % ↑	1 % ↑	1 % ↑	
23	1 %↓				1 %↓	1 % ↑			

[a] Only taxa showing a consistent response (NOECs calculated for two consecutive sampling dates) are displayed. Blank cells indicate NOEC > 5%. ↓: populations were significantly reduced at concentrations above NOEC. ↑ :populations were significantly increased at concentrations above NOEC.

Phytoplankton and zooplankton

Over the experimental period, a total of 59 different zooplankton taxa were identified. The majority of the taxa (40) belonged to the Rotifera, followed by Cladocera (13), Copepoda (3), Ostracoda (1), and Diptera (3). Copepoda and Ostracoda were not identified to species level. Rotifera were the most abundant. Of the total variance, 18% could be attributed to the treatment regime by the PRC analysis (Figure 6). This percentage is in line with results from other experimental ponds (see Sanderson 2002). The PRC diagram displayed a significant amount of the treatment variance using a threshold value of 0.10 for p ($p = 0.068$). It shows a clear trend, particularly for the 5% treatment level (Figure 6). Monte Carlo permutation tests showed significant reductions at this level from week 8 through week 14 after application (Table 8). The first effects on zooplankton followed the application of the insecticide *lambda*-cyhalothrin. The period in which effects on zooplankton were apparent also overlapped with the application period of the fungicide chlorothalonil. Recovery had already started within the application period of the fungicide fluazinam. Statistical calculations resulted in $NOEC_{community}$ for the zooplankton at the 1% treatment level (Table 8).

A number of Rotifera, including *Polyarthra remata* and *Hexarthra* sp., showed high negative weights in the PRC (Fig 6; see species weights bk), indicating a treatment-related decline. Cladocera had positive weights in the PRC, indicating treatment-related increases in abundance. Treatment-related responses for individual taxa were statistically analyzed and resulted in a range of NOECs (Williams test, p 0.05) for three zooplankton taxa (Table 9). For *Hexarthra* sp. and *Daphnia* group *galeata*, a NOEC of 0.2% was calculated on an isolated sampling date as well as a NOEC of 1% on several other sampling dates. For *P. remata*, a NOEC of 1% was calculated.

The dynamics of zooplankton populations showing treatment-related responses after application of the pesticide package are presented in Figure 7. Significant long-lasting reductions in numbers occurred in 2 populations of Rotifera species. In the population of *Hexarthra* sp., reductions were observed at the 1% and 5% levels (Figure 7A). In the population of *P. remata*, reductions were observed at the 5% level (Figure 7B). Within the group of Calanoida, reductions were observed at the 5% level (Figure 7D). Short-term increases were observed in the population of *Daphnia* group *galeata* at the 1% and 5% levels (Figure 7C). Both positive and negative effects were apparent from week 8 onward.

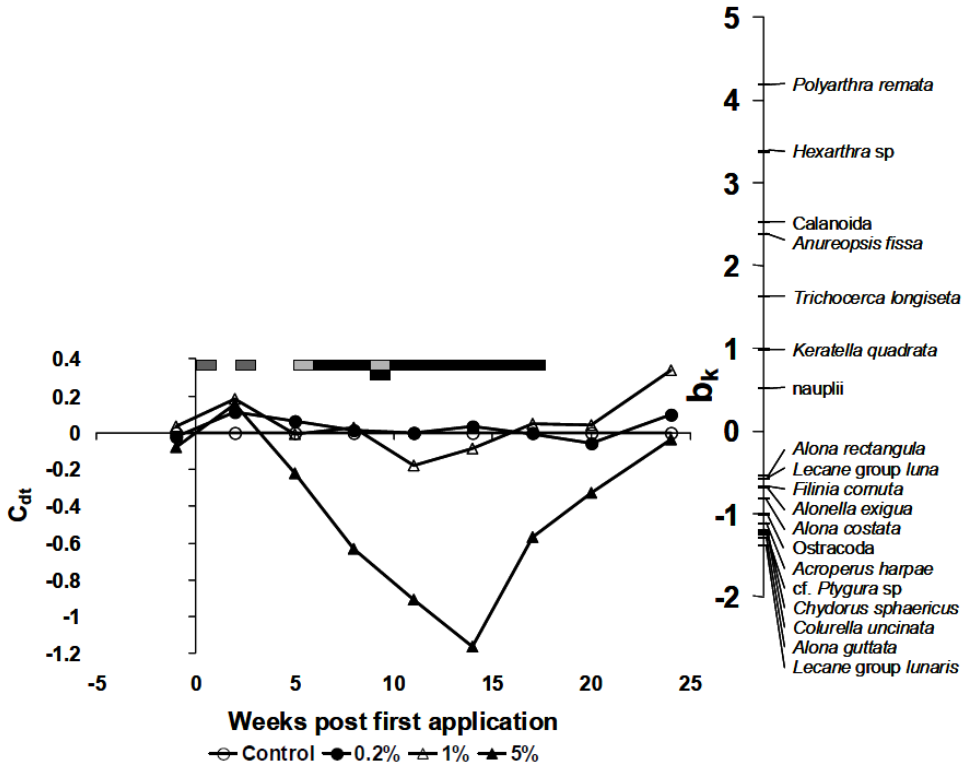

Figure 6. **Principal response curve (PRC) with species weights (bk) for the zooplankton data set, indicating the effects of applications of compounds of the pesticide package. Of the variance, 44% could be attributed to sampling date and is displayed on the horizontal axis. Differences between replicates accounted for 38% of all variance. Eighteen percent of the variance could be attributed to treatment scenario. Of this percentage, 24% is displayed on the vertical axis. The species weight can be interpreted as the affinity of the taxon to the PRC. The PRC diagram does display a moderate significant amount of the treatment variance (p = 0.068). Application of herbicides, medium shading; application of insecticide, light shading; application of fungicides, solid**

A total of 92 phytoplankton taxa were identified. Dominant groups were green algae, mainly Chlorococcales, Desmidiaceae, and Diatoms. The PRC diagram for the phytoplankton did not display a significant amount of the treatment variance and, thus, is not shown. Statistical calculations resulted in a $NOEC_{community}$ for the phytoplankton at the 1% treatment level (Table 8), which was calculated for 1 sampling date (week 5). The data also suggest a trend of an effect in week 2, although not statistically significant (p = 0.08; Table 8).

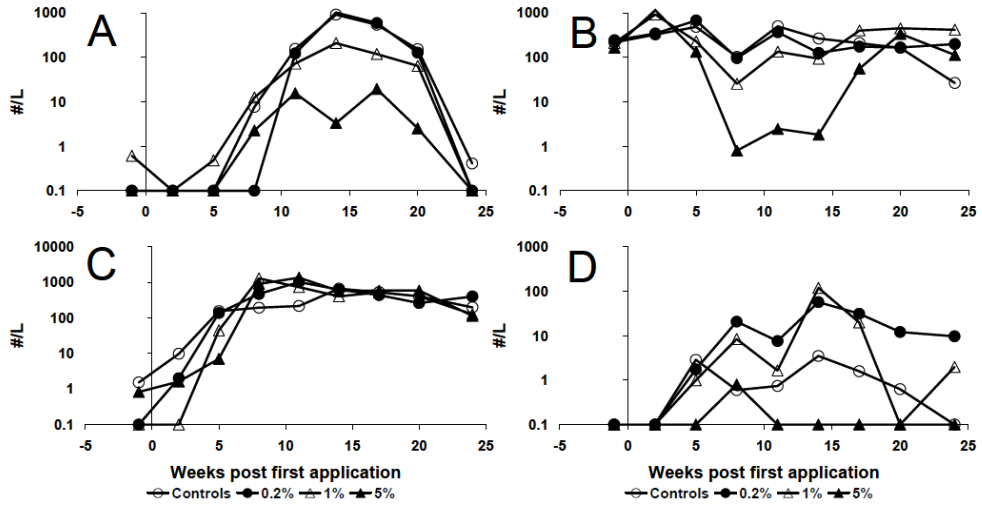

Figure 7. **Dynamics of zooplankton populations showing consistent treatment-related responses (except for Calanoida, Table 9) after applications of a pesticide package containing prosulfocarb, metribuzin, *lambda*-cyhalothrin, chlorothalonil, and fluazinam. Numbers are geometric mean abundance numbers of Hexarthra sp. (A), Polyarthra remata (B), Daphnia group galeata (C), and Calanoida (D)**

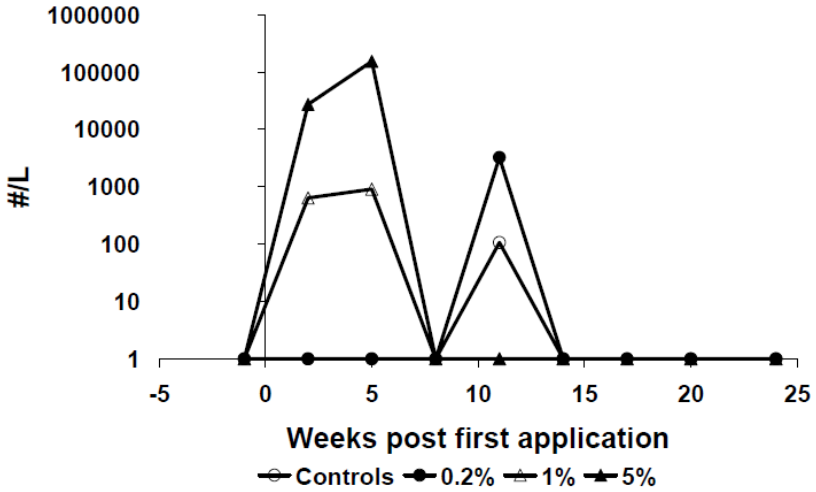

Figure 8. Dynamics of Flagellatae sp. population belonging to the phytoplankton. This taxon is the only taxon that showed a consistent treatment-related response (Table 9).

Of the phytoplankton taxa, only Flagellatae showed a consistent response (increase in numbers) at the 5% treatment level in weeks 2 and 5 (Table 9). For Flagellatae, a NOEC of 1% was calculated. Figure 8 presents the dynamics of the population of Flagellatae. Chlorophyll-α concentrations of phytoplankton did not show a consistent treatment-

Macrophytes

The aquatic vegetation in the ditches was dominated by *Chara globularis* ssp. *virgata*. Other abundant macrophytes were *Elodea nuttallii* and *Sagittaria sagittifolia*. Submerged and floating algal beds were locally abundant. Of the percentage cover/abundance data of macrophyte taxa, only floating filamentous algae showed a consistent response (Table 9) after the application period of the pesticide package. Floating filamentous algae developed in controls and 0.2% treatments and resulted in significantly higher percentage cover compared with that of (Table 10) the 1% and 5% treatments. Dynamics of submerged filamentous algae showed a consistent treatment-related response as well (Table 10). There were no significant treatment-related responses in four *in situ* bioassays performed with *M. spicatum* (NOECs > 5%). Total macrophyte biomass in bioassays varied from 0.68 ± 0.19 to 0.84 ± 0.25 g in week 5 and from 1.42 ± 0.35 to 1.98 ± 0.02 g in week 22.

Table 10. Dynamics of submerged and floating filamentous algae expressed as mean cover percentage per treatment level per sampling date plus associated standard deviation. Asterisks indicate significant differences (Williams test, p < 0.05).

Submerged filamentous algae				
Week	**Control**	**0.20%**	**1%**	**5%**
-1	29.47 ± 25.54	6.94 ± 7.51	20.83 ± 36.08	6.67 ± 3.57
7	21.91 ± 25.42	4.19 ± 5.92	1.06 ± 1.06	2.31 ± 2.31
13	21.78 ± 19.50	21.38 ± 19.27	9.58 ± 16.06	31.00 ± 24.00
19	14.50 ± 7.12	19.81 ± 4.68	4.38 ± 5.56	8.25 ± 7.72
23	26.75 ± 13.74	20.31 ± 1.33	4.88 ± 4.28 *	3.13 ± 1.44 *
Floating filamentous algae				
Week	**Control**	**0.20%**	**1%**	**5%**
-1	8.44 ± 7.67	2.38 ± 3.01	10.42 ± 16.87	0.83 ± 1.44
7	5.97 ± 4.37	6.04 ± 4.71	9.56 ± 9.56	2.19 ± 2.19
13	15.06 ± 11.01	14.25 ± 4.95	8.21 ± 14.00	4.94 ± 1.81
19	19.88 ± 6.84	21.06 ± 4.33	6.79 ± 6.37 *	5.54 ± 0.71 *
23	14.41 ± 3.65	23.06 ± 6.28	8.46 ± 9.90	2.54 ± 1.50 *

Leaf litter decomposition

Significant treatment-related effects on leaf litter decomposition were not observed in the seven decomposition experiments performed (NOECs > 5%). Only at week 8 was a single NOEC of 1% calculated. The decay rate varied from 19.29 ± 2.24% in 5% treatments to 21.78 ± 1.75% in 0.2% treatments over a 2-week period. In the control ditches, the decay rate was 20.63 ± 2.46% over a 2-week period.

Discussion

Experimental design

The treatment regime of the ditch mesocosms was considered a worst case, despite the absence of herbicide application at the end of the season. Instead of this, mechanical removal was simulated because of limitations set by the ditch mesocosms (no persistent compounds could be applied) and limitations set by the compounds (compounds should be permitted for use in The Netherlands at that moment and in near future). Because ditch mesocosms were not connected to each other during the experiment, the absence of recovery via water contributed to the worst-case situation. Recovery from neighbouring ditches via air was, of course, possible and reflected more or less the field situation.

Exposure concentrations

Except for prosulfocarb, the dissipation of other pesticides from the water of the ditch mesocosms was very rapid, and concentrations generally reached detection limits before the next application was made (Figure 2 and Table 5). The field dissipation measured covers the combined aqueous losses owing to photolysis, hydrolysis, sorption, volatilization, and biodegradation. Dissipation DT_{50} values from the literature (where available, field values) were compared to those measured in this experiment. For prosulfocarb, no other field studies were available and in the standard laboratory tests DT_{50} is 1 d in water and 150 to 380 d in sediment in a sediment–water study (data from Syngenta). For metribuzin, a dissipation half-life of 5 d was found in experimental pond mesocosms (26), whereas a range of 6 to 9.4 d (mean, 7.1 d) was reported by Brock et al. in a ditch enclosure study. In the present study, the dissipation of metribuzin from the water layer was faster than reported elsewhere. One possible explanation for this could be differences in photolysis, because this process is reported to be important for the dissipation of metribuzin (27, 28).

For *lambda*-cyhalothrin, DT_{50} values ranged from 0.7 to 1.1 d in laboratory microcosms (8) and from 0.24 to 0.27 d in ditch enclosures (29, 30). Wendt-Rasch et al. (31) calculated a mean DT_{50} in water of 0.86 d in outdoor *Elodea*-dominated microcosms and a mean water DT_{50} of 2.4 d in outdoor *Lemna*-dominated microcosms. Hand et al. (32) found a DT_{50} of less than 0.125 d for dissipation from the water column in an indoor macrophyte-dominated microcosm study and a DT_{50} of less than 0.125 d for the whole system. The dissipation of *lambda*-cyhalothrin in the present study was in the same range as reported by Van Wijngaarden et al. (8) and in the *Elodea*-dominated microcosms reported by Wendt-Rasch et al. (31) but was slower than that reported by Leistra et al. (29), Roessink et al. (30), and Hand et al. (31). Alkaline hydrolysis in the water near the surface of macrophytes is considered to be the main dissipation process for *lambda*-cyhalothrin (Leistra et al. 2003).

Available water DT_{50} values for fluazinam range from 1.5 to 3.3 d in indoor microcosms (8) and from 0.9 to 1.1 d in outdoor microcosms (31). The dissipation of fluazinam in our study fell within these ranges. For chlorothalonil, field DT_{50} values in water range from 0.17 (33) to 1.25 d (34). In the present study, the dissipation of chlorothalonil was somewhat slower than that reported by Davies (33).

In conclusion, when compared with the field DT_{50} values reported in other studies, the fate of the compounds was overall very comparable. The relatively fast dissipation of metribuzin, *lambda*-cyhalothrin, chlorothalonil, and fluazinam from the water of the ditch mesocosms suggests that in our test systems these pesticides cause short-term stress only, despite the repeated application of some of these compounds. However, the longer exposures to the herbicide prosulfocarb may have also led to chronic stress on the phytoplankton community.

The second application of *lambda*-cyhalothrin, which was approximately 50% lower than intended, had no effects at the 5% treatment. Concentrations still were above threshold levels (Tables 1 and 11). It might have had consequences for the 1% treatment, because achieved nominal concentrations were around threshold concentrations. If the target concentration (just above threshold concentration) would have been achieved instead, the effect at the 1% treatment level might have been more consistent between weeks 5 and 9 (community-level $NOEC_{macroinvertebrates}$ at the 0.2% treatment level [Table 8] on both dates and the effects would have been short lived). However, this would not have changed the results and conclusions of this experiment: a NOEC for the community of 0.2% and clear but transient effects at the 1% treatment level. Effects of *lambda*-cyhalothrin are in line with effects found in other mesocosm experiments (8, 30).

Secondary effects and interaction

In addition to direct effects on sensitive species, pollutants may exert effects on tolerant species by a number of ecological mechanisms. Such effects are called indirect (or secondary) contaminant effects. Competitive release and trophic cascades are indirect effects that are very common (35). Only in studies at the population, community, or ecosystem level can indirect contaminant effects be detected. Because indirect effects may be an important part of contaminant effects at these levels, we will consider some important indirect effects that were detected in the experiment more profoundly.

The trend of an effect on phytoplankton after the application of prosulfocarb in week 2 (Table 8), the observed effect on phytoplankton in week 5 (Table 8), and effects on Flagellatae (increase) in weeks 2 and 5 suggest that the effects observed can be attributed to herbicide application and not to the application of *lambda*-cyhalothrin. It seems most likely that these effects were a result of exposure to prosulfocarb (Table 12). The ecological reason for the increase in Flagellatae is difficult to explain from the data derived in the experiment. An increase in numbers usually indicates the occurrence of an indirect effect, such as a result of a decrease in other taxa. This primary effect, however, could not be demonstrated by the data. Chlorophyll-α concentrations of phytoplankton did not show a consistent treatment-related response, despite the temporal increase in Flagellatae. The decreases in pH and dissolved oxygen described above suggest that prosulfocarb decreases the net photosynthesis of the community of primary producers at the 5% treatment level in particular. However, consistent declines in populations of primary producers could not be observed during the first 5 weeks after prosulfocarb application. In addition, significant declines in zooplankton and macroinvertebrate taxa were also not observed in this period. A decrease in pH was also observed in the 8th week of the experiment (Table 7). A decrease in pH suggests a decrease in the net photosynthesis of the primary producers. Increases in oxygen levels, turbidity, and chlorophyll-α in water in weeks 14 to 16 (Table 7) suggest an increased algal production. However, effects on algae community structure were not observed in the 3 examples mentioned above. One possibility might be that effects on functional endpoints last longer and can be detected, whereas effects on structural endpoints might be too transient to detect with the chosen sampling frequency. A second possibility might be the filtration of the samples through a net with 40-µm mesh size. As a consequence of this, the smallest species (among others, nanoplankton) are not considered in this experiment. Therefore, effects on phytoplankton in this experiment are limited to the larger species. The decrease in filamentous algae from week 13 onward and the persistence of this effect

at the 5% treatment level may be another secondary effect, of which the cause is not clear.

Some Rotifera showed clear direct effects (Figure 6). The decrease in the population of *P. remata* may be due to direct effects of chlorothalonil, but indirect effects may also play a role. Because of the observed indirect effect of increasing daphnid abundance owing to a decrease in its predator *Chaoborus*, competitive exclusion may have led to a decline in Rotifera populations.

The significant reductions in *G. pulex* at the 5% treatment level did not influence the extent to which leaf litter was decomposed in the ditch mesocosms consistently. Only in week 8 was a single NOEC of 1% calculated. A reduced decomposition can probably be attributed to the effect of *lambda*-cyhalothrin, but the effect was not consistent. Obviously, other shredders such as *Proasellus meridianus/coxalis*, which were unaffected by the pesticide treatments, were able to maintain this function.

The adverse effect on *Chaoborus obscuripes* (Figures 4 and 5B) contributed to several indirect effects. In the 0.2% and 1% treatments, *C. obscuripes* was eliminated. Hence, predation of *C. obscuripes* did not reduce the numbers of Calanoida at these treatment levels (Figure 7D). At the 5% level, Calanoida show a strong decrease owing to direct effects after treatment with *lambda*-cyhalothrin and chlorothalonil. In the controls, numbers of Calanoida were reduced by predation of *C. obscuripes*. It may be due to such nonlinearity in the observed responses that apparent effects were absent.

Significant reductions in the abundance of the mayfly *C. dipterum* by *lambda*-cyhalothrin also resulted in several indirect effects. As a result of competitive release, other water column inhabitants like *S. lacustris*, *Dero* sp., and Orthocladiinae/Chironomini may have increased.

Other indirect effects observed in the ditch mesocosms were an increase of the flatworms *Dugesia lugubris* and Mesostoma and an increase of the snail *Lymnaea stagnalis*. The latter phenomenon has been observed in other mesocosm experiments (8, 30) and is probably owing to competitive release and subsequent higher food availability. Farmer et al. (36) found higher algal biomass and higher abundances of copepod nauplii and Rotifera as indirect effects of *lambda*-cyhalothrin at levels above the highest concentration in our study. Effects were probably owing to effects on Asellidae and Gammaridae. In the present study, Asellidae did not decrease significantly.

Summary of effects and ecological threshold concentrations of individual compounds

In the present study, the total pesticide package resulted in short-term responses of pH, oxygen, and phytoplankton and in long-term effects on zooplankton and macroinvertebrates at the 5% treatment level. At the 1% treatment level, only slight, transient effects were observed on a limited number of zooplankton and macroinvertebrate species and on pH. At the 0.2% treatment level, no consistent treatment-related effects were observed.

The effects at the community level were compared with known ecological threshold concentrations (NOEC and Class 2 LOEC values) for the individual compounds based on microcosm and mesocosm studies with these individual compounds (Table 11).

Table 11. Threshold levels (NOEC and CLASS 2 LOEC values) for several endpoints in microcosm and mesocosm studies with individual compounds (metribuzin (16), *lambda*-cyhalothrin (8, 30) (macrophyte-dominated mesocosms); chlorothalonil: unpublished data Syngenta; fluazinam: unpublished data Alterra). [a]

	Ecological threshold concentrations (NOEC – CLASS 2 LOEC values)			
	Metribuzin	*Lambda*-cyhalothrin	Chloro-thalonil	Fluazinam
Endpoints	µg/L	ng/L	µg/L	µg/L
Micro-crustaceans	18 – 56 ↓	10 – 25 ↓*	3 – 10 ↓*	2 – 10 ↓
Rotifers	18 – 56 ↓↑	10 ↓↑*	10 ↓*	2 – 10 ↓
Phytoplankton	5.6 – 18 ↓	> 250	10 ↓↑*	10 – 50 ↑
Macrophytes	> 180	> 250	100	> 250
Macrocustaceans	-	10 – 25 ↓*	> 300	10 – 50 ↓
Insects	-	10 ↓*	100 – 300	-
Other macroinvertebrates	-	100 – 250 ↓↑	100	50 - 250
pH, DO	5.6 – 18 ↓	> 250	100	> 50

[a] Values below nominal concentrations used in this study are marked with an asterisk.. CLASS 2 effects are slight effects and LOEC values are the Lowest Observed Effects Concentrations causing these effects. ↓ = populations were significantly reduced at concentrations above the thresholds. ↑ = populations were significantly increased at concentrations above the thresholds; ↓↑ = populations were both significantly reduced and significantly increased at concentrations above the thresholds.

This table shows that the effects of metribuzin and fluazinam at the community level are unlikely at the concentrations applied in the experiment. The treatment concentrations in the experiment lie below or in the range of the threshold concentrations for these compounds. Effects of chlorothalonil might be expected at the 5% treatment level but are unlikely at the 1% treatment level. The treatment concentrations of *lambda*-cyhalothrin lie above the thresholds, indicating that clear effects would be expected at both the 1%

and 5% treatment levels. For prosulfocarb only the First Tier Uniform Principles Standard is available (8.6 µg/L), indicating that probably effects would be expected at both the 1% and 5% treatment levels.

Table 12 provides an overview of the effects of the treatments on the various endpoints studied. In this table, observed effects were summarized into effect classes described by Brock et al. (17, 18). At the 5% level, a strong decline was observed for *G. pulex*, *Chaoborus*, and mayfly larvae, most likely caused by direct toxic effects of *lambda*-cyhalothrin (Tables 1 and 11). Also Haliplidae decreased at the end of the experiment. Increase in abundance was observed in populations of *Dero* sp., *L. stagnalis*, Orthocladiinae/Chironomini, and *S. lacustris*. This may be, at least partly, a result of competitive exclusion due to the strong negative effect on the mayfly *C. dipterum* by *lambda*-cyhalothrin. During the period of chlorothalonil application, decreases in population densities of Rotifera and Calanoida were observed.

Table 12. Summary of effects observed in ditch mesocosms treated with pesticides used in potato cultivation. [a]

Endpoint	Treatment level		
	0.2 %	1 %	5%
Microcrustaceans	1	2↑	2↑
Rotifers	1	2↓	3↓
PRC Zooplankton	1	2↓	3↓
Phytoplankton (Flagellata)	1	2↑	3↑
Macrophytes (Filamentous algae)	1	3↓	4↓
Macrocrustaceans	1	1	4↓
Insects	1	2↓	3↓
Other macroinvertebrates	1	2↓↑	4↓
PRC macroinvertebrates	1	2↓	4↓
pH/DO	1	2↓	3↓

[q] **Treatment** levels **are equal to 0.2, 1 and 5 % spray drift emission of label-recommended rates. The numbers in the table refer to effect classes described by Brock et al. (17, 18). 1 = No effect, 2 = slight effect, 3 = clear short-term effect, full recovery observed, 4 = clear effect, no full recovery observed at the end of the experiment, 5 = clear long-term effects. ↓ decrease of endpoint. ↑ increase of endpoint. ↓↑ both decrease and increase of endpoint is observed**

At the 5% treatment level, the ecological threshold concentrations for chlorothalonil were exceeded (Tables 1 and 11). The effects on Rotifera and Calanoida can therefore most probably be attributed to exposure to chlorothalonil. Although based on laboratory data,

effects of fluazinam on the zooplankton might have been expected. However, they were not observed in this experiment. At the community level, effects of fluazinam are unlikely (Tables 1 and 11). Effects on the higher levels might be absent because of functional redundancy between species. At the 5% level, a short-term decrease in pH and DO and an increase of Flagellata were observed in the first weeks of the experiment. Because effects of metribuzin at the community level are unlikely at the concentrations applied in the experiment (Tables 1 and 11), these effects can be attributed to prosulfocarb. Filamentous algae were lower than were the controls from week 13 onward. In weeks 14 to 15, algal productivity was increased. So, at the 5% treatment level, pronounced effects were observed and could be attributed to 3 of the 5 compounds within the pesticide package. Most of these effects persisted until the end of the experiment (effect class 4 in Table 12). Recovery was observed within the group of insects (Figure 5B to D), which may be attributed to the multivoltine character of their propagation and their recolonization abilities by air from neighboring control ditches. Recolonization by other routes was not possible in this experiment because of the hydrological isolation of the ditches. Hence, recovery of the population of *G. pulex* could not occur because of a lack of external supply. Other macroinvertebrates without a terrestrial stage in their life cycle were also hindered to recover. Rotifera and Calanoida recovered rapidly as a result of survival in less-affected microhabitats or production of resistant eggs. The importance of life cycle characteristics to the recovery potential of affected habitats and ditches has also been stressed by other authors (37, 38).

At the 1% treatment level, transient decreases were observed in populations of *Chaoborus* and *C. horaria*, most likely caused by *lambda*-cyhalothrin (Tables 1 and 11), and on *Hexarthra* sp., likely caused by *lambda*-cyhalothrin and chlorothalonil (Table 9). A clear adverse effect was observed on Haliplidae at the end of the experiment. Clear increases of population numbers of *L. stagnalis* and Orthocladiinae/Chironomini were observed, although abundance values of these invertebrates were small (Figure 5G and H). Another clear indirect effect is the temporary increase of Cladocera, most likely caused by the direct toxic effects of *lambda*-cyhalothrin on the predator *Chaoborus*. Prosulfocarb most probably caused a short-term decrease in pH in the first weeks of the experiment. Floating filamentous algae decreased from week 13 onward but were not significantly different any more from controls and 1% treatments by the end of the experiment. In weeks 14 to 15, algal production is increased. We can conclude that at the 1% level only slight, short-term effects were observed.

For macroinvertebrates, a NOEC was calculated at the treatment level of 0.2%. The NOECs for other invertebrates (zooplankton) and phytoplankton were equal to a treatment level

of 1%. For the pesticide package, the most sensitive organisms within the community were the macroinvertebrates, with *Chaoborus* and *C. horaria* as the most sensitive species and *lambda*-cyhalothrin as the most toxic compound. Hence, overall, we consider the 0.2% treatment level as the NOEC for the community.

Toxicity of the pesticide package

Effects on primary producers are likely at $TU_{algae} > 0.1$ (17). At the 5% level, a temporal exposure peak up to 0.9 TU_{algae} was observed (Figure 3), which resulted in short-term responses of pH, oxygen, and phytoplankton (Table 7). For algae, effects of treatment predominantly resulted from prosulfocarb treatments and resulted in short-term responses of pH, oxygen, and phytoplankton.

After repeated application, effects on invertebrates are likely to occur at $TU_{Daphnia} > 0.1$ (18). At the 5% level, exposure concentrations amounted to 0.4 $TU_{Daphnia}$ and resulted in long-term responses (see Figures 4 and 6). For invertebrates, effects were probably mainly caused by *lambda*-cyhalothrin and chlorothalonil (Table 11). Effects on zooplankton were most apparent from week 8 onward, after the application of *lambda*-cyhalothrin in week 5 and applications of chlorothalonil in weeks 6 and 7 (Figures 3 and 6). The effects on zooplankton continued into the period in which fluazinam was sprayed. However, some zooplankton populations were already showing recovery within this period (Figure 7B and C). The calculations of TU show that treatment concentrations for fluazinam lie in the range in which effects are border line. This is in accordance with Tables 1 and 11, which show that effects of fluazinam are unlikely.

Effects based on multi- and repeated stress

The exposure concentrations in toxic units (Figure 3) may indicate potential risks owing to multi or repeated stress. During the experiment, two periods of multi-stress can be recognized. The first is the application period of the two herbicides, in which multi-stress of the two herbicides seems to exhibit a potential risk. However, it is not an actual risk, because effects of metribuzin at the community level are unlikely at the concentrations applied in the experiment (Tables 1 and 11) and effects can be attributed to prosulfocarb. The second is the simultaneous application of *lambda*-cyhalothrin and chlorothalonil as indicated by Figure 3. Experimental data seem to confirm an effect by the two stressors. The maximum TU was reached after week 9 (Figure 3) after the application of both *lambda*-cyhalothrin and chlorothalonil. This was followed by a clear, but not significant, effect on zooplankton at the 1% level (Figure 6). This effect was not observed after the

application of one of the two compounds separately in the weeks before. Similar effects at the 1% level are also clear at the population level within the population of *Hexarthra* sp. (Figure 7 and Table 9). At the 5% level, the effects proceed until recovery starts, which was not until week 15 (Figure 6). It can be concluded that at the 1% level minor effects on zooplankton were observed, which might result from multistress by *lambda*-cyhalothrin and chlorothalonil. These effects on zooplankton and macroinvertebrates seem to be more severe at the 5% level.

During the experiment repeated stress was potentially caused by the multiple applications of *lambda*-cyhalothrin and chlorothalonil. Repeated stress might have played a role in the effects of chlorothalonil on zooplankton and in the effects of *lambda*-cyhalothrin on macroinvertebrates, although in the experiment these effects cannot be separated from the effects of the combined application of chlorothalonil with *lambda*-cyhalothrin on the fourth chlorothalonil application. We can conclude that multi- and repeated stress played a small role within the applied pesticide package. This was probably mainly because most of the substances rapidly dissipated and the absence of many simultaneous applications.

Conclusions

Comparing the findings from previous experiments with those observed in the present study, it can be concluded that the patterns of effects observed from the application of the pesticide program were in line with the effects we could deduce from the thresholds and concentrations for the individual compounds, although repeated applications were used. This may be because of the short dissipation time of most compounds.

If no effects on aquatic ecosystems are accepted and if spray drift is considered the only emission route, emission reduction measures to values below 1% spray drift are necessary. The current aquatic risk assessment procedure, based on individual compounds, the Uniform Principles, and a drift emission of 1%, may sufficiently protect aquatic ecosystems if slight and transient effects are accepted. The crop approach is a promising approach for risk assessment and evaluation of effects of realistic pesticide stress. Moreover, a crop approach is also promising from a risk management point of view, because it will improve the practicality and acceptance.

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Chapter 5: Aquatic fate and effects of *lambda*-cyhalothrin in model ecosystem experiments[4]

Summary

The fate and effects of the synthetic pyrethroid *lambda*-cyhalothrin in aquatic model ecosystem experiments are reviewed. In laboratory studies, *lambda*-cyhalothrin is highly toxic to fish and invertebrates. Its physico-chemical and laboratory fate properties indicate that it will dissipate rapidly from the water phase, reducing exposure for organisms in the water-column. For European aquatic risk assessments, where exposure models predict that spray drift is the main entry route from agricultural uses, this has been a key factor in refining higher-tier risk assessments for water-column organisms. Modified exposure studies in the laboratory confirmed that rapidly reduced exposure mitigates effects on fish and invertebrates. Eight aquatic model ecosystem experiments have been conducted with *lambda*-cyhalothrin in a variety of indoor and outdoor test systems. These were of differing trophic status, and ranged in size from 0.43 to 450 m3. The timing of application of test substance also varied between studies. The fate of the compound in the various experiments was consistent, typified by rapid dissipation (and degradation) in the water phase with median dissipation times (DT_{50}) of typically a day or less. Only 5-22% (where quantifiable) of the chemical applied to the water column reached the sediment, and it was not possible to calculate sediment DT_{50} values. Effects in the studies were driven by population responses of macrocrustacea and certain insects, along with zooplanktonic microcrustacea. Considering the range of test systems, the variety of locations, different trophic status of the test systems, differences in season of application, and differences in numbers of applications, the effects thresholds observed in the studies were remarkably consistent, with no to slight effects occurring consistently at initial nominal treatment concentrations up to 10 ng/L. The effects threshold values for clear effects with recovery were more variable than the no to slight effects, but still reasonably consistent, with thresholds between initial nominal treatment concentrations of 16 and 50 ng/L. This gives considerable confidence in the potential to extrapolate the effects

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observed in one study to a different situation, at least in this case where effects tend to be of an acute nature, and the dissipation and degradation of the compound is rapid.

Introduction

Synthetic pyrethroids are highly toxic to aquatic invertebrates and fish in the laboratory (1, 2). However, under field conditions, their rapid dissipation from the water column is cited as a mitigation of potential for effects under field conditions (1). For this reason, over the last three decades, many aquatic field studies have been performed on a variety of synthetic pyrethroids to measure their effects on populations and communities of aquatic organisms (3, 4). Beginning in the late 1970s and continuing into the early 1980s, the first field studies on pyrethroids were conducted in farm ponds, mainly in the USA (5). While these studies had the advantage of being realistic because they were conducted in natural water bodies, they had a number of disadvantages. The experimentation was difficult (e.g. finding appropriate sites of adequate similarity for a treated and untreated system) and expensive, there were no treatment replicates so there was a lack of statistical power, there was high temporal and spatial variability, and there was no dose-response relationship since only one treatment was applied. Consequently, it was difficult to establish cause and effect.

From these early field studies, the mesocosm evolved in the late 70s and mid 1980s. Mesocosms are defined (6) as bounded and partially enclosed outdoor experimental units that closely simulate the natural environment. During the late 1980s and early 1990s, mesocosm studies were required by the United States Environmental Protection Agency (EPA) for synthetic pyrethroid registration submissions. The mesocosms used for these studies were large, using experimental ponds of around 400 m3, and a range of endpoints were evaluated including plankton, macroinvertebrates, macrophytes, and fish growth and reproduction (7). Though mesocosms arguably moved the science forward from farm pond studies, it was generally recognized that the results were still difficult to interpret for a number of reasons (8). The ponds were stocked with adult bluegill sunfish (Lepomis macrochirus) whose progeny of up to 20 000 young-of-year fish at the end of the study could themselves have a substantial effect on the ponds. The influence of these young fish could be seen in the control and treatment data, as numbers of arthropods consistently declined through the course of the study, presumably due to predation. Also, since the ponds used for such studies were large, there were often environmental gradients across study sites, leading to quite high variability for some endpoints. The studies were also very expensive, typically taking two to three years to complete, and costing several million dollars.

In 1992, the EPA introduced the 'New Paradigm' in which it was recognized that evaluation of ecotoxicological field studies was problematic and time intensive. Since that time, mesocosm data have not been routinely used in pesticide registration in the USA, though they remain a higher-tier option. In Europe however, mesocosms, and their smaller counterparts, microcosms, continued to be an option for higher-tier risk assessment under the European Union (EU) plant protection product directive, 91/414/EEC. During the 1990s, a variety of different test systems were established, and the methodologies for microcosms and mesocosms developed substantially, resulting in a number of reviews and guidance documents (9, 10, 11) and ultimately leading to the production of an Organisation for Economic Cooperation and Development (OECD) guidance document in 2006 (12). The use and interpretation of aquatic model ecosystem experiments in the EU continue to be the focus of discussions (13).

Though the methodology for conducting aquatic model ecosystem studies was well-established by the late 1990s, a number of questions remained regarding their interpretation and implementation in risk assessment (11). Four uncertainties that were identified were the extent to which aquatic model ecosystem data generated in one location could be applied to another situation; the potential influence of mixtures of chemicals or stressors; whether the timing (season) of application would influence the outcome of the study; and whether differences in ecosystem properties (e.g. trophic status) might influence the results. Here we review the fate and ecological threshold levels of *lambda*-cyhalothrin in eight indoor and outdoor aquatic model ecosystem experiments under a wide range of experimental conditions in light of these uncertainties.

For *lambda*-cyhalothrin aquatic risk assessment in Europe, concerns for aquatic organisms have focused on the potential exposure of water bodies by spray drift. Based on the results of European exposure modeling approaches, spray drift is identified as the major route of entry of *lambda*-cyhalothrin. from agricultural uses. This review therefore focuses on water-column endpoints. For other regions or uses, different sources or routes of exposure may also be important for pyrethroids (14, 15).

Summary of laboratory fate and effects profile

In common with other pyrethroids, *lambda*-cyhalothrin (1:1 mixture of Z(1R,3R,αS) and Z(1S,3S,αR), esters of α-cyano-3-phenoxybenzyl 3-(2-chloro-3, 3, 3-trifluoroprop-1-enyl)-2, 2-dimethylcyclopropane-carboxylate) has a range of physico-chemical characteristics that can have a substantial influence on its fate in the environment, particularly in aquatic ecosystems. It has a low water solubility of 5 µg/L and a high octanol:water partition

coefficient (log Kow = 7.0), resulting in highly lipophilic properties (16). This means that the compound readily adsorbs to soils and sediments, and soil- and sediment-water partition coefficients normalized for organic carbon content (K_{oc}) are reported to be typically in the range 200 000 to 350 000 (16), and partition coefficients to humic substances in water have been reported in the range 400 000 to 800 000 (17). Consequently, under field conditions, *lambda*-cyhalothrin would be expected to partition rapidly and substantially from the water phase to sediment and other organic materials. In laboratory soil and water-sediment degradation studies, *lambda*-cyhalothrin has been shown to be readily degraded. Average soil and aquatic sediment half lives under aerobic laboratory conditions have been reported to be 43 and 22 days respectively (16).

Another important physico-chemical property of *lambda*-cyhalothrin is its lability under alkaline aqueous conditions. At higher pH values, the ester bond is readily hydrolysed, and the mean degradation time (DT_{50}) at pH 9 is reported to be 8.7 days (16). In small edge of field surface waters that are the protection aim for European risk assessment, pH values of this order are not uncommon (see for example the UK National Pond Survey (18)). It therefore might be anticipated that hydrolytic degradation may play a role in the fate of *lambda*-cyhalothrin in such water bodies.

In standard laboratory tests (with maintained exposure concentrations), *lambda*-cyhalothrin is highly acutely and chronically toxic to fish and aquatic invertebrates, but of low toxicity to algae, indicating negligible risks to aquatic plants (Figure 1). When the standard European uncertainty factors of 100 and 10 for acute and chronic assessments respectively are applied to these data, comparison to exposure concentrations triggers further refinement of the risk assessment (19).

Three principal factors have been proposed that could be investigated to refine the assumptions made in the lower tier risk assessments (19). Firstly, as mentioned above, it has long been noted that the fate properties of pyrethroids mean that standard, maintained exposure laboratory studies are likely to somewhat overestimate the potential for effects in the field, where the compounds will tend to dissipate rapidly. Secondly, the preliminary risk assessments with Daphnia magna and fish in Europe include the use of an uncertainty factor to account for potentially more sensitive species. In the European Union this value is 100 for acute and 10 for chronic assessments. Consequently, if these species are at the sensitive end of the species sensitivity spectrum, the assessment may be conservative. Thirdly, standard laboratory tests do not take into account important processes such as population recovery through reproduction and re-invasion, or avoidance (which may be important for larger organisms that can move quickly).

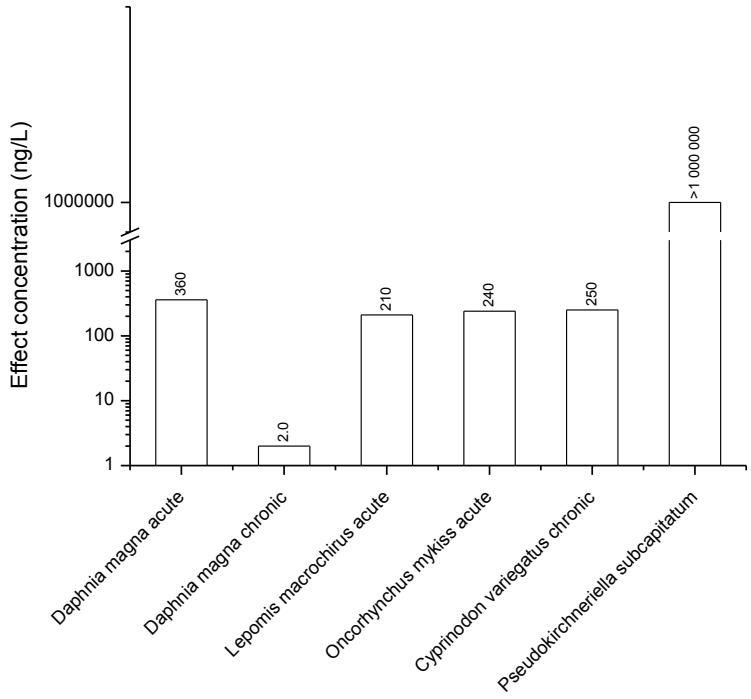

Figure 1. Acute and chronic toxicity of *lambda*-cyhalothrin to standard test species. Labels on the column are the effect concentration in ng/L based on measured concentrations (19) .

The rapid dissipation of *lambda*-cyhalothrin from the water phase in water-sediment systems has been shown to reduce apparent toxicity compared to water-only studies (19). In studies where cyhalothrin (the unresolved form of which *lambda*-cyhalothrin is one of the paired isomers) was applied to static water-sediment systems, there was a three- to four-fold reduction in toxicity compared to water-only for fish and Daphnia. Schroer et al. (20) also found differences in the shape and steepness of laboratory and field species sensitivity distributions.

Similarly, short durations of exposure have been shown to result in substantially less severe effects than maintained, long-term exposures. In studies with a sensitive malacostracan crustacean species Gammarus pulex (19), there was a significant reduction in toxicity with decreasing exposure times, with one hour exposures to a certain

concentration being around eighteen times less toxic than those after ninety-six hours of exposure.

Species sensitivity distributions of fish and aquatic arthropods invertebrates (Figure 2) with *lambda*-cyhalothrin have been reported by several authors (19, 20, 21). Broadly speaking, fish tend to be less sensitive to *lambda*-cyhalothrin than arthopods. Within the invertebrates, generally speaking, crustacean and certain insect arthropod taxa tend to be among the most sensitive, with non-arthropod invertebrates being at the less sensitive end of the distribution.

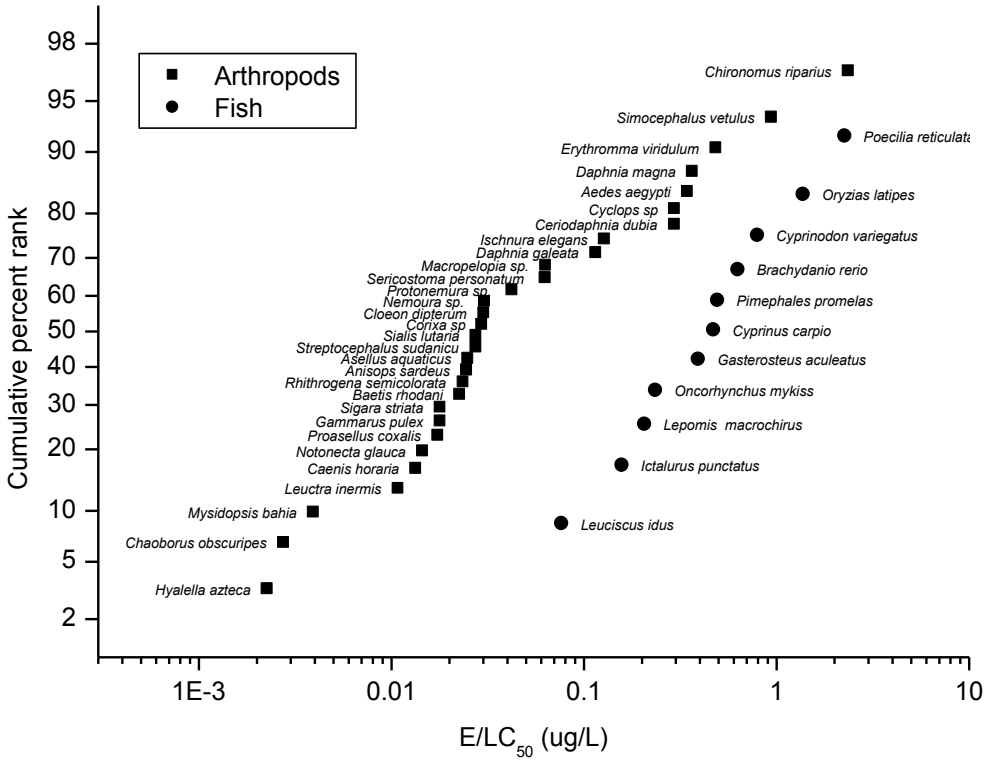

Figure 2. Species sensitivity distributions based on acute laboratory toxicity data for aquatic arthropods (48 h EC_{50}) and fish (96 h LC_{50})

The results of these studies lead to some important implications for the anticipated effects of *lambda*-cyhalothrin under field conditions. Firstly, short-duration exposure may mitigate effects predicted on the basis of standard laboratory data. Considering that exposure is likely to be of short duration in the aqueous phase, potential for recovery for organisms that can recolonize or have resting stages is likely to be high. The organisms that are most likely to be affected in the field are crustacea and certain insect taxa. Further discussion of these aspects as studied in the field can be found below.

Review of Aquatic Model Ecosystem Studies with Lambda-cyhalothrin

An overview of the studies reviewed here is shown in Table 1, and each study is numbered in order to facilitate comparisons in later tables. A brief summary of each study design is provided below, along with an overview of the fate and effects of *lambda*-cyhalothrin in subsequent sections. Analytical residue data from the studies are not presented in detail here. In each aquatic model ecosystem study, treatment solutions were analyzed to confirm that the required amount of test substance had been added to the test system. Due to incomplete mixing at the time of application and subsequent rapid dissipation, initial measured concentrations may be a poor indication of what was actually applied (11). Treatments were therefore confirmed by measuring the application solution (which was then completely emptied into the test system), and then expressing the results as the initial nominal treatment concentration. All of the studies described below were considered to have been treated as intended by this approach, and so results are expressed as the initial nominal treatment concentration in the water phase. The initial concentration decreases over the course of the experiment due to dissipation and degradation processes. It is therefore important to recognize that this description of the treatment concentration is different from laboratory studies where measured concentrations over the course of the experiment are usually used to express the effect concentration.

Summary of experimental design for studies simulating spray drift and runoff

The first mesocosm study with *lambda*-cyhalothrin was carried out in Greensboro, North Carolina, USA during 1985 and 1986 (22, 23). A total of sixteen 15 x 30 m variable depth (0.15 to 2.0 m) pond mesocosms (total water volume of 450 m3) were used and each of three treatments was replicated four times with four cosms used as controls. Each pond

had a 10 cm deep sandy loam sediment and was filled with water from an established pond. Twenty-five adult bluegill sunfish (Lepomis macrochirus) were added to each mesocosm. As was the case for most early mesocosms, the experiment followed a 'simulation' (11) type of experimental design, with treatments attempting to simulate spray drift and runoff entry into farm ponds.

Table 1. Overview of aquatic model ecosystem studies performed with *lambda*-cyhalothrin

Study number	Location	Test system	Initial nominal treatment conc. range (ng/L)	No.of applications	References
Lambda-cyhalothrin applied alone					
1	USA	Pond mesocosm (450 m^3)	1.6 – 160[a] 4.7 – 470[b]	12[a] 6[b]	22, 23
2	UK	Pond mesocosm (25 m^3)	17 -170	4	24
3a	NL	Phyto-plankton dominated ditch enclosure (0.43 m^3)	10 – 250	3	25, 26
3b	NL	Macrophyte-dominated ditch enclosure (0.43 m^3)	10 – 250	3	25, 26
4	NL	Macrophyte-dominated ditch enclosure (0.43 m^3)	10 – 250	3	26, 27
Lambda-cyhalothrin applied with other pesticides					
5	NL	Indoor microcosm (0.6 m^3)	10 – 240	5	28
6	NL	Ditch mesocosm (55 m^3)	4 – 85	2	29
Lambda-cyhalothrin fate only studied					
7	UK	Indoor microcosm (0.60 m^3)	2300	1	30

[a] Spray drift [b] Run-off

A total of twelve applications with an emulsifiable concentrate formulation simulating spray drift were made at one week intervals. Six runoff-simulating applications (*lambda*-cyhalothrin mixed into a soil-water slurry) were made to the same mesocosms at two-week intervals, with the first one three days after the first spray drift application. The application rates were:

- 12 x 0.017 g ai/ha (spray-drift) + 6 x 0.05 g ai/ha (run-off slurry).
- 12 x 0.17 g ai/ha (spray-drift) + 6 x 0.5 g ai/ha (run-off slurry).
- 12 x 1.7 g ai/ha (spray-drift) + 6 x 5 g ai/ha (run-off slurry).

Assuming total mixing in the water column, these applications would have resulted in initial nominal water concentrations of 1.7 and 4.7 ng/L; 17 and 47 ng/L; and 170 and 470 ng/L respectively. However, the results of these studies can be difficult to interpret as standard 'toxicological' (11) effect concentrations since treatment to the water surface probably would have resulted in a concentration gradient during the early part of the study (higher than nominal water concentrations in the upper layers of the water column). For the purposes of comparison with later 'toxicological' studies, the exposure concentrations from this study were expressed as the median of the nominal concentration from the spray drift and run-off applications.

A second spray drift 'simulation' study was conducted in 1986 in Bracknell, Berkshire, UK (24) using outdoor experimental ponds. Each pond was 5.0 m x 5.0 m and 1.3 m deep, and contained 1.0 m depth of water over 0.15 m of sediment (total volume 25 m3). An emulsifiable concentrate formulation was applied with a spray boom at 0.17 and 1.7 g ai/ha on four occasions at two-week intervals. Resulting nominal treatment concentrations (again, potentially underestimating exposure in the upper water layers soon after treatment) were equivalent to 17 and 170 ng/L.

Both of these early US and UK mesocosm studies were 'simulation' type studies, where the test compound was applied to the water surface of the mesocosms either as a spray or as a slurry application in order to simulate spray drift and/or runoff entry. Interpretation of these data as concentrations (as opposed to application rates in mass per unit area) can be problematic. While the simplest approach to interpreting this is to calculate a nominal concentration based on the total loading divided by the water depth, this ignores the fact that for a short time after application, concentration gradients may exist while the test compound mixes through the water column. Indeed, previous studies have shown that for pyrethroids, application to the water surface may (depending on the method of application) result in concentrations in the upper layers that are higher than those based on a calculation of amount nominally applied divided by water depth (3, 31). Some evidence of concentration stratification with water depth was reported by Farmer et al. (24). Consequently, for organisms inhabiting the upper layers of the water column, the use of nominal concentrations to assign effect concentrations from these studies may be quite conservative (i.e., the effects attributed to a lower nominal concentration actually occurred due to exposure to a higher stratified concentration).

Summary of experimental design for studies evaluating the influence of trophic status and season of application

The studies reported by Roessink et al. (25) and Schroer et al. (20) investigated the influence of the trophic status of the test system on the effects of *lambda*-cyhalothrin. Experimental ditches were used that had been established under different regimes of macrophyte growth and nutrient supply over several years to produce distinctive, stable ecosystems: one "macrophyte dominated" and the other "phytoplankton dominated". Van Wijngaarden et al. (27) conducted studies in the macrophyte-dominated ditches and compared the effects of *lambda*-cyhalothrin on aquatic communities following different application regimes, one in spring and one in late summer.

In all of these experiments, multiple applications of *lambda*-cyhalothrin were made to enclosures (cylinders of 1.1 m diameter and 0.90 m height) placed in the ditches and embedded in the sediment (sandy loam) to a depth of about 0.15 m. The water depth was 0.50 m resulting in a water volume of 0.43 m3. Two replicate enclosures in each ditch were dosed directly with *lambda*-cyhalothrin ('toxicological' design) as an aqueous solution of a 10% capsule suspension formulation at initial nominal treatment concentrations of 10, 25, 50, 100, and 250 ng/L. Additionally, two enclosures in each ditch were used as controls and dosed with water only. Each enclosure was dosed three times at one-week intervals. The applications were made to the enclosures in macrophyte- and phytoplankton-dominated ditches in spring (May 2000) and to a further macrophyte-dominated ditch in late summer (August 2000).

Summary of experimental design for studies with applications of multiple pesticides

An indoor microcosm study including applications of *lambda*-cyhalothrin was performed at Alterra, Wageningen University and Research Centre, The Netherlands to investigate the ecological impacts of pesticides used in a typical crop protection programme for tulip culture (28). Twelve indoor microcosms (simulating aquatic communities typical of macrophyte-dominated Dutch drainage ditches) were used in the experiment. Each microcosm was 1.1 m x 1.1 m x 1.0 m deep, with a sediment layer (sandy loam) of 0.10 m, a water column of 0.50 m, and a water volume of approximately 0.6 m3. The microcosms were maintained in a climate room with a daily photoperiod of 14 h and a constant temperature of approximately 20oC and acclimatised for two months, during which time the water was circulated through all twelve systems. Multiple applications of *lambda*-

cyhalothrin and the three other pesticides (fluazinam, asulam and metamitron) were made to the microcosms at four application rates, equivalent to spray drift entry of 0.2%, 0.5%, 2% and 5% of the label recommended use rates and at the recommended frequencies for each pesticide. In this case, the treatments were mixed into the water column in a 'toxicological' design. Lambda-cyhalothrin was applied five times at one week intervals, resulting in mean initial nominal treatment concentrations of 10, 24, 90 and 250 ng/L (based on measured dosage concentrations for each of the applications divided by the water volume of the microcosm). Since *lambda*-cyhalothrin was the most toxic to invertebrates of the four pestsicides applied in the study, it was considered that results could be attributed to the test concentrations of *lambda*-cyhalothrin with reasonable certainty.

In 2002, Arts et al. (29) also used a 'crop-based' treatment regime to investigate the effects of different spray drift rates from a typical crop protection treatment programme for potatoes in The Netherlands. The experiment was performed in twelve large ditch mesocosms which were 40 m long, 3.3 m wide at the water surface and 1.6 m wide at the sediment surface, and had a water depth of 0.5 m, a sediment (sandy loam) depth of 0.25 m, and a total volume of approximately 55 m3. In addition to *lambda*-cyhalothrin, the herbicides prosulfocarb and metribuzin, and the fungicides fluazinam and chlorothalonil were applied at rates equivalent to spray drift at 0.2, 1 and 5% of label-recommended rates, with the lowest treatment duplicated, the two higher treatment triplicated, and four untreated control ditches. Applications were made by spray boom and then gently mixed following treatment in a toxicological design. Lambda-cyhalothrin was applied twice in the study at five and nine weeks after the start of treatment resulting in initial nominal treatment concentrations of 4, 16 and 85 ng/L. Again since *lambda*-cyhalothrin was the most toxic compound to invertebrates applied in the study, it was considered that results could be attributed to the test concentrations of *lambda*-cyhalothrin with reasonable certainty.

Summary of experimental design for indoor, radiolabelled, aquatic model ecosystem fate study

In 1998, Hand et al. (30), studied the dissipation and degradation of 14C-radiolabelled *lambda*-cyhalothrin in an indoor aquatic microcosm. Within a glasshouse, a large glass tank (2 m x 1 m x 0.5 m high) was placed in a surrounding tank through which water cooled to 13oC was pumped. Sediment (sandy clay loam) and pond water were obtained from an established ponds at Jealott's Hill Research Station, Bracknell, UK. Sediment was added to the microcosm to a depth of 10 cm, and over this a water column of 30 cm was

added. The total volume of the test system was approximately 0.60 m3. Plant and animal communities were allowed to establish prior to treatment. Lambda-cyhalothrin was applied evenly across the water surface drop-wise with a pipette to provide an initial nominal treatment concentration of 2.3 µg/L. Subsequent to application, radiochemical residue measurements were made in samples of water, plants and sediment taken at intervals from the test system.

Overview of the Fate Profile of *Lambda*-cyhalothrin in Aquatic Model Ecosystem Studies

A summary of the fate profile of *lambda*-cyhalothrin in the various aquatic model ecosystem experiments is presented in Table 2. In all studies, the dissipation of *lambda*-cyhalothrin from the water column was rapid. Results between the different test systems were consistent, with water phase DT_{50} values of approximately a day or less. In most cases, residues in the water phase declined to detection limits within a period of four to five days after treatment, and there was no accumulation of residues in the water column resulting from multiple applications. This therefore indicates that under field conditions, water column exposure resulting from spray drift is likely to be of a short-pulsed nature, with residues declining rapidly from their peak values.

Measurements of *lambda*-cyhalothrin in sediments were made less often in the reported studies than those in the water column. Only a small proportion of the compound applied ever reached the sediment (see Table 2) in the studies where residues were measured. In two cases, none reached the sediment, and in the remainder where values were reported, only 5 to 22% was detected in the sediment. Because of the low concentrations, and also the short period after application for which residues were measured in most cases, sediment DT_{50} values could not be estimated reliably and were typically not reported.

The results of these studies with *lambda*-cyhalothrin are consistent with those observed for other pyrethroids in aquatic model ecosystem studies. A review of pyrethroid studies (3) concluded that of the 38 aquatic model ecosystems reviewed, there was also a rapid decline in water residues, with the DT_{50} in most cases being less than two days. Similar dissipation profiles were noted irrespective of the type or size of test system. Sediment data were also generally not sufficient to make calculations.

Table 2. Summary of dissipation and distribution of *lambda*-cyhalothrin in aquatic model ecosystem Studies

Study No. [a]	Test System	Water DT_{50} (days)	Max..% of Applied in Sediment	Whole System DT_{50} (days)
1	Pond mesocosm (450 m^3)	c. 1 (20-25% of applied after 2 d)	- [b]	-
2	Pond mesocosm (25 m^3)	< 1 (23% after 24 h)	22	-
3a	Phytoplankton dominated ditch enclosure (0.43 m^3)	< 1 (37% of applied after 24 h)	< limit of quantification	-
3b	Macrophyte dominated ditch enclosure (0.43 m^3)	< 1 (23% of applied after 24 h)	< limit of quantification	-
4	Macrophyte dominated ditch enclosure (0.43 m^3)	< 1 (3-4% after 24 h)	17	-
5	Indoor microcosm (0.60m^3)	0.7 – 1.2	-	-
6	Ditch mesocosm (55 m^3)	0.9 – 1.2	-	-
7	Indoor microcosm (0.60 m^3)	< 0.13	5	< 0.13

[a] Study numbers refer to the studies listed in Table 2.

[b] a dash indicates that data were not reported.

In the studies described above, plants seem to have played a significant role in the dissipation and degradation of *lambda*-cyhalothrin, perhaps explaining why a relatively small proportion of the applied residue reached the sediment. Another study confirms these fundings. Wendt-Rasch (32) observed 50% dissipation times for *lambda*-cyhalothrin of 0.87 and 2.4 days in Elodea (submerged macrophyte) dominated and Lemna¬ (floating macrophyte) dominated experimental ponds of similar dimensions (coated concrete walls). This observation again indicates that the presence of submerged macrophytes is important in the dissipation of *lambda*-cyhalothrin from water. The method of application of pyrethroid solution to the water column may have had an influence on the dissipation profile of *lambda*-cyhalothrin observed in the studies reviewed here. Due to its highly hydrophobic nature, the compound will adsorb readily onto any available surface such as plants. A different picture may have emerged if the compound was applied as runoff, i.e. already bound to soil particles. In this case, higher sediment residues might be expected unless there was substantial desorption.

Comparison of effects of *lambda*-cyhalothrin in aquatic model ecosystem experiments

Characteristics of observed effects

The patterns of effects that emerge in the different test systems are reasonably consistent, considering the differences in size, location and experimental design of the studies. In all studies, malacostracan crustaceans and certain insect species (Diptera and Ephemeroptera) were among the most sensitive, reflecting the distribution of sensitivities that were seen in the laboratory. Recovery of affected insect species tended to be reasonably rapid, most probably due to reseeding of aquatic model ecosystems by flying adult stages. For the Malacostraca, at higher concentrations where effects were substantial, recovery tended to be slow or did not occur. However, considering that these organisms would usually recover by immigration from unaffected sites (none present in these enclosed test systems), this result is not too surprising, and has also been observed with a range of insecticides where crustaceans are sensitive (33). Effects on zooplankton species tended to occur at higher concentrations than those that affected macroinvertebrates, and effects on zooplankton tended to be followed by rapid recovery, due to the presence of resting stages and the short life-cycle of these organisms. As would be expected, there were generally no direct effects on aquatic plants in these studies, although occasionally, indirect effects (short-term blooms of algae) could be observed due to decreases in grazing pressure from the direct effects of the chemical.

Effects thresholds

In order to compare the ecological effects thresholds observed in these studies, an effect classification system was used (34). The measured endpoints in the studies were assigned to one of eight groups. These groups comprised one functional category (community metabolism) and seven structural categories (microcrustaceans; macrocrustaceans; insects; fish; other zooplankters; other macroinvertebrates; algae and macrophytes). The functional category community metabolism refers to dynamics of dissolved oxygen (DO), pH, inorganic carbon and nutrients in the water column or decomposition as studied by a litter bag technique. The structural categories refer to changes in species composition or population densities and biomass.

To facilitate comparisons, the most sensitive endpoint within each category was selected for each exposure concentration studied, resulting in a more or less worst-case evaluation of the studies. The classification of the categories above was mainly based on univariate

analysis of the measurement endpoints. The studies also provided a multivariate analysis of the data which allows an evaluation at the community level. The responses observed for the most sensitive endpoint within each category and at each exposure concentration were assigned to the following effect classes (Table 3) based on these criteria:

1. No effects demonstrated: No consistent adverse effects are observed as a result of the treatment. Any observed differences between treated test systems and controls do not show a clear causality.
2. Slight effects: Confined to responses of sensitive endpoints (e.g., partial reduction in abundance of sensitive arthropods). Effects observed on individual samplings only and/or of a short duration directly after treatment.
3. Clear short-term effects, lasting < 8 weeks: Convincing direct and/or indirect effects on measurement endpoints. Recovery, however, takes place within eight weeks after the last treatment. Transient effects reported on both sensitive and less sensitive endpoints. Effects observed on a sequence of samplings.
4. Clear effects, recovery not studied: Clear effects are demonstrated (e.g., severe reductions of sensitive taxa over a sequence of samplings), but the duration of the study is too short to demonstrate complete recovery within eight weeks after the last treatment.
5. Clear long-term effects, lasting > 8 weeks: Convincing effects on measurement endpoints that last longer than 8 weeks after the last application.

It is apparent from the aquatic model ecosystem experiments performed with *lambda*-cyhalothrin that regardless of type of test system, initial nominal treatment concentrations specifically in the range of no to slight and transient effects (Effect Class 1 - 2) are consistent (Table III). This consistency in the findings indicates that the threshold level for 'no to slight effects' can be used with confidence as an indicator of safe concentrations in the field under the exposure conditions investigated in the studies (at least, when studies contain representatives of sensitive taxonomic groups and when exposure regimes are more or less similar). The range of concentrations at which there were clear short-term effects with recovery were more variable than the no to slight effects category, but were still reasonably consistent, with effects concentrations ranging from 16 to 50 ng/L, around a factor of 3. Note however, that in studies 1 and 2, class 5 effects were observed at nominal concentrations of 27 and 17 ng/L, respectively. However, as discussed above, the effect thresholds determined for studies 1 and 2 should be treated with some caution, since the nominal treatment concentrations in these studies may have underestimated the actual exposure concentrations.

Table 3. Effects Threshold Concentrations (ng/L) from Various Aquatic Model Ecosystem Studies with *Lambda*-cyhalothrin. Results are Expressed as Initial Nominal Treatment Concentrations Applied to the Test System.

Study No.[a]	Test system	*Effect Class*				
		1	2	3	4	5
1	Pond mesocosm (450 m^3) [b]	2.7				27
2	Pond mesocosm (25 m^3)					17
3a	Phytoplankton dominated ditch enclosure (0.43 m^3)		10		25	
3b	Macrophyte dominated ditch enclosure (0.43 m^3)		10	50		
4	Macrophyte dominated ditch enclosure (0.43 m^3) [c]		10	25	50	
5	Indoor microcosm (0.60m^3) [c]	4.0		16		85
6	Ditch mesocosm (55 m^3)		10		25	

[a] Study numbers refer to the studies listed in Table I.

[b] Experiment was characterized by both spray drift and run-off applications. As exposure concentrations, the median between nominal spray drift and run-off applications was used.

[c] Several pesticides applied.

Considering that the variability in effect class 1-2 responses observed in aquatic model ecosystem experiments is comparable to that one might observe for between study variation in the laboratory, these data are remarkably consistent. Some differences in recovery would to be expected in these types of studies, depending on the type of organism affected and its life history. The margin between the effect classes 2 and 3 (i.e. giving some indication of the steepness of the dose response curve between slight and clear effects) is around a factor 3 to 5. As discussed above, the effect thresholds determined for studies 1 and 2 should be treated with some caution, since the initial nominal treatment concentrations in these studies may have underestimated the actual exposure concentrations.

The results observed in these studies are consistent with those observed for other pyrethroids in aquatic model ecosystem stuides. A 2005 review by Van Wijngaarden et al. (33) on the effect thresholds for a range of insecticides including pyrethroids

demonstrated a similar pattern of effects to those shown here, with microcrustaceans and insects among the more sensitive taxa.

Discussion

From the eight aquatic model ecosystem studies reviewed here, a consistent pattern of fate and effect of *lambda*-cyhalothrin emerges. As typifies synthetic pyrethroids, dissipation from the water phase was rapid, with DT_{50} values typically of around one day or less. The results were consistent irrespective of the size, location and type of system, trophic status, or season of application. Most of the studies were not designed to distinguish between dissipation and degradation, as only the concentration of the parent molecule was tracked through time in the environmental compartments. However, findings from the microcosm study of Hand et al. (30), with application of 14C-radiolabelled *lambda*-cyhalothrin demonstrated that degradation via ester hydrolysis was occurring in the test system. It was proposed that one reason for this rapid degradation was the influence of plants, providing a substrate for partitioning of *lambda*-cyhalothrin from the water phase and possibly sites where degradation of the compound is facilitated. The precise mechanism by which this occurs has yet to be determined, but the study does emphasize the importance of considering the influence of aquatic plants on pesticide fate – an environmental component that is not usually considered in laboratory fate experiments or modeling. Aquatic plants have also been demonstrated to be an important factor in the fate of *lambda*-cyhalothrin in agricultural drainage ditches (15)

Generally speaking, only a small percentage of the applied *lambda*-cyhalothrin was detected in sediments, probably due to a combination of adsorption to plants and degradation in the water column and the method of application of the test substance (as solution into the water column). Since sediment residue levels were low, and sampling was only carried out for a short time, it was not possible to calculate sediment half-lives in any of the studies. Degradation rates of pyrethroids in laboratory aquatic systems have been reported to range from 7 to 80 days (16). Future studies to better define the fate of pyrethroids in sediment under field conditions would be a useful addition to the current database. Catchment monitoring studies indicate that pyrethroid residues can be found in sediments (14, 35), but in mixed landuse catchments it can be difficult to establish the relative importance of the various potential sources of these residues.

The effects observed in field studies with *lambda*-cyhalothrin are consistent with those observed in studies with other pyrethroids (3, 4, 33), with population responses being driven by effects on macrocrustacea and certain insects, followed by zooplanktonic

microcrustacea. Considering the range of test systems, the range of locations, different trophic status of the system, differences in season of application, and differences in numbers of applications, the effects thresholds (no to slight effects) observed in the studies were remarkably consistent. This gives considerable confidence in the potential to extrapolate the no to slight effects observed in one aquatic model ecosystem study to a different situation, at least in this case where effects tend to be of an 'acute' nature, and the dissipation and degradation of the compound is rapid. The effects threshold values for clear effects with recovery were more variable than the no to slight effects, but still reasonably consistent, considering the different ecosystems that were studied.

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Chapter 6: The influence of simulated immigration and chemical persistence on recovery of macroinvertebrates from cypermethrin and 3,4-dichloroaniline exposure in aquatic microcosms[5]

Summary

Chemical dissipation and organism immigration are considered important factors that influence recovery potential from perturbation of aquatic macroinvertebrates. We experimentally investigated the effect of simulated immigration on recovery of aquatic macro-invertebrates exposed in outdoor microcosms to ecotoxicologically similar concentrations of the rapidly-dissipating pyrethroid insecticide cypermethrin (70 ng/L) or the more persistent herbicide intermediate and degradate 3,4-dichloroaniline (10 mg/L). Microcosms were covered with light-permeable mesh to prevent recolonisation. Immigration was simulated by the regular addition of organisms after treatment. Microcosms exposed to 3,4-dichloroaniline treatment suffered substantial loss of taxon-richness and by ten months after treatment had only recovered where invertebrates had been added. Those treated with cypermethrin underwent an initial decline in certain crustacean and insect populations. These populations showed some signs of recovery over a period of five months through internal processes alone. However, rate of recovery was further enhanced where immigration was simulated, and in this case recovery had occurred around 100 days after treatment. Although not the only factors involved, simulated immigration and chemical fate clearly influence the ability of communities to recover from chemical exposure. Consideration of immigration processes and development of models will help to increase the realism of risk assessments.

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Introduction

The potential long-term, ecological effects of xenobiotic chemicals on freshwater ecosystems depend not only on inherent toxicity (i.e. the potential to cause effects) but, ultimately, on the potential of the impacted community to recover from these effects. Recovery can be achieved either by internal (autochthonous) processes, such as growth and reproduction of surviving individuals or hatching of resting stages, and by external (allochthonus) processes like immigration of individuals from adjacent areas or other habitats. In aquatic ecotoxicology most interest in assemblage recovery has focused on autochthonous processes, and particularly on life history attributes, such as fecundity and reproductive period, which can influence internal recovery success (1, 2). Only a few studies have experimentally investigated the role that allochthonous processes might play in facilitating and directing community recovery (3, 4, 5, 6). The environmental fate properties of a chemical can also play a significant role in the rate at which populations recover from a chemical disturbance, with recovery potentially taking longer for chemicals which persist at effective concentrations for greater periods. Overall, recovery is a complex issue which should involve considerations of toxicological, ecological, and chemical dynamics (7).

In this study we investigated the effect of immigration on recovery of aquatic macro-invertebrate communities using outdoor pond microcosms. This paper describes in detail the ecological responses observed, while modelling approaches with these data are described elsewhere (7). Patterns of internal population recovery and immigration-enhanced recovery were evaluated in response to treatments of the pyrethroid insecticide cypermethrin and 3,4-dichloroaniline (DCA), an intermediate and degradate of several herbicides (8). These two compounds were selected for the study because of their differing fate and effects in aquatic systems, and because their aquatic ecotoxicology is well-studied. As would be expected for an insecticide, cypermethrin is highly toxic to freshwater arthropods, but generally of significantly lower toxicity to other macroinvertebrate phylla. The 48 h median lethal concentration (LC_{50}) of cypermethrin in the laboratory to amphipods, isopods, Diptera, Hemiptera and Coleoptera is generally in the range 1 to 100 ng/L, whereas for Mollusca, Planariidae and Oligochaeta, the acute toxicity is generally >100 µg/L (1, 9). Cypermethrin is lost very rapidly from pond water (with a half-life of one day or less) because it has a low water solubility and it sorbs rapidly to sediment and organic matter (10). The acute toxicity of 3,4-DCA is substantially less in

the laboratory to arthropods than that of cypermethrin, with values in the range 0.1-100 mg/L (11, 12) but toxicity is less selective, with acute effects on a range of invertebrate phylla (e.g. Mollusca, Planariidae, Oligochaeta) occuring at similar concentrations to those for arthropods. In the environment, the principal route of degradation is via photolysis, and consequently rates of degradation are likely to be variable but generally in the range of several days to several weeks (8).

The overall aim of the study was to compare the rates of recovery of aquatic macro-invertebrates following treatment with chemicals of differing toxicity and persistence, and then to compare recovery rates where only autochthonous processes occurred with treatments where organisms were added to simulate immigration. Exposure concentrations were based on acute laboratory toxicity data and were selected in the anticipation of causing similarly substantial effects on macro-invertebrate communities, and were not intended to be representative of environmental exposure concentrations.

Methods

Microcosm test system and treatment

The study site was at Syngenta Jealott's Hill International Research Centre, Bracknell, Berkshire, UK. The microcosms were constructed from cylindrical, fibre-glass tanks of 1.25 m diameter and 1.25 m height, each with an integral base. To regulate microcosm temperatures the tanks were maintained in 5 m x 5 m x 1.2 m outdoor concrete ponds filled with water to a depth of 1 m. The microcosms were established with sediment from a mature pond on site (added sediment depth 10 cm) and microcosms were filled to a depth of 1 m using a combination of pond water and potable mains water. Aquatic macrophytes were added to each microcosm which were either collected from nearby ponds or grew from propogules present in the added sediment. The dominant floating-leaved plant species was *Potamogeton natans* L.. The most abundant submerged species were *Ceratophyllum demersum* L. and *Myriophyllum spicatum* L.

The concentrations for treatment were selected to have a marked effect on invertebrate taxa, based on knowledge of laboratory acute toxicity data. Based on the available acute laboratory toxicity data (9, 11) species sensitivity distributions (SSD) were constructed for cypermethrin and 3,4-DCA (Figure 1 a and b respectively). Plots were generated by ranking toxicity values, calculating cumulative percent rank (dividing the rank position by the total number of observations plus 1), and plotting against log base 10 transformed effect concentration. For cypermethrin, certain of the taxa tested (e.g. molluscs and

oligochaetes) were not affected at the highest treatment concentrations. These data were included in the ranking but were not plotted (hence the curtailment at the upper end of the distribution).

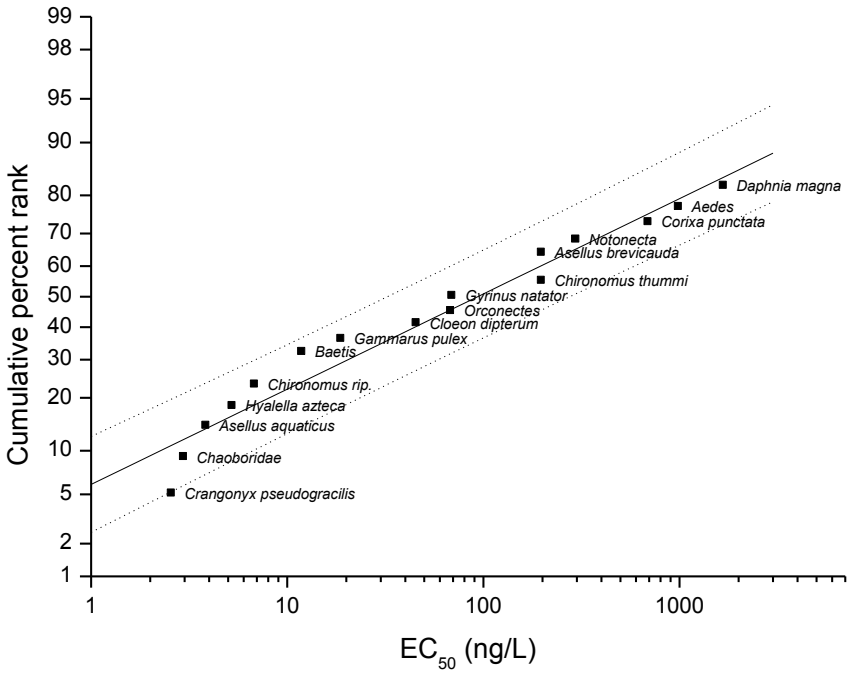


Figure 1a. Probabilistic distributions of acute laboratory toxicity data for cypermethrin with aquatic invertebrates. Fitted lines are apparent linear fits (solid line) as plotted by Microcal Origin v 6.0 and their respective 95% confidence limits (broken line).

The selected treatment concentrations broadly approximated the median (50th percentile) acute toxicity value from the SSD, and were 70 ng/L and 10 mg/L for cypermethrin and 3,4-DCA respectively. Thus, the treatment concentrations would be expected to be broadly equivalent in terms of their potential for effects on invertebrates (based on laboratory data), and would also be expected to potentially impact a significant number of the invertebrate taxa present in the microcosms (assuming similar responses to those used to derive the SSDs, these concentrations would be expected to result in approximately 50% effect for 50% of the taxa present). The exposure concentrations were

not intended to be representative of environmental exposure concentrations. The study site contained eighteen microcosms, of which ten were used for the current experiment. Selection of the ten study microcosms was made on the basis of their macro-invertebrate assemblage as established by sampling prior to treatment (data not shown). Two blocks of five microcosms were selected, and two replicates were assigned to each treatment. Treatments were assigned at random within these blocks to treatments of control (no chemical or organism addition), 70 ng/L cypermethrin with immigration, 70 ng/L cypermethrin without immigration, 10 mg/L 3,4-DCA with immigration, or 10 mg/L 3,4-DCA without immigration. Since the aim of the study was to assess the rate of recovery after disturbance, there was no untreated control with additions.

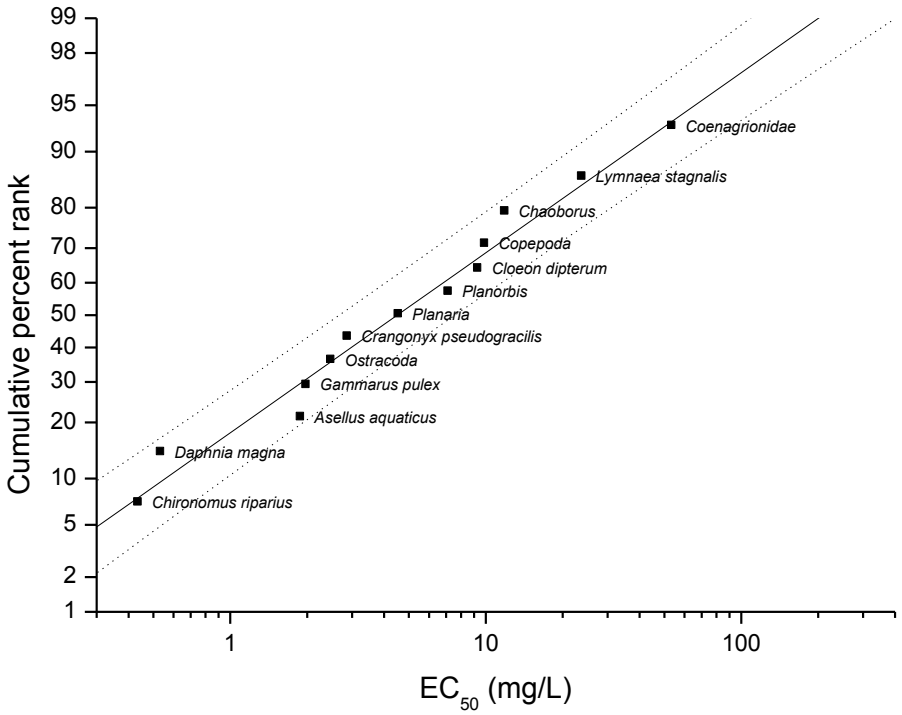


Figure 1b. Probabilistic distributions of acute laboratory toxicity data for 3,4-dichloroaniline with aquatic invertebrates. Fitted lines are apparent linear fits (solid line) as plotted by Microcal Origin v 6.0 and their respective 95% confidence limits (broken line).

The application solutions (including 100 ml methanol) were poured over the surface of the microcosm on 25 June 1996, and the microcosm was gently stirred to mix in the chemical. An equal volume of the solvent was added to each of the control microcosms. After treatment, each microcosm was covered with a removable net hood (0.45 mm x 0.78 mm mesh) which allowed water and 90% light penetration (as indicated by the manufacturer) but prevented entry of macro-invertebrates. Apart from the very short time in which samples were collected (c. two minutes per sample), the microcosms remained covered for the remainder of the experiment.

Chemical and biological sampling and analysis.

To determine exposure concentrations of cypermethrin and 3,4-DCA in the microcosms, depth-integrated water column samples were taken for analysis using a water column sampler. Four columns of approximately eight litres were collected from each mesocosm and combined. These were then subsampled as follows:

- 2 x 500 ml into 1 litre volumetric flasks (controls and cypermethrin treatments);
- 2 x 10 ml into glass vials (controls and 3,4-DCA treatments).

The remaining water was returned to the microcosm. Control and cypermethrin samples were extracted into 5 ml of hexane by shaking for approximately 1 minute. The hexane layer was then removed, evaporated to dryness and re-dissolved in 1 ml of hexane for analysis by gas chromatography (electron capture detector, RTx column: 5- 30 m x 0.32 mm internal diameter x 0.5 um film thickness). The limit of determination for cypermethrin was 10 ng/L. For 3,4-DCA samples, a 1 ml subsample was mixed 50:50 with acetonitrile and then analysed by high peformance liquid chromatography (Spherisorb ODS2, column length 15 cm, internal diameter 4.6 mm). The limit of determination for 3,4-DCA was 0.05 mg/L. Concentrations of cypermethrin at 2 days after treatment were close to the limit of determination and sampling ceased at this point. Samples for 3,4-DCA analysis continued at 8, 31, 56, 84, 114 and 150 days after treatment until the limit of determination was reached. Controls were analysed for cypermethrin on day 0 and day 2, and for 3,4-DCA on day 8 and confirmed that there was no cross-contamination.

Surveys of the macroinvertebrate fauna in the microcosms were undertaken on the day before and the day after addition of the chemicals (day -1 and day 1 respectively). Sampling continued on days 3, 7 and 14 after treatment and was then undertaken fortnightly until 16 weeks after treatment. Invertebrate sampling of the cypermethrin

microcosms ceased at this point. Sampling of 3,4-DCA and control microcosms continued monthly until 36 weeks after treatment.

Macroinvertebrates were collected from the microcosms using a small D-frame hand net with maximum dimensions of 15 cm x 17 cm and a net mesh of 1 mm diameter. On each sampling occasion, six sub-samples were taken from each microcosm (two from the top, middle and bottom respectively). Each sub-sample was collected by sweeping the net through the microcosm and scraping along the microcosm sides. The bottom sub-sample skimmed through the top of the sediment. Each sub-sample took approximately 15 seconds to collect. Each microcosm was therefore sampled for about 90 seconds on each sampling occasion. The total sample from each microcosm was estimated to sample approximately 40% of the water column and sides of the microcosm. Sampling equipment was washed between experimental treatments to avoid transfer of organisms between microcosms.

Samples were live-sorted in large white trays at the study site. To avoid progressive depletion of the microcosms, invertebrates were identified to the lowest taxonomic level possible in the field and returned to their original microcosm within 30 mins of sampling. In practice, all macroinvertebrate taxa, with the exception of Diptera (including Chaoboridae), Hydracarina, and Oligochaeta, were identified to genus or species.

From day 15 after treatment, selected invertebrates (collected from untreated ponds on the same study site, so assumed to be of similar composition as those in the microcoms) were added to half of the treated microcosms (i.e. two 3,4-DCA and two cypermethrin treated microcosms) to simulate immigration. Invertebrate taxa added to the treated microcosms were selected in order: (i) to represent the dominant taxa present before xenobiotic addition (ii) to include a range of invertebrate families with differing mobilities and life cycle strategies, and (iii) to include taxa which early results showed to have had a clear response to chemical addition. Invertebrate additions were made immediately after each invertebrate survey had been undertaken, and the periodicity followed the invertebrate sampling programme described above.

The number of invertebrates to add to each microcosm (Table 1) was calculated as follows:

1. The population size of each taxon was calculated on the assumption that 40% of invertebrates were netted from each microcosm (based on prior exhaustive sampling), using the combined six microcosm sub-samples.

2. The number of organisms added was determined based on the total number of organisms that would be needed to be added over an eight week period (i.e. the total pre-treatment population estimate divided by five) to replenish the pre-treatment populations (ten weeks after treatment), although additions would be continued until recovery (similarity to control conditions) was observed or the end of the study.

3. The 'original' pre-treatment populations were calculated from the overall mean populations sampled from the pre-treatment and control replicates on the day before treatment (i.e. the overall average conditions in the controls with those the pre-treatment microcosms for each chemical treatment).

Slightly different numbers of organisms were added to the 3,4-DCA and cypermethrin treatments because of differences in abundances prior to treatment. However, since one aim was to assess the time taken to return to pre-treatment conditions, this approach was considered appropriate.

Table 1: Number of organisms that were added at each addition interval to treated microcosms to simulate immigration.

	Cypermethrin	DCA*
Crangonyx pseudogracilis	33	20
Asellus aquaticus	36	32
Chaoborus sp.	5	7
Notonecta sp. (nymphs and adults)	1	2
Polycelis sp.	none†	10

* On 15th October 1996, cypermethrin additions ceased. DCA additions continued monthly at a similar rate (i.e. the additions were doubled to compensate for the greater period between additions).
† *Polycelis* sp. were not added to M06 and M09 because the species showed no decline following cypermethrin addition.

Statistical analysis

All abundance data were log base 10 (abundance + 1) transformed prior to analysis. Macroinvertebrate community responses were analysed using principle response curves (PRC) (13, 14) Population-level data for certain taxa were also analysed using univariate analysis methods (a separate analysis was performed for each sampling occasion). A two-tailed Dunnett's t-test was carried out on the data at each of the sampling occasions using the estimate of error from the analysis of variance. In these tests, comparisons were made between each treatment group and the control. All tests were carried out using a five percent type I error rate. Given the low number of replicates (two per treatment) and the typical variability of such test systems, there were often limited numbers of statistically significant differences in the univariate analyses. In such cases, patterns of abundance were also reviewed to assess any indications of treatment-related trends.

Results

Analysis of water samples taken immediately after chemical application showed that cypermethrin concentrations in the microcosms on day 0 ranged from 41 to 58 ng/L (58% to 83% of nominal). That these levels were somewhat lower than nominal probably reflects the very rapid loss of the chemical because of its lipophilic, highly adsorptive nature, and also the technical difficulties of analysis at low concentrations for a compound of such low water solubility. Cypermethrin water concentrations in the microcosms on day 2 were close to the limit of determination of 10 ng/L, indicating a median dissipation time (DT_{50}) from water of approximately one day or less. The 3,4-DCA water concentrations ranged from 10.8 to 11.6 mg/L (108% to 116% of nominal) immediately after application. Subsequent samples showed that 3,4-DCA levels fell progressively until day 150 by which time concentrations had fallen below the limit of determination (0.05 mg/L). The water DT_{50} of 3,4-DCA was approximately 30 days.

The mean total numbers of macro-invertebrate taxa sampled from the control and treated microcosms are shown in Figure 2. Variability between replicate was quite larget, so error bars are omitted from the graphs for clarity. As mentioned above, the variability between replicates meant the experiemental power was low. Prior to treatment, mean total taxa were similar in all treatments. Declines in taxon number following 3,4-DCA treatment were pronounced, and taxon numbers continued to be suppressed in the treatment without immigration for the remainder of the experiment. Where organism additions were made to the 3,4-DCA treatment, mean number of taxa gradually increased, and by the end of the experiment were similar to the controls (although lower than at the start of

the experiment, probably because of seasonal declines). Application of cypermethrin resulted in only a small decrease in total taxa, and there was little apparent difference between the control and treatments throughout the course of the experiment.

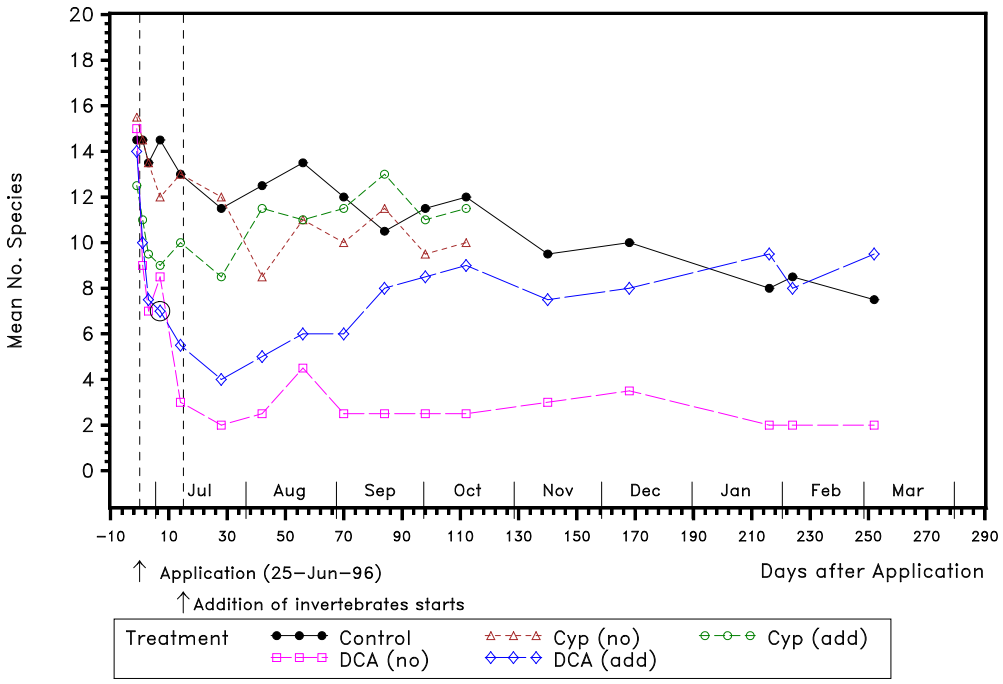


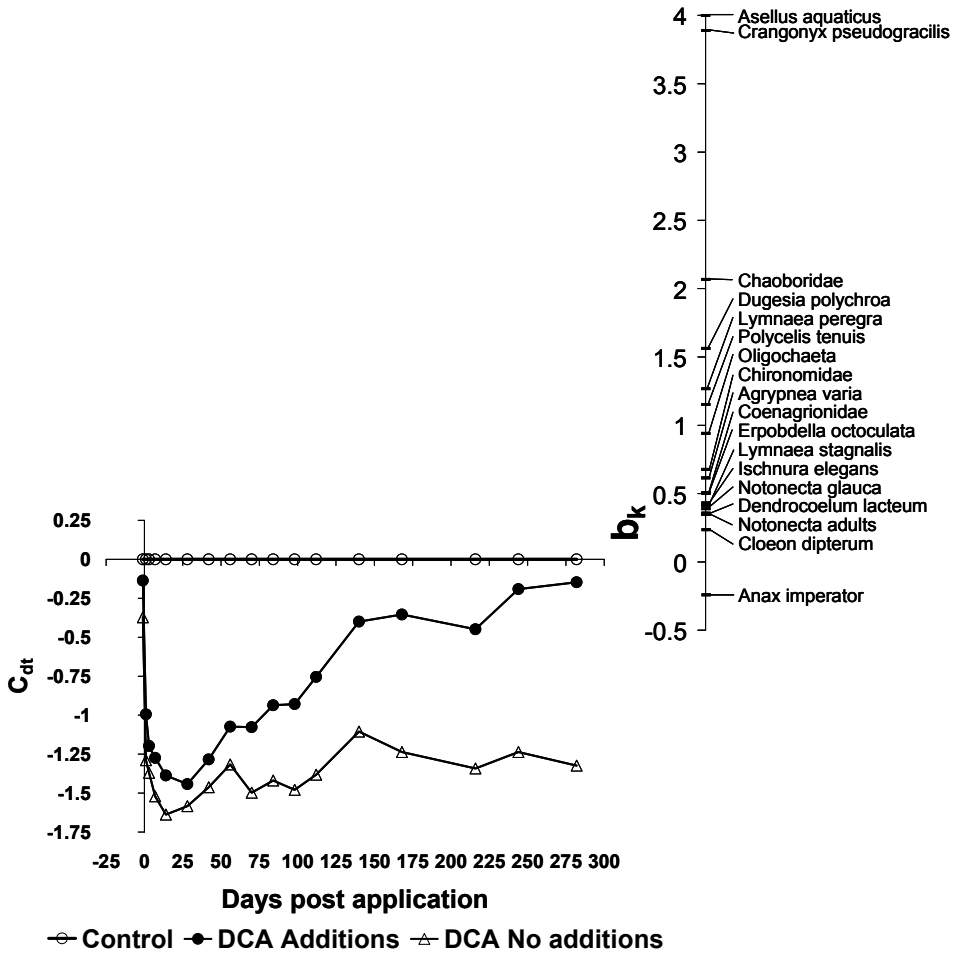
Figure 2. **Total mean number of taxa sampled in control microcosms and microcosms treated with cypermethrin (Cyp) or 3,4-DCA (DCA) with addition of organisms to simulate immigration (add) or without addition (no). Circled data points are significantly different to controls.**

Figures 3 a and b show PRCs that describe how the macro-invertebrate community structure responded to 3,4-DCA and cypermethrin treatments respectively. Control data are plotted as zero and the y-axis shows the relative dissimilarity between the treatments and the control through time. For the DCA treatments (Figure 3 a), 13% of the total variation in taxon composition could be attributed to differences between sampling dates, while 39% of the differences in taxon composition constituted of differences between the replicates. The remainder 48% can be attributed to differences in taxon composition between the different treatments; 61% of the latter is displayed in the diagram. For the

cypermethrin treatments (Figure 3 b), 14% percent of the total variation in taxon composition could be attributed to differences between sampling dates, while 54% of the differences in taxon composition constituted of differences between the replicates. The remainder 32% can be attributed to differences in taxon composition between the different treatments; 53% of the latter is displayed in the diagram. For both figures, the taxon weights shown in the right part of the diagram represent the affinity of each taxon with the response shown in the diagram i.e. a high positive weight shows a species related decline with treatment and vice versa (for clarity only species with a weight > 0.25 or < -0.25 are shown).

For the first week after application, the PRC indicated that after chemical application there was a marked divergence from the control for both of the 3,4-DCA treatments (Figure 3 a), with the pattern of response being very similar between the two treatments, even after the start of addition of invertebrates to the immigration treatments. Gradually as the experiment progressed, the 3,4-DCA treatment with organism additions appeared to return towards control levels more rapidly than the treatments where there were no additions, although recovery was generally slow, and did not begin in earnest until concentrations were around or below the limit of determination. However, by the last two sampling occasions, in this treatment macro-invertebrate communities were similar to the controls. The macro-invertebrate community in the 3,4-DCA treatment without organism additions remained markedly different from the control throughout the course of the experiment, and showed no signs of recovery even after the chemical determination limit had been reached. There is a suggestion that the recovery process slowed and even perhaps showed a slight reverse in both 3,4-DCA treatments, during the period between approximately day 140 and day 216 (November 12th to January 27th) coinciding with the harshest winter months of 1996/97. During most of January, in particular, the microcosms were covered with ice. For cypermethrin treatments (Figure 3 b), community responses were similar in both treatments until the start of organism additions, at which point the immigration treatment appeared to recover more quickly than that without additions. The community in the treatment with additions had recovered by around 100 days after treatment. The treatment without additions showed limited recovery by the time that sampling ceased.

(a)


Asellus aquaticus
Crangonyx pseudogracilis
Chaoboridae
Dugesia polychroa
Lymnaea peregra
Polycelis tenuis
Oligochaeta
Chironomidae
Agrypnea varia
Coenagrionidae
Erpobdella octoculata
Lymnaea stagnalis
Ischnura elegans
Notonecta glauca
Dendrocoelum lacteum
Notonecta adults
Cloeon dipterum
Anax imperator
b_k
C_{dt}
Days post application
Control
DCA Additions
DCA No additions

(b)

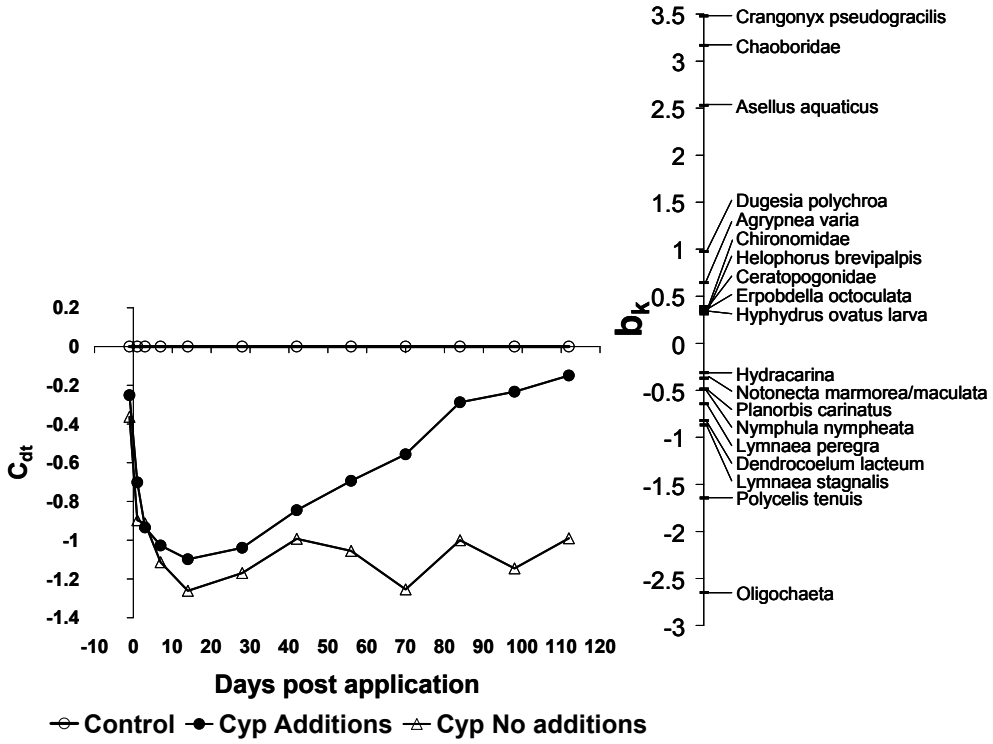


Figure 3: Principal response curves for macro-invertebrate communities in microcosms treated with (a) 3,4-DCA (DCA) or (b) cypermethrin (Cyp) with addition of organisms to simulate immigration (additions) or without addition (no additions).

Taxon weights from the PRC indicated that abundances of slaters *Asellus aquaticus* L., shrimps *Crangonyx pseudogracilis* Bousfeld and phantom midges Chaoboridae had the greatest influence on the community structure. Population level data for these three taxa are presented in Figures 4, 5 and 6 for all treatments along with those for flatworm *Polycelis tenuis* Ijima (Figure 7) – the other taxon that were added to the microcosms. Abundances of the water boatman Notonecta sp. proved too low to provide meaningful data.

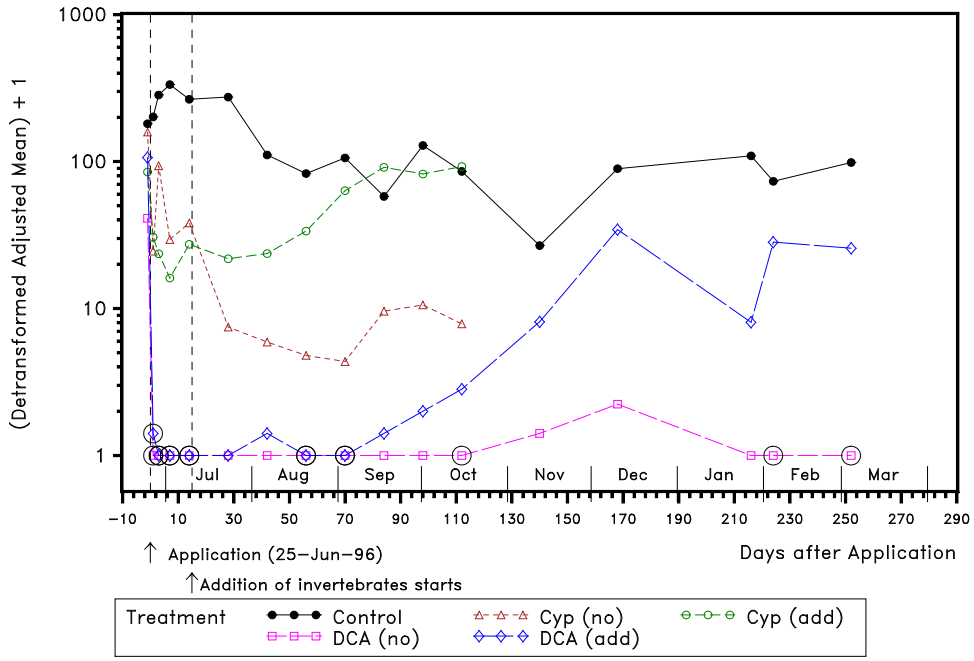


Figure 4: Abundance of *Asellus aquaticus* in control microcosms and microcosms treated with cypermethrin (Cyp) or 3,4-DCA (DCA) with addition of organisms to simulate immigration (add) or without addition (no). Circled data points are significantly different to controls.

The abundance of *A. aquaticus* (Figure 4) followed the pattern for the overall community effects, with abundances decreasing dramatically and significantly in the 3,4-DCA treatments, and only recovering slowly in the 3,4-DCA with immigration treatment. In treatments without organism additions, no recovery was observed by the end of the experiment. Although cypermethrin-treated populations of *Asellus* were not significantly different, there did appear to be a difference between the microcosms with and without simulated immigration. Abundances in the treatment with additions had reached pre-treatment and control abundance levels of around 100 individuals per microcosm around ten weeks after treatment, whereas treatments without additions remained at an abundance level of around 10 individuals per microcosm until the end of the sampling period.

Abundances of *C. pseudogracilis* (Figure 5) followed a very similar pattern to that of *Asellus*. However, populations both the cypermethrin and 3,4-DCA treatments were significantly reduced after treatment to very similar levels of abundance in all four

treatments. There was clear evidence of more rapid recovery in cypermethrin treated systems, and those where organisms were added also appeared to recover more rapidly. Recovery in the 3,4-DCA systems took longer, but again there was clear evidence of more rapid recovery in treatments where organisms were added. In the 3,4-DCA without organism additions, there was no sign of recovery at the end of the experiment.

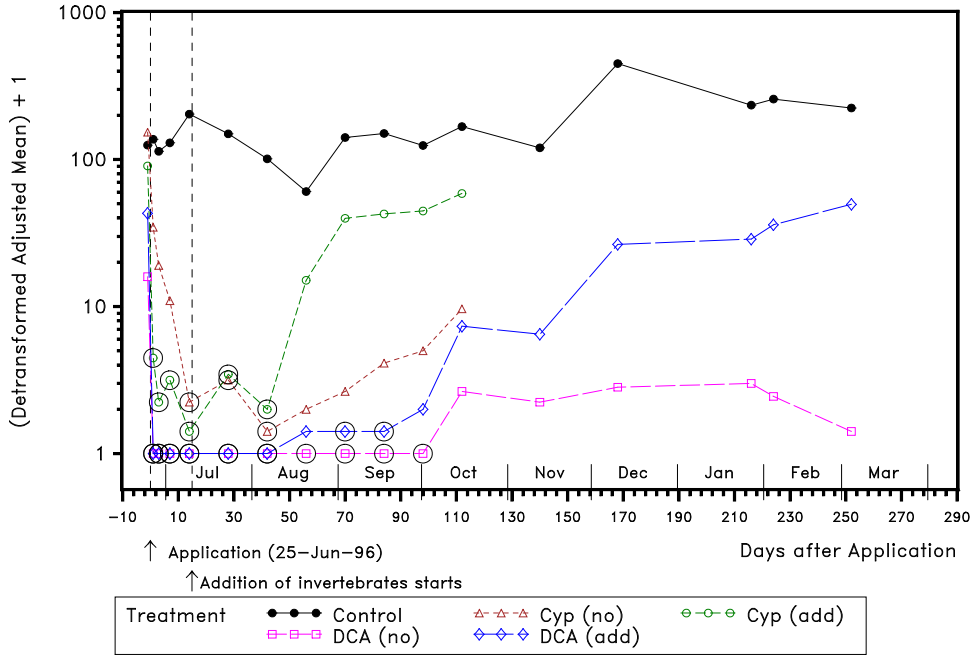


Figure 5: Abundance of *Crangonyx pseudogracilis* in control microcosms and microcosms treated with cypermethrin (Cyp) or 3,4-DCA (DCA) with addition of organisms to simulate immigration (add) or without addition (no). Circled data points are significantly different to controls.

For the Chaoboridae (Figure 6), there were clear effects of chemical treatment in all test systems, with abundances in all treatments significantly reduced to similarly low levels following treatment. There was a pronounced difference in the recovery of abundance, with only those systems to which organisms were added recovering from the effects of treatment. For both 3,4-DCA and cypermethrin treatments with no additions, there was no evidence of recovery by the end of the experiment. There is some suggestion in the data that the 3,4-DCA treatment took a little longer to recover than the cypermethrin treatment, but there were no statistically significant differences.

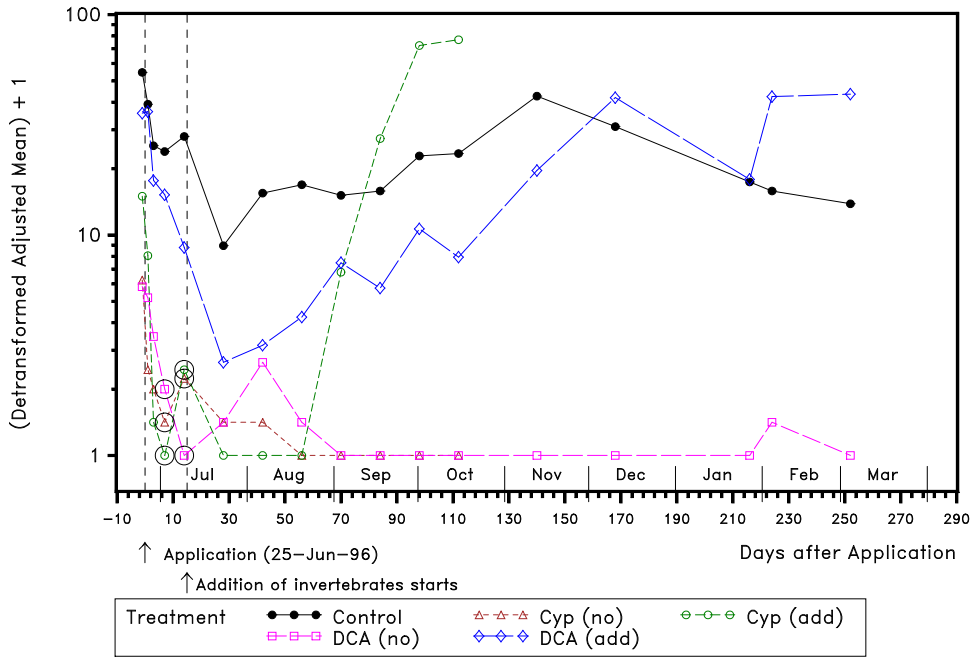


Figure 6: Abundance of Chaoboridae in control microcosms and microcosms treated with cypermethrin (Cyp) or 3,4-DCA (DCA) with addition of organisms to simulate immigration (add) or without addition (no). Circled data points are significantly different to controls.

Populations of *P. tenuis* (Figure 7) responded quite differently to cypermethrin and 3,4-DCA treatments. There appeared to be a treatment-related increase in abundance in the cypermethrin treatments though this returned to similar levels to the control by the end of the sampling. In the 3,4-DCA treatments, there were substantial effects on *Polycelis* after treatment, with no recovery in 3,4-DCA treatments without additions, but an indication of recovery in treatments with additions, although the overall abundances in control and treatment were q uite low making it more difficult to draw conclusions.

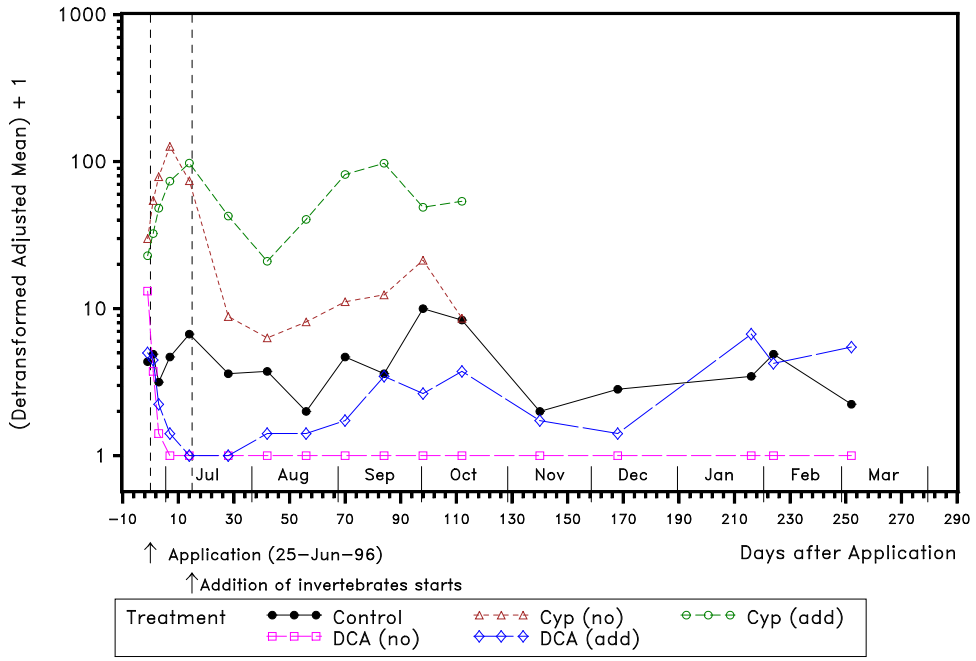


Figure 7: **Abundance of *Polycelis tenuis* in control microcosms and microcosms treated with cypermethrin (Cyp) or 3,4-DCA (DCA) with addition of organisms to simulate immigration (add) or without addition (no). Circled data points are significantly different to controls.**

Discussion and Conclusions

Effects and recovery

The effects of 10 mg/L 3,4-DCA on the invertebrate assemblages were substantial, and by two weeks after application had resulted in significant effects on macro-invertebrate taxon diversity and abundance. Considering that the applied concentration was approximately equivalent to the median acute laboratory toxicity values, the effects observed were perhaps somewhat greater than would have been predicted. This may have been because of the much longer duration of exposure compared to standard laboratory exposures of 48 to 96 h, coupled with slow dissipation. Indeed, the slower dissipation of 3,4-DCA clearly had an influence on the recovery profile of the test systems. In treated systems where no organisms were added recovery was slow or did not occur at

the community or population level, most likely because ecotoxicologically significant concentrations were present for several months after treatment. By the time sufficient dissipation had occurred, seasonal factors such as low temperatures and food supply, meant that further recovery was probably constrained by low numbers of survivors and low reproductive rates. Even when organisms were added to the 3,4-DCA treatment, recovery was relatively slow, again probably hampered by residues of the chemical causing effects on the organisms that were added. However, the difference in recovery rates between the two treatments did show that simulated immigration had a substantial influence on recovery potential of macro-invertebrates at both the population and community level.

For cypermethrin treatments, the effects were not perhaps as pronounced as would have been expected on the basis of the laboratory toxicity data, with only minor reductions in taxon richness. There were however major effects on certain arthropod taxa including *Crangonyx pseudogracilis*, *Asellus aquaticus* and Chaoboridae as would have been anticipated at this treatment concentration. Effects on these organisms also resulted in an indirect increase of the flatworm *Polycelis tenuis*, probably through an increase in food supply via dead or moribund organisms (abundances in cypermethrin treatments increased from 10-20 individuals to around 100 or more individuals, whilst the controls remained fairly constant). The probable reason that the observed effects of cypermethrin were not as great as might be predicted on the basis of laboratory toxicity data was likely associated with the very rapid dissipation of the chemical, and the specific mode of action of the chemical (targeting arthropods). Acute laboratory toxicity tests are normally conducted under conditions of relatively constant exposure, typically over a period of 48 to 96 h. Since the majority of the chemical had dissipated during the first 1 to 2 days of the study, this amelioration of exposure could explain why the toxicity observed in the microcosms was less than expected. The results observed in this study with cypermethrin are consistent with those observed by Caquet *et al*. (6) in a study with the pyrethroid deltamethrin. In that study, some ponds were left uncovered and other were covered after treatment, resulting in differences in recovery rates for certain taxa, notably winged insects.

Ecological relevance of the study

In this study, macro-invertebrate immigration into treated water bodies was simulated by the regular addition of a pre-selected number of individuals and taxa. The taxa added were all invertebrates which were common in the pre-treatment microcosms and which had shown a clear decline in response to chemical application. The taxa specifically

included genera with a range of differing life cycle strategies and mobilities including potentially "high mobility" species with winged adults, such as Chaoboridae and *Notonecta*, and relatively "low mobility" species such as *C. pseudogracilis* and *P. tenuis*.

In terms of natural analogues, our simulated immigration pattern is most likely to resemble immigration within a water body only partly impacted by a toxic chemical, where colonisation can occur by migration of similar species from unaffected parts of the same water body. A second analogue might be an impacted pond or lake where a mixture of potentially 'low' and 'high' mobility taxa colonise from an inflow stream. Considering that organisms were added at a rate to allow potentially full recovery 10 weeks after treatment, this experiment probably can be considered to simulate situations in which there is relatively high levels of recovery potential.

The simulated immigration rationale is less likely to be an appropriate analogue for small, hydrologically isolated water bodies which have been severely impacted. Under such circumstances, colonisation might be expected to be consistently weighted towards high mobility taxa, particularly species with a strong potential for flight (e.g. many adult aquatic insects), taxa which produce abundant easily wind-borne resting stages (e.g. some crustaceans), or taxa which are frequently translocated by animals like water fowl. The colonising community resulting from such immigration could, in such cases, be substantially different to the pre-impact assemblage.

Implications for assessing recovery potential

The results of the study suggest that the process of immigration will potentially have a strong influence on community recovery after xenobiotic chemical effects, and that the speed and extent to which this recovery occurs can be influenced by both toxicological (species sensitivities), ecological (reproduction and dispersal rates) and chemical dynamics (dissipation rate). Whether the results have valid practical implications depends largely on whether immigration does indeed act as a strong force in the natural environment. Empirical studies provide evidence that, in the aquatic environment at least, this often the case. As Darwin initially recognised (15), many freshwater animals are highly mobile, moving not only within water bodies, but between them, through possession of a free-flying adult stage or through adaptation to dispersal by wind or animal translocation. Studies of new and physically perturbed freshwater systems show how rapid and effective such dispersal processes can be. Thus flood-scoured areas of river channels are often capable of recovering their former richness and abundance within a few weeks (16, 17) and completely new channels colonise in six months to two years (18, 19). More

hydrological isolated water bodies also colonise effectively. In a new pond complex in Oxfordshire, England, for example, rich assemblages with over 60 macro-invertebrate species were recorded from new water bodies 12 months after creation, and within 5 years, 180 species (approximately 20% of all Britain's freshwater macro-invertebrates in the groups studied) had been recorded on this site (20).

If immigration is a potentially important factor influencing aquatic community recovery from physical disturbance then it is also likely to be important in recovery from chemical perturbation. This suggests that greater understanding of immigration processes may bring considerable insights, both in terms of the relative significance of xenobiotic impacts to different freshwater habitats and in the development of strategies for minimising their detrimental effects.

Whilst this study has demonstrated that recovery potential is clearly influenced by chemical fate properties and simulated immigration, it would be impractical to perform such experiments for the range of permutations of chemical dissipation and organism immigration rates. Recently, modelling approaches have been developed that allow an exploration of recovery times under a range of conditions (21, 22). It has been recommended that such modelling approaches could be usefully applied to the interpretation and extrapolation of microcosm and mesocosm studies in the future (23).

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Chapter 7: Probabilistic Risk of Cotton Pyrethroids: Combining Landscape-level Exposures and Ecotoxicological Effects Data to Characterize Risks[6]

Summary

Since their introduction, synthetic pyrethroid insecticides have generated regulatory concerns regarding their toxicity to fish and aquatic invertebrates. In this paper we assess the potential for risks to aquatic ecosystems in cotton-growing areas, focusing on cypermethrin as a suitable representative of the pyrethroid class and static water bodies (ponds and lakes) as worst-case water bodies because of low levels of dilution. Reviews of cypermethrin effects under laboratory and field conditions have characterized the potential aquatic effects of the chemical. Also, a landscape-level exposure characterization has been conducted in a worst-case cotton-growing county, Yazoo County, Mississippi, USA, to provide a more realistic exposure characterization than is possible using standard model scenarios. Risks were characterized using the standard tier I and II approaches of the U.S. Environmental Protection Agency. In addition, a probabilistic risk assessment was conducted by comparing landscape-level exposure calculations for ponds and lakes in Yazoo County (modified tier II analysis) with distributions of laboratory effect concentrations and with data from field studies. Risk characterization using tier I and tier II models demonstrated a level of concern for certain aquatic organisms. However, modified tier II analysis showed that exposure concentrations are unlikely to exceed concentrations that might cause ecologically significant effects. Indeed, in the vast majority of cases, concentrations in the modified tier II analysis were several orders of magnitude lower than those at which effects would be predicted on the basis of laboratory and field data. The conclusion of minimal potential for adverse ecological effects was also supported by field studies, which showed that impacts on aquatic systems were negligible, even at concentrations many times higher than the modified tier II exposure concentrations.

[6] Published in Environmental Toxicology and Chemistry, 20, 687-692. Maund SJ, Travis KZ, Hendley P, Giddings JM, Solomon KR.

Introduction

Pesticide risk assessment relies on a basic framework where exposure and effects profiles are described and compared—the process of risk characterization (1–4). At the lowest tiers, this usually involves single worst-case estimates of exposure and effect to determine a risk quotient (RQ). If this indicates a concern, more realistic (higher-tier) estimates of exposure and effect are generated to refine the risk assessment. Approaches to higher-tier risk assessment can include the use of probabilistic approaches to characterizing exposure and effects, the inclusion of landscape-level data (e.g., remote sensing data from satellite or aerial imagery), and ecotoxicological field studies. Such higher-tier assessments have been conducted for cotton pyrethroids (5–8). In this paper we consolidate these assessments into a landscape-level probabilistic risk characterization, using cypermethrin as a representative compound for the pyrethroid class as a whole.

The focus of the risk characterization reflects the concern of the U.S. Environmental Protection Agency (U.S. EPA) for static water bodies—the Georgia, USA, farm pond scenario—based on their potential for worst-case exposure and their importance as a recreational amenity (fishing). Lakes and ponds are considered the worst case for exposure because they lack the rapid dilution mechanism that would substantially reduce exposure in flowing waters.

Effects Characterization

Laboratory toxicity in water alone

Large amounts of laboratory acute toxicity data are available for cypermethrin, including both standard regulatory studies and those published in the scientific literature. Distributions of acute toxicity values (median lethal concentrations (LC_{50}s) and median effect concentrations (EC_{50}s)) were used to estimate the concentration at which a given percentage (or centile) of species are likely to be affected by cypermethrin (5). Analysis showed that estuarine and marine arthropods are generally more sensitive to pyrethroids than are freshwater arthropods, perhaps because of effects on osmotic balance or to enhanced bioavailability. However, for cypermethrin, estuarine and marine arthropods were included in the distributional analysis to increase the conservatism of the effects characterization.

More limited laboratory chronic toxicity data are available for cypermethrin, and include the standard chronic regulatory studies (9). Distributional analysis cannot be applied

reliably to such small data sets. Also, the approach is less readily used with chronic effects data because the studies are not entirely comparable because of differences in test designs, particularly with regard to differences in exposure concentrations, test durations, and study endpoints. However, the chronic effect concentrations can be compared with probabilistic exposure data to determine the likelihood of exceedence of the effect concentration (see below).

In general, chronic toxicity data are less useful in assessing the potential risks of pyrethroids because of significant differences between exposures in the laboratory and those in the field. Because pyrethroids dissipate rapidly (dissipation half-life in the water column is generally less than 1 d), it is difficult to reconcile field exposures with those used in laboratory chronic studies, which maintain constant concentrations for tens to hundreds of days. Complicated dynamics will occur in the field, involving uptake and depuration in relation to changing chemical concentrations. The implications have been demonstrated in a study with the amphipod *Hyalella azteca* (10). In this study, the effects of short-term exposures to cypermethrin were evaluated by exposing organisms for periods from 1 h (and then returning to clean water) up to 96 h, and recording toxicity after 96 h. As would be expected for a compound that can be depurated as well as taken up, the effects of short-term exposures were considerably less than those of constant exposure (Fig. 1). For example, the toxicity measured at 96 h after a 1-h exposure of *Hyalella* to cypermethrin was two orders of magnitude less than after a constant 96-h exposure. Whether such short exposures may result in longer-term sublethal effects on an aquatic organism's life history is not readily addressed in laboratory toxicity studies. However, field studies that simulate realistic exposure and then measure effects on populations and communities for weeks to months will indicate whether initial exposures may have longer-term effects. Such data are available for cypermethrin (6) and are discussed in more detail below.

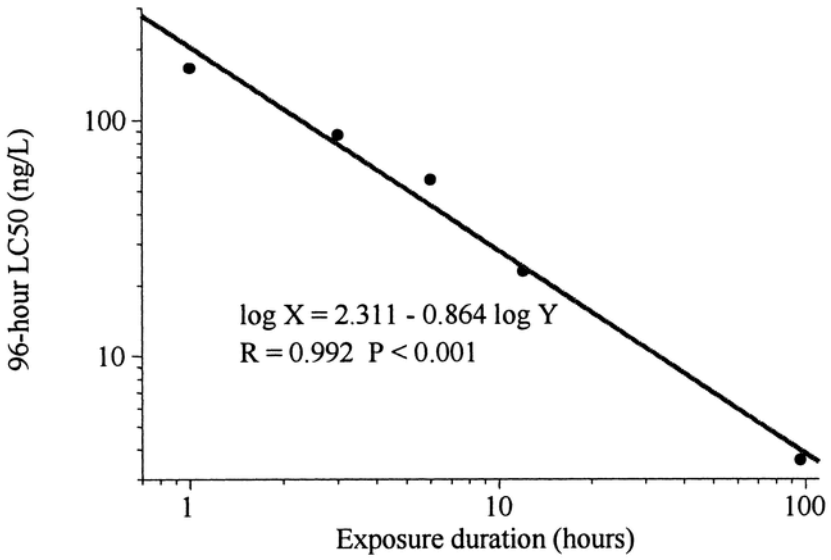

Figure 1. Relationship between exposure duration of cypermethrin and mortality at 96 h for *Hyalella azteca* [13]. Test organisms were exposed for the duration indicated on the x axis and then returned to clean water. Mortality was then measured after 96 h

Toxicity to aquatic organisms in sediments

The laboratory toxicity database for cypermethrin includes a range of important benthic taxa that might be expected to be sensitive to pyrethroids, such as midges, amphipods, isopods, and mayflies. The range of sensitivities of these organisms seems to be comparable to that of non-benthic arthropod taxa. Consequently, if appropriate sediment exposure concentrations are used, assessment with the laboratory data for water alone may also provide a useful indication of likely impacts on sediment organisms.

Much debate has taken place recently on how sediment toxicity data and exposures should be included in risk characterization (11). In sediments, organisms can theoretically be exposed through the sediment pore water, ingestion of sediment particles, and direct contact with contaminated sediment particles. Sediment organisms that inhabit burrows can also be exposed to overlying water. The relative importance of each of these exposure routes depends principally on the properties of the chemical and the sediment. For nonpolar organic compounds such as pyrethroids, it has been proposed that exposure in sediment (bioavailability) is principally determined from exposure to the chemical in the pore water. This is determined by the adsorption of the chemical and its partitioning

between the sediment and pore water. In the case of nonpolar organics, binding apparently is mainly determined by the organic carbon content of the sediment (12).

To investigate these considerations for pyrethroids, a number of sediment toxicity and bioavailability studies and assessments of adsorption kinetics have recently been performed with cypermethrin (13). These studies confirmed that bioavailability of cypermethrin in sediment was principally related to the sediment organic carbon content. Bioavailability from sediments to water-column organisms and to sediment organisms was very similar. This suggests that ingestion of sediment particles or direct contact with sediment-contaminated particles did not significantly increase the bioavailability of cypermethrin (13).

In studies that measured the toxicity of cypermethrin in sediment, effects also were demonstrated to be related to the amount of chemical predicted to be in the water phase (13). Comparison of sediment LC_{50}s (expressed as pore-water concentrations) with toxicity in water alone showed that the inherent toxicity of pyrethroids in water or in the sediment was very similar, given the different experimental conditions (particularly study duration) (13).

Considering these findings, comparing effects measured in studies in water alone with calculated exposure concentrations in the sediment pore water would be appropriate to characterize risks to sediment organisms. Expressing sediment toxicity as pore-water concentrations also normalizes the apparent differences in effect concentrations when the data are expressed as bulk sediment concentrations.

Therefore, this approach allows sediment risks to be characterized in a manner consistent with existing exposure models and risk characterization methods. In the risk characterization below, potential risks to sediment organisms were assessed by comparing sediment pore-water concentrations with acute and chronic toxicity measured in water alone. Although this approach may not be suitable for all chemicals, the available data support the use of this method for pyrethroids. The approach is also consistent with the approaches proposed by the U.S. EPA for developing sediment quality criteria (14).

Effects of cypermethrin in field studies

The ecological relevance of effects characterizations based on laboratory data should ideally be evaluated with data generated under field conditions. For pyrethroids, a considerable number of field studies (farm ponds, mesocosms, and microcosms) have been conducted (6). The advantage of using field data in a risk assessment for pyrethroids

is that they more realistically simulate the exposure that would occur in the environment (i.e., a rapid decline in exposure through time). Field studies also include many of the important ecological processes that can modify the effects of chemicals in the field (such as avoidance, reproduction, recovery, immigration, and so on), which cannot readily be included in laboratory studies.

The lowest-observed-adverse-effect concentration for cypermethrin for the overall ecosystem was 100 ng/L and the no-observed-adverse-effect concentration was 30 ng/L. The lowest-observed-adverse-effect concentration was equivalent to the 54th centile of acute toxicity values derived in laboratory studies with arthropods (6). Therefore, these data support the hypothesis that risks from pyrethroids will be substantially lower in the field than would be predicted from laboratory data. This reduction in impact is due to the rapid dissipation of pyrethroids and ecological factors that mitigate effects on exposed populations.

Exposure Characterization

Tier I and II exposure characterization

As with effects characterization, exposure characterization followed a tiered approach, using models of increasing sophistication and realism and decreasing conservatism as the tiers progressed. As a starting point, we estimated exposure concentrations of cypermethrin in aquatic systems following U.S. EPA tier I and tier II procedures (8). Under the U.S. EPA scheme, tier I and II assessments are conducted using the models GENEEC (15) and PRZM-EXAMS (16,17), respectively. Both approaches incorporate conservative assumptions about the compound, its use and fate, and the agronomic and environmental conditions, as is appropriate for lower-tier characterizations. The U.S. EPA has selected Yazoo County, Mississippi, USA, as a standard scenario for pesticide use on cotton. Yazoo County is equivalent to approximately the 90th centile of cotton-producing counties in terms of cotton acreage and water. Soil and weather conditions in the U.S. EPA Yazoo County scenario represent a worst case for pesticide runoff. Therefore, conclusions based on Yazoo County will be protective for the vast majority of cotton uses in the United States.

Modified tier II exposure characterization

The tier II scenario assumes that a 1-ha pond 2 m deep is surrounded by a 10-ha catchment containing only cotton that is entirely treated with the pesticide. Such a

scenario will overestimate exposures that may occur under more agriculturally and environmentally realistic conditions.

One approach to refining the scenario at tier II is to develop environmental and agricultural data to modify the assumptions of the tier II model. Recent developments in technology (e.g., land use/land cover analysis using satellite or aerial imagery) have enabled the collection of this type of data. Land use/land cover data for Yazoo County have been generated to modify the tier II scenario for cotton (7). To increase the environmental realism of the tier II scenarios, certain exposure mitigating factors reflecting distance and land cover between treated field and water were included in the modified tier II analysis (8).

As well as expressing the exposure data as the 90th centile concentration for a given site over multiple years (the standard tier II approach), exposure distributions can be developed over multiple sites (8). Distributions of 90th centile concentrations in all 597 ponds and lakes in the Yazoo County remote sensing study are shown in Figure 2 for certain exposure phases and durations. More than 80% of the ponds in Yazoo County have effectively zero exposure, mainly because they are at a large distance from cotton fields. The exposure concentrations in the modified tier II scenario were substantially lower than the concentrations in the standard tier II scenario.

The modified tier II scenario accounted for only a small number of the factors that would be expected to mitigate the exposure of water bodies to cypermethrin under environmentally realistic conditions. The effects of other factors individually on drift and runoff entry and on exposure concentrations are discussed in more detail by Travis and Hendley (8). Taken as a whole, these additional factors are estimated to possibly reduce exposure by 5 to 100 times.

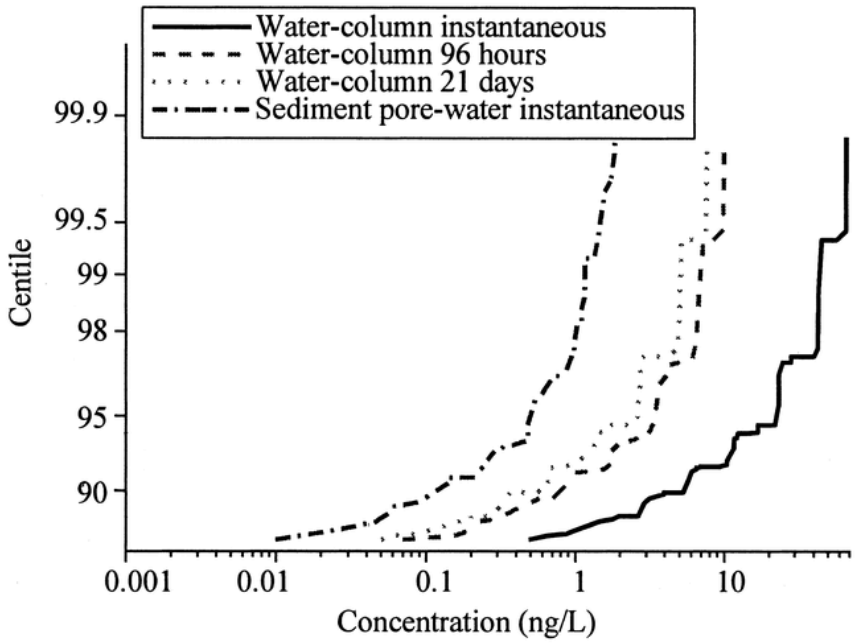

Figure 2. Modified tier II exposure distributions for Yazoo County, Mississippi, USA

Risk Characterization

Tier I and II risk characterization

Standard tier I and II risk characterization under the U.S. EPA regulatory scheme currently compares the exposure and effect concentrations across a range of exposure times to arrive at an RQ. In simple terms, if a difference of 10-fold or more exists between acute exposure and effect concentrations (i.e., RQ ≤ 0.1), the compound is assumed to be safe. For chronic exposure and effects, the compound is considered to be safe if the exposure concentration is less than the effect concentration (i.e., RQ ≤ 1). A major difficulty of this approach is selecting the appropriate exposure and effect concentrations, because values across a range of times are available for both effects and exposures.

For acute toxicity to invertebrates and fish, the basic approach would use the Daphnia acute toxicity value and the lowest available fish toxicity value from the species tested. For cypermethrin, this would ignore the large toxicity database that is available. As an alternative, the 10th centiles from the acute sensitivity distributions for fish and invertebrate species can be used as the effect concentrations. This endpoint is considered

conservative because ecologically significant effects are observed under field conditions only at concentrations higher than this for cypermethrin and esfenvalerate (6) and for other pesticides (18–21). For water-column organisms, estimated exposure concentrations in the water column were compared with the 10th centiles for fish and invertebrates. For sediment organisms, sediment pore-water exposures were compared with the 10th centile for invertebrates in general because differences in the effects measured in sediment and water-column exposures were negligible (see above).

Selection of an appropriate exposure period for comparison is critical, because of the significant difference in exposure duration between standard laboratory toxicity tests and field exposures. Consequently, both instantaneous and 96-h exposure values were used in the risk characterization. Because the acute toxicity data were based on constant exposure up to 96 h and short-term exposures do not have the same effects as longer exposures, the use of instantaneous exposure concentrations for risk characterization will overestimate the potential for effects.

For chronic toxicity, results from standard toxicity tests for fish and Daphnia were compared to 21-d water-column exposure concentrations. These will likely overestimate effects on fish, for which chronic toxicity tests are longer than 21 d. For chronic toxicity to sediment organisms, 21-d pore-water concentrations were compared to the lowest 10-d LC_{50} (expressed as the pore-water concentration) from sediment toxicity tests with *Chironomus* and *Hyalella*. Risk quotients for tier I and II risk characterization are shown in Table 1. Tier I risk characterization indicated an acute RQ greater than 0.1 for fish and invertebrates, and a chronic RQ greater than 1 for invertebrates. Tier II risk characterization for fish indicated a level of concern for instantaneous exposures, but no concerns for 96-h or chronic exposures. A level of concern was identified for both water-column and sediment invertebrates at tier II.

Modified tier II risk characterization

As a first step in characterizing risks using the modified tier II scenario, a quotient approach similar to the above can be used by comparing the 10th centile effect concentrations with the modified 90th centile exposure concentrations (Table 1). This analysis indicates no level of concern for fish or sediment invertebrates, and only a marginal level of concern for water-column invertebrates. Although the quotient approach can give an indication of potential areas of concern, it does not fully utilize the probabilistic aspects of the available data (i.e., the distributions of exposure and effect concentrations). Only one exposure concentration (the 90th centile pond in the 90th

centile year) and one effect concentration (the 10th centile effect concentration) are compared. To better understand the likelihood of impacts in ponds in Yazoo County, comparing the effects and exposure distributions across their range is possible. This can be done initially by visually examining the distributions on the same axes (Fig. 3).

Table 1. Tier I and II risk characterization—quotient approach. Acute effect concentrations are 10th centiles of species sensitivity distributions [5]. Chronic effect concentrations are *Pimephales promelas* 300-d maximum acceptable toxicant concentration [9], *Daphnia magna* 21-d LOEC [9], and *Hyalella azteca* 10-d LC_{50} based on pore-water concentration [13]a

		Tier I		Tier II		Modified Tier II	
	Effect conc. (ng/L)	EEC	RQ	EEC	RQ	EEC	RQ
Fish							
Acute: instantaneous	380	422	1.1	216	0.57	5.4	0.014
Acute: 96 h	380	121	0.32	35	0.092	0.8	0.002
Chronic: 21 d	110	29	0.26	25	0.23	0.6	0.005
Water-column invertebrates							
Acute: instantaneous	6.4	422	66	216	34	5.4	0.8
Acute: 96 h	6.4	121	19	35	5.5	0.8	0.13
Chronic: 21 d	7	29	4.1	25	3.6	0.6	0.086
Sediment invertebrates							
Acute: instantaneous	6.4	- [a]	-	6.4	1.0	0.1	0.016
Acute: 96 h	6.4	-	-	6.3	0.98	0.1	0.016
Chronic: 21 d	1	-	-	6.0	6	0.1	0.1

EEC = estimated exposure concentration in ng/L (8). RQ = risk quotient (EEC/effect concentration).

[a] The model GENEEC does not calculate sediment exposure concentrations

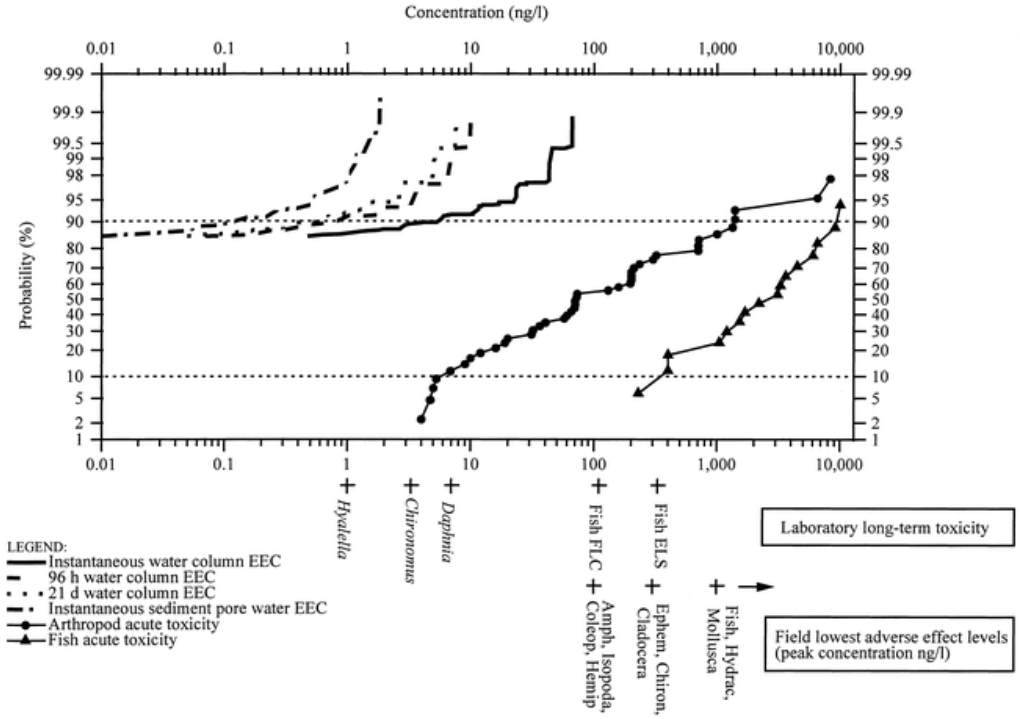

Figure 3. Comparison of modified tier II exposure distributions with acute toxicity (LC_{50} or EC_{50}) distributions [5], long-term laboratory effect concentrations [5,13], and field study lowest-adverse-effect levels [6] for cypermethrin. (Long-term laboratory toxicity endpoints (see Tables 2 and 4 for further information) included lowest-observed-effect concentrations for Daphnia magna 21-d reproduction (Daphnia) and Pimephales promelas 30-d early life stage (Fish ELS), maximum acceptable toxicant concentration for a 300-d P. promelas life cycle study (Fish FLC), and geometric mean 10-d LC_{50} values (expressed as pore-water concentrations) for Hyalella azteca (Hyalella) and Chironomus tentans (Chironomus). For the field lowest-adverse-effect levels, abbreviations are as follows: Amph = Amphipoda; Coleop = Coleoptera; Hemip = Hemiptera; Ephem = Ephemeroptera; Chiron = Chironomidae; Hydrac = Hydracarina. EEC = estimated exposure concentration)

To quantify the degree of overlap and thereby generate a truly probabilistic risk characterization (i.e., to develop an understanding of the proportion of ponds in Yazoo County in which exposure concentrations might exceed effect concentrations based on the landscape characteristics of the ponds), distributions of RQs were generated. By comparing the 90th centile exposure value (water column and sediment pore water) for each of the 72 pond categories in Yazoo County (8) with each acute and chronic laboratory

toxicity datum (5), distributions of RQs were generated for fish (Fig. 4), water-column invertebrates (Fig. 5), and sediment invertebrates (Fig. 6). For acute characterization, instantaneous and 96-h exposure values were compared to acute toxicity data (LC_{50}s). For chronic characterization for fish and invertebrates, 21-d exposure concentrations were compared to the appropriate chronic toxicity data. For invertebrates, data on sediment organisms (the 10-d LC_{50} for Chironomus and Hyalella expressed as the pore-water concentration) were also included in chronic assessments for both the water column and sediment.

These results (Figs. 4 to 6) represent the range of RQs predicted for the various taxa in ponds in Yazoo County. The likelihood of exceeding RQs of 0.1 and 0.5 for acute and 1.0 for chronic comparisons are shown in Table 2. This table (based on laboratory toxicity data) shows that in only a small number of ponds would concentrations be expected to exceed levels of concern. In the majority of ponds, predicted exposure concentrations will be several orders of magnitude less than laboratory effect concentrations.

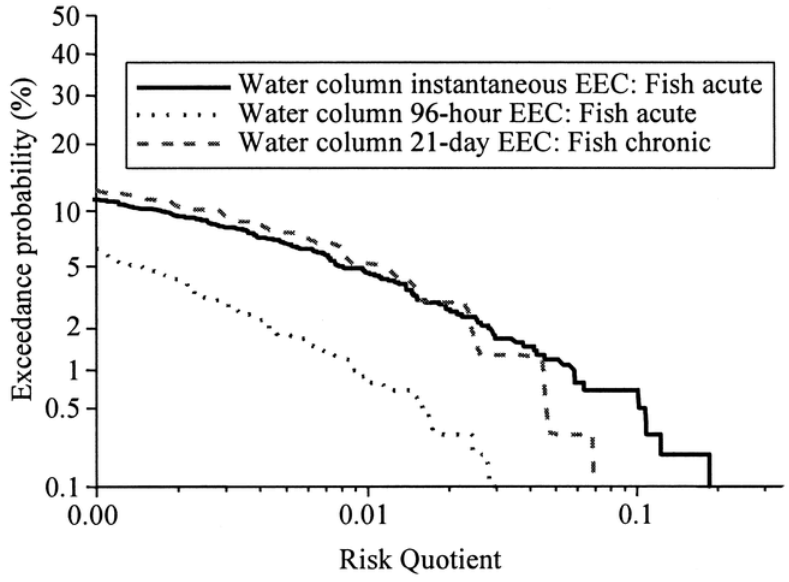

Figure 4. Distributions of acute and chronic risk quotients for fish. EEC = estimated exposure concentration

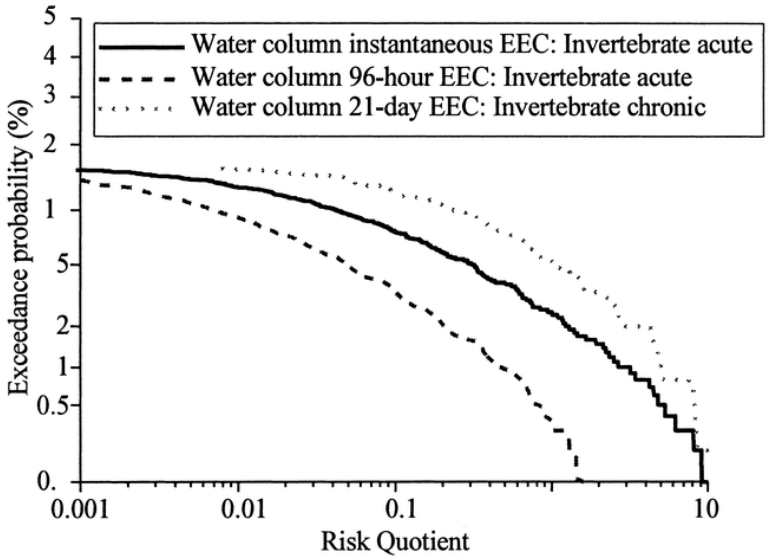

Figure 5. Distributions of acute and chronic risk quotients for water-column invertebrates. EEC = estimated exposure concentration

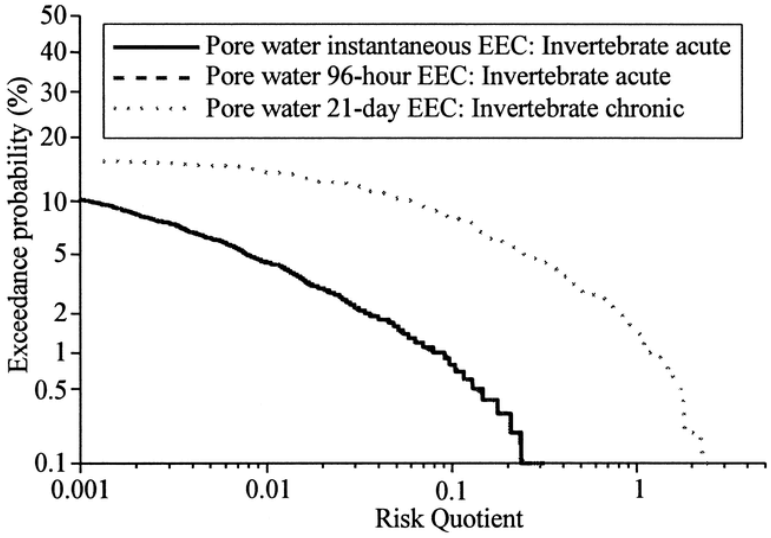

Figure 6. Distributions of acute and chronic risk quotients (RQs) for sediment invertebrates. Instantaneous and 96-h estimated exposure concentrations are very similar for pore water, so RQ distributions overlap. EEC = estimated exposure concentration

In addition to using the laboratory toxicity data for risk characterization, modified tier II exposure estimates can be compared with concentrations that have been observed to generate effects under field conditions (shown in Fig. 3). The lowest-adverse-effect concentration on the overall ecosystem was 100 ng/L (6). This effect concentration is many times higher than the maximum predicted exposure concentrations in Yazoo County ponds (Fig. 2). This suggests that cypermethrin concentrations are unlikely to reach levels that might cause adverse effects in ponds in Yazoo County.

Table 2. Modified tier II risk characterization—probabilistic approach based on laboratory toxicity data. Values in the main body of the table indicate the percentage of ponds in which the risk quotient (RQ) values in the column header are predicted to be exceeded.

	Percentage of ponds exceeding indicated RQ				
	Instantaneous EEC[a]: acute toxicity		96 h EEC: acute toxicity		21 d EEC: chronic toxicity
Taxon	RQ > 0.1	RQ > 0.5	RQ > 0.1	RQ > 0.5	RQ >1
Fish	0.7	0	0	0	0
Water-column invertebrates	7.7	3.9	3.4	1.0	5.2
Sediment invertebrates	0.8	0	0.8	0	1.4

Conclusions of Overall Risk Assessment

Considering the information presented in the preceding papers (5–8) and the arguments outlined above, our review of the laboratory and field effects data for cypermethrin and other synthetic pyrethroids, and our development of a landscape-level probabilistic analysis for cotton uses of cypermethrin in Yazoo County, Mississippi, we arrive at a number of conclusions. The first is that standard tier I and II assessments for cypermethrin indicated a level of concern for invertebrates, particularly those that inhabit the water column. Only marginal concerns existed for fish at tier II. The second conclusion is that modifications to the tier II model using landscape-level data based on a reasonable worst-case cotton county showed that under more environmentally realistic conditions, exposure will be substantially lower than predicted by the lower tier models. The third

conclusion is that additional landscape factors not included in the modified tier II assessment (especially buffer vegetation) would substantially further reduce exposure. The fourth conclusion is that, based on laboratory toxicity data, the likelihood of generating levels of concern in ponds (worst-case water bodies) for fish and invertebrates was very small. In the majority of cases, large margins of safety exist for aquatic organisms in ponds. The final conclusion is that comparison of the modified tier II exposure concentrations with field effects data confirmed that substantial margins of safety exist.

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Chapter 8: Summary, Reflections and Concluding Remarks

Overview of the aquatic ecotoxicity of synthetic pyrethroids

Early studies with the SPs demonstrated that aquatic invertebrates and fish were relatively sensitive compared to other taxa, with effect concentrations in the range of 1 ng/L to 10 µg/L (1, 2). A substantial database of the laboratory and field ecotoxicology of pyrethroids is now available (3, 4, 5, 6, 7, the earlier chapters of this thesis), and it confirms the patterns of sensitivity observed. From the available laboratory and field data for freshwater organisms, the Crustacea and certain species of Insecta appear to be among the most sensitive organisms to SPs. The general pattern that emerges for organisms from lentic and lotic habitats is that the amphipods and isopods are among the more sensitve Crustacea, while the Diptera and Ephemeroptera are among the most sensitive insect taxa, followed then by other crustacean groups, the Cladocera and Copepoda. Non-arthropod invertebrates including Mollusca, Rotifera, and Oligochaeta tend not to be particularly sensitive to SPs.

The sensitivity of the Arthropoda to SPs is perhaps not surprising considering their close taxonomic relationship to the target insect species. However, the reasons for the observed differences in toxicity between different aquatic arthropod groups are not clear. One contributor to this issue is that standard approaches to measuring aquatic toxicity normally express the effects as a function of the concentration in the testing medium (water as g/L or sediment as g/kg). This approach tells us little about the actual dose that causes the effect in the organism (as g/kg body weight). Whilst the standard approaches allow comparisons to exposure concentrations (because they are in the same units) and thereby facilitate risk assessment, they usually offer little insights about the mechanistic reasons for such observed differences. Furthering our knowledge of why there are differences in sensitivity between species is a key need for the further development of ecotoxicology. Until now, ecotoxicology has been principally mensurative (i.e. measuring effects) – in the future in should become more mechanistic (predicting effects) in order to increase our understanding of the likely impact of toxicants in ecosystems. Some authors have recently begun to explore the potential for these concepts for invertebrates (8).

The expression of toxicity in aquatic organisms will depend on a number of factors. Probably first and foremost will be the amount of the chemical that is taken up into the

organism (uptake). This is then affected by the distribution of the chemical within the body (kinetics), and whether it is able to reach particular organs or receptor tissues which then elicit the response (dynamics). Metabolism and excretion may then also modify the response, depending on the metabolic lability of the compound and the metabolic activity of the organism. Consequently, the morphology and physiology of aquatic invertebrates may play a role in their apparent susceptibility to SPs.

Several hypotheses can be proposed to provide a starting point for further investigation of these apparent differences in sensitivity. Firstly, since the mode of action of pyrethroids is via the nervous system, it may be that invertebrates with well-developed nervous systems are more sensitive (e.g. Crustacea, Insecta) compared to those with simple or rudimentary nervous systems (e.g. Mollusca, Rotifera, Oligochaeta). Secondly, invertebrates with external gills also seem to be sensitive. This may be due to the fact that the gills offer a large surface for uptake, or that exposure of the gills to the toxicant results in respiratory malfunction. Indeed, as well as their primary mode of action via the ion channels in the nerve axon, pyrethroids have also been reported to interfere with certain ATPase enzymes associated with maintaining ionic concentrations across membranes (9). It has been previously speculated that this may explain the sensitivity of certain aquatic insect taxa (10), and would also explain why crustaceans with gills seem to be more sensitive than those without (e.g. amphipods compared to cladocerans). This may also account for the observed differences in sensitivity between freshwater and saltwater arthropods, e.g., for fenvalerate and permethrin (6), it has been observed that saltwater arthropods are more sensitive than those from freshwater, possibly due to the added stress of interference with osmotic regulation, although there may be other explanations such as differences in bioavailability or inherent sensitivity (note also that in marine environments nearly all arthropods are crustaceans, while in freshwaters, the majority are insects, so differences in taxonomic composition may play a role (11). Finally, the degree of 'permeability' of the organism (which would include both consideration of whether it was an air or dissolved oxygen breather; can close off its respiratory system; the degree of chitinisation of the body; the surface area to volume ratio) will influence the rate at which the compound is taken up. This might explain why large, heavily chitinised arthropods that can close their respiratory system to the water (e.g. Odonata) would be less sensitive than those that that are small, soft-bodied and take up oxygen through the body surface or via specialised respiratory surface that cannot be closed (e.g. Diptera). At this stage, such hypotheses are conjecture, but further research into causes for differences in sensitivity of arthropods to pyrethroids could provide some interesting mechanistic insights into their ecotoxicology. Studies with chlorpyrifos (12) have explored a number of these considerations for aquatic insects. Similar methodologies, perhaps supplemented by autoradiography to study the

distribution of the pyrethroid in the organisms might be rewarding topics for future research.

For fish, there do not appear to be such large differences in sensitivity between species, or differences between freshwater and saltwater species. I speculate that similarities in morphology probably result in similar uptake kinetics between species principally via the gills. Expression of toxicity would then be based on inherent susceptibility and dynamics at the receptor, and the ability of the fish to metabolise the compound. The close taxonomic relationship of fishes (in comparison to the diversity present in invertebrate taxa) might perhaps explain why there are not such large differences in sensitivity. Where differences are observed, I speculate that the proposed secondary mechanism of toxicity of ATPase interference may provide a rationale. Salmonids and other fish typical of flowing water which have low tolerance to low levels of dissolved oxygen are less tolerant of SPs than cyprinid and centrarchid species which tend to be more tolerant to low dissolved oxygen levels.

Understanding the mechanisms of toxicity for pyrethroids could be helpful in further refining aquatic risk assessments. This will be particularly important in the future, particularly in the EU, where the introduction of the FOCUS surface water scenarios (13) means that exposure time courses are more complex than those used at present. Pyrethroid exposures are characterised by a range of peaks with different return times (varying according to the use pattern and scenario). The development of toxicokinetic models (14) could help to refine predictions of toxicity under these circumstances. For example, it has been demonstrated experimentally that short-duration exposures elicit lower levels of effects, even if initial exposure concentrations are similar (15, 16). Considering that uptake into organisms is generally rapid, this suggests that effects may be reversible, indicating that metabolism or depuration may be taking place.

Sediment toxicity and bioavailability

Despite their inherent toxicity to aquatic arthropods via water column exposure, the general pattern that emerges from the work presented here (see Chapter 2) and elsewhere is that SPs associated with sediment are of low bioavailability, and that the bioavailability can be predicted on the basis of the sediment properties (16, 17, 18, 19). This is undoubtedly due to the very great affinity of SPs for sediments, brought about by their extremely lipophilic nature. There are very few pesticides that are as lipophilic as the pyrethroids, so from this perspective they perhaps present an extreme case. However, the research presented here, even for this extreme case, has provided further evidence

that for non-polar organic pesticides, Equilibrium Partitioning Theory – EqP (20) is a suitable tool for predicting sediment toxicity. This is encouraging because it means that there is the potential to allow the prediction of effects across a broad range of sediment types – something that would not be logistically feasible to do experimentally. Since sediment type can vary substantially, and hence result in significant differences in bioavailability and hence toxicity, this is a very important principle to have established in order to develop sediment risk assessments for pyrethroids (see Chapter 7).

Although EqP does seem to adequately predict sediment effects of SPs (within a factor of 3), perhaps further refinements to predictions of the bioavailable fraction of SPs could be developed by assessing the influence of sediment texture and the nature of the organic carbon fraction. The data presented here suggest that these may be significant covariables for the adsorption and hence bioavailability of SPs, and the development of further data and models would help to further refine predictions of potential effects from sediment-associated residues. Interesting work by Xu and colleagues (21) has also shown that ageing of sediment is important because with time adsorption increases, leading to decreased bioavailability.

The limited extent of effects in sediments predicted on the basis of the laboratory studies is also reflected in the results observed in field studies. Generally speaking, at environmentally relevant concentrations, few effects have been observed on benthic communities, most likely reflecting the low levels of exposure, despite the relative sensitivity of certain benthic taxa like the Chironomidae. Laboratory studies have also shown that population density also plays a significant role in the response of Chironomidae to SP exposures in sediment. Hooper *et al*. (22) demonstrated that at higher levels of population density (as would be expected in the field), cypermethrin provoked stimulatory effects on the population growth rate of *Chironomus riparius*, probably by increasing the available resources for organisms that survived initial exposures. This provides a further explanation of why in general there appear to be limited effects of pyrethroids associated with sediment.

Considering these findings, and other work which demonstrates the potential to predict effects of insecticides in sediments from the effects observed on water column organisms by taking into account the partitioning of the molecule (23, 24, 25), the future value of extensive laboratory sediment testing programmes seems questionable (at least for non-polar organic pesticides). Perhaps resources could be better spent on testing a broader range of organisms to better define the species sensitivity distribution, and then applying

these data to calculated values of pore-water exposure in sediments (as presented in the approach described in Chapter 7).

The data presented here regarding bioavailability also raise issues about the potential applications of chemical monitoring to assess potential risks. Due to their highly lipophilic nature, it is extremely challenging to effectively sample pyrethroids at very low concentrations. It is also extremely difficult to establish the provenance of extracted residues, particularly from the water phase where SPs may be associated with both particulate and dissolved organic materials (26). Considering the future emphasis on chemical monitoring in Europe via the Water Framework Directive, the development of analytical methods which permit a realistic estimate of bioavailable SPs is a key research need. The application of semi-permeable membrane sampling devices may offer some possibilities in this direction, and recent studies have shown some encouraging developments (27).

Environmental and ecological factors that influence the effects of SPs under field conditions

There is a substantial body of data in which the impacts of SPs under field conditions have evaluated (6). In general, the initial effects that are observed in these studies are consistent with predictions based on the preliminary risk assessment – those organisms that are observed to be among the most sensitive in the laboratory, are also among the most sensitive in the field (see Chapter 4). Similarly, the reductions in toxicity observed in laboratory toxicity tests where exposure is modified (either through the addition of sediment or by removal to clean water) are also apparent in the field. Field effect concentrations are generally observed to occur at concentrations around 3 to 10 times those based on standard laboratory data. Dissipation and degradation are therefore clearly the critical factor in mitigating effects of pyrethroids under field conditions. This provides reassurance that preliminary risk assessments based on laboratory data (with safety factors applied) will be protective. Similar findings have been observed with a range of insecticides and herbicides (28, 29). Indeed, even for mixtures of compounds that dissipate rapidly, as shown in Chapter 4, this approach is likely to provide adequate margins of safety for aquatic organisms.

It is noteworthy that although microcosm and mesocosm studies have been conducted with a range of different communities, in a range of geographic locations, under a variety of trophic conditions, in different seasons and by different research teams, the exposure concentrations at which responses are seen are generally similar (see Chapter 5). When

the application of mesocosm studies in risk assessment was discussed at the CLASSIC workshop (30), concerns were raised about the repeatability of mesocosms and their application in different geographic regions. The data for SPs at least indicate that mesocosms are a robust tool for predicting the potential for effects under a variety of field conditions.

Although mesocosm studies provide a reliable method for predicting effects in the field, there are limitations to their ability to predict the longer-term consequences of those effects, particularly for organisms which do not have non-aquatic dispersal mechanisms. This is because the closed nature and absence of untreated areas in the test system means that ecological processes which may be important influences on recovery under natural conditions (e.g. avoidance, autochthonous or allocthonous immigration) are often not well-represented (see Chapter 3). One particular case in point is for the amphipod crustaceans, which have often been shown to be affected in SP mesocosm studies, whilst showing little signs of recovery (see e.g. Chapter 4, 5 and 6). From a regulatory perspective, this limitation is an important one. The European risk assessment process does not requires that there are no effects on aquatic ecosystems, but that there are no *unacceptable* effects, implying that short-term disturbances of certain organisms may be acceptable if it can be demonstrated that the longer-term effects are not great (31).

Better estimates of recovery potential can be made either through empirical or modelling studies. The data presented here showed (not surprisingly due to the rapid dissipation of the compound) that re-introducing amphipods (and other organisms) to microcosms following exposure to cypermethrin (Chapter 5) resulted in significantly more rapid recovery than systems where there was no reintroduction. Similar results have been observed elsewhere in mesocosms with the pyrethroid deltamethrin (32, 33) and also in natural systems (34). While such experimental studies are useful for demonstrating this point, they are limited by the logistical constraints of experimentation, in that usually only a limited number of recovery scenarios can be investigated. Consequently, modelling approaches may provide an alternative tool for investigating likely recovery rates under a range of conditions. Even relatively simply modelling approaches have been demonstrated to provide much better estimates of recovery potential than predictions based on toxicity alone (35).

More recently, further developments have taken place to implement ecological models to refine predictions of recovery rates under a range of environmental conditions (36). One disadvantage of such models is that they require detailed life-history information that is usually only readily available for a limited number of organisms. However, efforts are

underway to begin to collect and catalogue such data (37). The implementation of more ecological modelling in ecotoxicology would also see a furthering of collaboration between ecotoxicologists and ecologists, something that has been called for by the scientific community for many years (38, 39).

As well as developing models for specific organisms, perhaps one approach that could be developed in the future is to develop categories of life-history for aquatic invertebrates that are reasonably representative of the range of life histories seen in nature. For example, such categories could include scenarios that consider amongst other things generation time, reproductive rate, and dispersal ability. By developing a limited number of such life-history scenarios it would be possible to identify those life-history categories that might be particularly sensitive to the exposure scenario of a particular pesticide. This could then be used to guide further model development or experiments. Baird et al. (40) have begun to explore and scope out the potential for such an approach with macroinvertebrates – so-called trait-based ecological risk assessment (TERA).

The feasibility of such approaches has been a point of much debate amongst ecologists and ecotoxicologists, but we should perhaps draw a parallel to the fate and exposure area. The range of environmental and climatic properties that influence pesticide exposure are probably at least as variable as the range of life-history strategies of aquatic organisms. However, by judicious reductionism, and selecting suitably representative worst-cases, the development of exposure scenarios in both the USA and Europe has provided wider options for the refinement of aquatic risk assessments by identifying those areas of use where risks may be highest, which can then be the focus of further study - one such approach was used to derive the exposure values in Chapter 7 (41). Similar developments in ecotoxicology would be welcome.

Landscape-level Considerations

The preliminary assumptions that are included in exposure assessments in the EU and USA are by design conservative because their aim is to investigate potential for effects under worst-case conditions. Since this worst-case approach is designed to approximate the highest 10^{th} percent of exposure conditions, it therefore follows that in most cases the exposure of aquatic ecosystems under normal agricultural uses will be less (perhaps substantially so) than those predicted at lower tiers. In refining the risk assessment for SPs, it is therefore important to evaluate the potential for exposures that may cause effects under more realistic conditions.

The study presented in Chapter 7 evaluated exposures of cypermethrin (used as a suitably representative SP) for a worst-case crop, cotton, from aerial and ground applications in a worst-case weather county in Mississippi, USA. The data clearly demonstrated that even by only taking into account the distance of the crop to surface water, exposure conditions that were likely to occur were substantially lower than those predicted at the lower tiers, indicating that risks in practice were likely to be acceptable.

In the interests of developing refined risk assessments for pesticides, and therefore achieving more sustainable uses (i.e. not being under- or over-protective), such landscape-based assessments are beginning to receive greater attention in both the USA and EU. For example, ECOFRAM (42) identified landscape approaches as a potentially useful tool for refining risk assessments. Similarly, in the EU the FOCUS surface water scenarios group (43) has recommended that regional or local assessments that take into account the cropping patterns and surface water distributions are a suitable way to develop higher-tier exposure estimates. This work has been further developed by the FOCUS landscape and mitigation group (44), and a variety of options for developing landscape level assessments are now supported. Over recent years, due to the increasing availability of landscape level data and the application of geographical information systems (GIS), such approaches are beginning to receive wider attention.

The development of landscape and GIS-approaches not only offer higher-tier tools for exposure assessment, but may also provide options for refining effects assessments at the landscape level. For example, it may be possible to adapt the ecological modelling approaches described above by considering the influence of meta-population processes on the recovery of affected organisms. By understanding the dispersal ability of organisms and the proximity of potential sources of recolonising organisms, it may be possible to develop estimates of likely effects and recovery in a more complex landscape which includes both intensive areas of agriculture with those that are unexposed. There are only limited published examples of this approach to date (e.g. 45, 46), but even these studies demonstrate that meta-population considerations are an important factor in better understanding the impacts of pesticide perturbation at the landscape level.

Some studies are also beginning to demonstrate that complexity of the landscape may have a significant influence on communities in agricultural areas. For example, in a case study in Lower Saxony, Germany, it has been observed that the presence of wooded areas upstream in a catchment effectively negates the impact of intensive agriculture when compared to areas where there is no upstream woodland (47). These complex interactions at the landscape scale require further study, but they offer great potential for

the future by perhaps allowing the development of landscape management techniques that will allow the co-existence of intensive agriculture and aquatic ecosystems of high ecological quality.

Moving from the laboratory to the landscape will not only help us to refine our risk predictions, but will also feed back into the fundamental assumptions of the risk assessment. For many years now an often-asked, but seldom-answered question has been 'What is it that we are trying to protect?' By identifying the aquatic ecosystems that are associated with agriculture, it will be possible to define the ecological characteristics of those systems, focus the risk assessment on the organisms of concern, and develop targeted mitigation measures to protect those organisms. Recent studies in the UK and EU to develop such approaches in are encouraging (48, 49). Combining this sort of information with the often substantial effects databases that are available for pesticides like the SPs will provide new approaches that permit a more realistic assessment of the likely impact of pesticides.

Importantly, such approaches will also help to define mitigation measures that can be differentiated on the basis of ecological considerations. For example, at present, most mitigation schemes in the EU recommend buffer zones of fixed distances to all types of water bodies. In the future, these could be differentiated on the basis of the type of water body concerned. For example, a seasonally dry drainage ditch may require lesser mitigation measures (especially when it is dry) than a pond. The development of such differentiated mitigation measures (along with improvements in application technology such as drift reducing nozzles) offers without doubt great potential for the future development of sustainable agriculture and management of the agricultural landscape.

Moving to the landscape level should also bring in considerations of other ecological stressors and their potential interaction with pesticides. From a regulatory perspective, this is a difficult issue to tackle, because pesticide regulation is based on individual active ingredients. Perhaps the answer to this is to move beyond the regulatory arena and begin to consider the broader issue of landscape management and stress ecology. Van Straalen (37) and van den Brink (50) have discussed such matters with suggestions that resound with the views discussed above for SPs. Given the increasing regulatory pressure on pesticides, against which needs to be weighed the increasing need for agriculture to feed a burgeoning world population, these integrative approaches seem to me to be the way ahead for pesticide ecotoxicology in the future. I look forward to participating in their development.

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Nederlandse Sammenvatting

Synthetische pyrethroïden (SPs) hebben voor een doorbraak in de bestrijding van insectplagen gezorgd door hun breed-spectrum werkzaamheid bij lage doseringen, terwijl ze een relatief lage toxiciteit hebben voor zoogdieren. Bezorgdheid is gerezen omtrent de hoge toxiciteit van SPs voor aquatische organismen in laboratoriumstudies, met name voor vissen en aquatische geleedpotigen. Echter, als gevolg van haar zeer lipofiele eigenschappen zal een groot deel van het pyrethroïde snel uit de waterfase aan organische stof in het sediment gebonden worden, waardoor de blootstelling voor de organismen in de water-kolom relatief laag zal zijn. Het is eerder aangetoond dat de door sorptie veroorzaakte verlaging van de blootstelling de effecten op aquatische organismen onder veldomstandigheden vermindert.

De in het proefschrift opgenomen publicaties behandelen drie specifieke vragen met betrekking tot de aquatische ecotoxicologie van de SPs, namelijk:

- Wat is de toxiciteit en de biologische beschikbaarheid van het pyrethroïde cypermethrin in het sediment, en kunnen er methodieken worden ontwikkeld voor het beoordelen van de risico's van pyrethroïden in het sediment?
- Hoe beïnvloeden milieufactoren en ecologische factoren de effecten van SPs op aquatische organismen onder veldomstandigheden, hoe kunnen deze factoren worden onderzocht in semi-veldexperimenten, en wat zijn de implicaties voor de hogere-trap risicobeoordeling?
- Hoe kunnen landschapsfactoren, zoals het samen voorkomen van gewassen en oppervlaktewater, de blootstelling aan pyrethroïden in een landschap beïnvloeden, en hoe kunnen deze factoren geïntegreerd worden in hogere-trap risicobeoordelingsmethodieken?

De experimenten evalueren een aantal van de belangrijkste factoren die bij de ontwikkeling van hogere-trap aquatische risicobeoordelingsmethodieken voor pyrethroïden in ogenschouw genomen moeten worden. Over het algemeen geven de resultaten aan dat het mogelijk is om de landbouwkundige voordelen van SPs (werkzaamheid, breed-spectrum bestrijding van insectplagen) te verenigen met aanvaardbare potentiële risico’s voor het aquatische milieu.

Vanwege hun zeer lipofiele aard, is er bezorgdheid geuit over de mogelijke effecten van SPs op sediment gebonden organismen. Uit de laboratoriumgegevens van cypermethrin, zoals gepresenteerd in hoofdstuk 2, blijkt dat de biologische beschikbaarheid in het sediment laag is. De effecten op aquatische macro-evertebraten kunnen bovendien adequaat worden voorspeld door rekening te houden met het partitioneringsgedrag van de stof en de effectconcentraties die gebaseerd zijn op de waterfase.

In hoofdstuk 3 worden ecologische factoren die van invloed zijn op de effecten van SPs onder veldomstandigheden besproken, en in de daaropvolgende hoofdstukken 4, 5 en 6 is een aantal van deze factoren experimenteel onderzocht. Hoofdstuk 4 evalueert de effecten van toepassingen van een combinatie van bestrijdingsmiddelen, die een normaal landbouwkundig gebruik van bestrijdingsmiddelen voor een aardappelgewas simuleren. Uit de resultaten blijkt dat de effecten in voldoende mate kunnen worden voorspeld op basis van de effecten van de afzonderlijke bestrijdingsmiddelen (althans in dit geval, waar de verdwijning van de stof redelijk snel was). Uit een literatuurstudie van acht micro- en mesocosm studies, uitgevoerd met een *lambda*-cyhalothrin (hoofdstuk 5), blijkt dat als gevolg van hun snelle verdwijning, de effecten van pyrethroïden waargenomen in micro- en mesocosm studies opmerkelijk consistent zijn, ongeacht de grootte, de samenstelling, de trofische status of locatie van het testsysteem. In hoofdstuk 6 wordt het belang van immigratie voor het herstel van macro-evertebraten na sterfte als gevolg van blootstelling aan cypermethrin aangetoond. Onder veldomstandigheden kunnen de dieren uit onaangetaste regio's binnen het waterlichaam of uit aangrenzend oppervlaktewater migreren naar het aangetaste deel van het watersysteem. Dit leidt waarschijnlijk in veel gevallen tot sneller herstel dan geobserveerd in standaard cosm studies.

In hoofdstuk 7 zijn laboratorium- en veldeffectgegevens gecombineerd met een blootstellingskarakterisering op het landschapsniveau (ontwikkeld met behulp van geografische informatiesystemen) voor de risicobeoordeling van het gebruik van het SP cypermethrin in de katoenteelt. Hiervoor is een County in de Verenigde Staten, die qua risico's als worst-case bestempeld werd, als case-study gekozen. Door de inschatting van de blootstelling te verfijnen met gegevens over de nabijheid van oppervlaktewater en de teelt van katoen, kon aangetoond worden dat negatieve effecten op organismen in het water (gebaseerd op laboratorium- en veldgegevens) in de overgrote meerderheid van de gevallen onwaarschijnlijk zijn. De steeds grotere beschikbaarheid van gegevens op landschapsniveau en de verdere ontwikkeling van methoden op dit gebied zullen een ruimere toepassing van een dergelijke aanpak in de toekomst toelaten.

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As I get closer to finishing off the final draft of this thesis, I am sat on a 747 headed across the Atlantic to Washington DC. I am part way through one long journey, and yet also feel that I am finally coming to the end of another even longer one. The circumstances of writing these acknowledgements reflect those that have accompanied me in completing the thesis and its papers – long-haul flights, weekends, holidays... Of course, using all this free time has only been possible with the understanding indulgence of my family. My wife Christina and my children Isabella and Laurens have been enormously patient and supportive while Dad went into the office again for the umpteenth weekend or day off, and I would like to thank them for standing by me and keeping me going when I was feeling that it was all a bit too much. I love you very much. I would also like to thank all the rest of my family, and the many friends and colleagues who encouraged me to keep going.

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Curriculum Vitae

Stephen John Maund was born to Richard and Margaret Maund on Tuesday 14th June 1966 in the town of Solihull, in the English Midlands. He is brother to Philip, Helen, and Rachel. He married Christina Maria Brewster in 1993, and they have two children, Isabella Celeste (1996) and Laurens Eades Brewster (1998), two rabbits, Pixie and Fairy (2003), and a very cute cat called Smudge (2008).

Steve was educated at Tudor Grange School (1977-1982) and Solihull Sixth Form College (1982-1984) before attending the University of York, where he graduated in 1987 with a Bachelor's Degree in Biology. He then studied for a Master's Degree in Applied Hydrobiology at the University of Wales Institute of Science and Technology in Cardiff, graduating in 1988.

From 1988 to 1991, Steve worked at the University of Wales College of Cardiff on an EU framework research project, developing laboratory chronic toxicity testing methods with aquatic invertebrates, and validating them in pond (at GSF Munich, Germany) and stream (at Shell, Sittingbourne, UK) mesocosms. In 1991, he was employed by ICI Agrochemicals at Rocky Mount, North Carolina, USA to work on microcosms and mesocosms evaluating pesticide effects on aquatic ecosystems. In 1993, he returned to the UK to lead the ICI Agrochemicals and subsequently Zeneca Agrochemicals aquatic ecotoxicology team at Jealott's Hill Research Station, Bracknell.

On the formation of Syngenta (a merger of Zeneca and Novartis) in 2000, Steve became the Global Lead for Aquatic Ecology. In 2001, he was appointed a Syngenta Science and Technology Fellow, a position that recognizes contributions to scientific leadership. In 2002, Steve relocated to Basel, Switzerland, firstly leading the aquatic ecotoxicology team there, and then subsequently providing advice to research and development projects on environmental risk. In 2006, he moved to Global Product Registration where he now leads the fungicide section. Much of Steve's work in Syngenta and its predecessor companies has contributed to the development of new active ingredients, including the fungicides azoxystrobin, picoxystrobin, mandipropamid and isopyrazam. He was also closely involved in the EU and US re-registrations of the synthetic pyrethroid insecticide *lambda*-cyhalothrin. He and his team are currently working on the development of three further new fungicidal molecules.

Steve has actively participated in the development of aquatic risk assessment of pesticides in Europe over the last decade. He was an organizing committee member of the SETAC

HARAP, CLASSIC, EPiF, AMPERE and TERA workshops, and also of the EU-funded EUFRAM and EUPRA projects. He was a member of the FOCUS Surface Water Scenarios group, secretary to the FOCUS Landscape and Mitigation group, and secretary to the FOCUS Steering Committee. Steve was the industry representative for the development of the 2002 EU pesticide aquatic guidance document. From 2000 to 2007, he was chairman of the annual Fresenius ecotoxicology conferences, and was also a founding member of FreshwaterLife (www.freshwaterlife.org).

The main research interest of Steve's career to date has been aquatic microcosms and mesocosms. He dug his first pond in 1976 (see photo below), and since then has worked on more than twenty five 'cosm studies of various shapes and sizes in several different countries (USA, Germany, UK, Netherlands, Switzerland). Steve has also had a variety of research projects on themes around mechanisms of effects on aquatic organisms, and 'ecologizing' aquatic risk assessment and management. He has co-superivsed several PhD and postdoctoral researchers in these areas, and is an author on more than 80 publications.

In his spare time Steve enjoys music, reading, cooking and nature, preferably accompanied by a nice pint of beer.

So this is where it all started - the first pond, summer of 1976, 4 Thornby Avenue, Solihull, UK. Must have been cut out for a career in industry - already got the tie on by the age of 10…

Mum and Dad always encouraged me to have a go, and to love nature – thanks so much for all that precious nurturing… S xxx

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