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Immunochemical Detection of Pesticides and their Metabolites in the Water Cycle

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10 The Model Ecosystem Approach in Ecotoxicology as Illustrated with a Study on the Fate and Effects of an Insecticide in Stagnant Freshwater Microcosms

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10.1 Introduction

Hazard assessment of pesticides in freshwater ecosystems usually starts with estimating their immission rates, environmental transport, rates of transformations, and biological effects from data obtained from laboratory toxicity tests. Although these laboratory tests can be considered as a good first step in toxicity testing it is generally recognized that the extrapolation of results of these tests to the complex "real world" is not fully realistic since they do not incorporate the interactions that may exist between the pesticide and the physical, chemical and biological properties of the ecosystem, and between organisms and their biotic and abiotic environment [1-3]. When assessing the impact of pesticides in the natural environment, however, it has to be emphasized that the complexity and variability within and between freshwater ecosystems is enormous. This raises the problem that studies with pesticides in complex unreplicated freshwater ecosystems are difficult to perform and to interpret. In an attempt to bridge the gap between laboratory tests and whole ecosystem studies, research with model ecosystems is performed to assess the fate and effects of chemicals, indicating their potential hazards in the aquatic environment.

10.2 Model Ecosystems

Model ecosystems (dependent on their size also mentioned microcosms or mesocosms) are bounded systems that are constructed artificially with samples from, or portions of, natural ecosystems, or that consist of enclosed parts of natural ecosystems. Although model ecosystems usually are characterized by a reduction in size and complexity when compared with natural ecosystems, they have to include an assemblance of organisms representing several trophic levels and that is in equilibrium with its ambient environment [4-6].

The diversity in types of freshwater model ecosystems used in ecotoxicology is large. A major division is that in "generic" and "semi-realistic" freshwater model ecosystems. The "generic" or "defined" model systems do not mimic any natural ecosystem in particular, but rather exhibit some basic properties common to all ecosystems, such as species interaction, production, decomposition and nutrient cycling. These "generic" systems intend to contain only certain defined species and defined abiotic qualities chosen by the experimenter, and they are relatively simple and well standardizable [7-9]. Many freshwater model ecosystems used in ecotoxicology are of the "semi-realistic" or "derived" type in that they attempt to mimic real ecosystems. They can be classified according to the type of natural freshwater system that they represent, and whether they are situated indoor or outdoor. In outdoor freshwater model ecosystems a distinction can be made between newly constructed systems (e.g., concrete tanks in which sediment and water are introduced and that serve as ponds) and enclosed parts of existing ecosystems (e.g., by means of plastic bags, plastic or metal cylinders, or artificially lined limnocorrals).

The most frequently used freshwater model ecosystems are those that mimic more or less shallow, stagnant freshwater habitats (e.g., ponds, ditches, littoral zones of lakes). This can be explained by the fact that the physical features of shallow stagnant freshwater are much easier to incorporate in model ecosystems than those of rivers and deep lakes.

10.3 Advantages and Disadvantages of Model Ecosystems

Microcosms and mesocosms should be considered as models rather than substitutes of natural systems. Therefore, when predicting potential hazards in natural ecosystems by means of model ecosystems it is important to be aware of both the advantages and drawbacks of these systems.

10.3.1 Model ecosystem studies versus laboratory tests

10.3.1.1 Advantages

When compared with laboratory fate and effect studies the major advantage of model ecosystem studies is that the results are more directly applicable to nature. In model ecosystems interactions are incorporated between various species of a more or less natural assemblage of organisms, between the biotic and abiotic components, and between the pollutant and the various environmental compartments. This e.g., allows the study of the partitioning of the

chemical over various ecosystem compartments and of indirect effects on biological populations. Other advantages of model ecosystem studies are the possibility to obtain data for species that are not easily maintained in the laboratory. In addition to providing insight on community resistance to chemical stress, microcosm and mesocosm tests can provide information on recovery of systems. This is information single species tests are expected to provide. Furthermore, it is argued [5] that in model ecosystems the behaviour of organisms is more natural and that long-term and seasonal studies are possible.

10.3.1.2 Disadvantages

A disadvantage of model ecosystem studies over laboratory tests is that results are harder to interpret. Model ecosystems give information about the overall fate and effects of a chemical in a whole system, integrating individual processes, and thereby obscuring the route of cause and effects [4, 5]. Individual processes need to be studied under more controlled conditions in simpler laboratory tests. Another disadvantage of model ecosystems over laboratory single species tests is that it is much harder to obtain more or less identical replicates in model ecosystems because of the complexity of the several environmental compartments and types of organisms involved. Another drawback is that in model ecosystem studies more space, time and costs are involved than in laboratory single species tests. Particularly, newly constructed "semi-realistic" model ecosystems are relatively large and complex and require at least several months to reach maturity [10]. Furthermore, a proper investigation of both the fate and the ecological impact of chemicals in a series of model ecosystems requires a team of more or less specialized researchers. Because of the space, time and money available, the experimental design of model ecosystem studies usually includes fewer replicates than that of laboratory bioassays, which makes a statistical interpretation of the data harder.

10.3.2 Model ecosystem studies versus whole ecosystem studies

10.3.2.1 Advantages

When compared with whole ecosystem studies in nature the major advantage of model ecosystem studies is that they allow for experimental control and replication. Model ecosystems provide the opportunity to do research at the ecosystem level under conditions where certain parameters can be varied by the experimenter, while the results can be interpreted statistically by comparing control and treated systems [11]. In this respect it can be argued that usually more experimental control and better replicates can be obtained in relatively small, structurally less complex laboratory model ecosystems than in large and

complex outdoor model ecosystems. Another advantage of model ecosystems is that they can be more or less standardized so that ecotoxicological studies performed with different stressors in these systems are easier to compare and to interpret. In addition, the major factors that govern the structure and function of „standardized" model ecosystems are better understood. This again may be an advantage when coupling model ecosystem studies with computer simulation models in order to predict ecosystem responses to chemical stress or to generate new hypotheses concerning stressed ecosystems.

10.3.2.2 Disadvantages

When compared with field studies in natural freshwater ecosystems a disadvantage of model ecosystems is that they differ from natural ecosystems, e.g., in lacking large predators and input of nutrients and organic matter from adjacent terrestrial systems [4, 12]. Ideal model ecosystems, that e.g. simulate lakes in an appropriate way, are too large to have good experimental control and replication, and they are in general too costly. Furthermore, they are too complex, making it more difficult to interpret results and to isolate causes and effects. In contrast, smaller model ecosystems are limited in the size and number of organisms and in the types of microhabitats that can be maintained, while the problem of adequate sampling, without disturbing the system, may also arise. In addition, a smaller size enhances effects caused by the presence of walls, such as periphyton growth and the slowing down of water movements [5].

10.4 A Case Study on the Fate and Effects of an Insecticide in Macrophyte-dominated and Macrophyte-free Freshwater Microcosms

To gain insight in the types of information that can be obtained with model ecosystems some results on the fate and biological effects of the organophosphorus insecticide Dursban® 4E (active ingredient chlorpyrifos) in two types of indoor freshwater microcosms will presented. Both types of model ecosystem comprised biotic communities obtained from drainage ditches, but differed in the presence or absence of aquatic macrophytes. In particular we will address the question whether results of single species toxicity tests can be extrapolated to more complex microcosms and whether the results of ecotoxicological research performed in one type of ecosystem can be extrapolated to another type of ecosystem. The single species tests were performed with species that also inhabited our microcosms [13]. The data presented here on the fate and

effects in the microcosms are largely based on the studies described in Brock et al. [14, 15].

Twelve model ecosystems were constructed in a climate room of the Department of Nature Conservation at the Agricultural University Wageningen. Each system consisted of a glass aquarium (length 110 cm, width 110 cm, height 70 cm) in which a sediment layer of 10 cm thickness, a column of overlying water of 50 cm, and some artificial sample substrates were introduced. Many organisms were introduced in the model ecosystem together with the natural sediment and overlying water. The macro-invertebrates introduced comprised several taxonomic groups and representatives of various trophic levels. A daily photoperiod of 14 h and a constant temperature of 20 °C were maintained in the climate room. In the first experiment, which started in September 1988, *Elodea nuttallii* shoots were planted in the bottom substrate which soon changed the model ecosystems into macrophyte-dominated ones. In the second experiment, which started in September 1989, the model ecosystems were kept free of macrophytes. During an acclimation period of three months a biocoenosis developed in the model ecosystems of both experiments. In this period all systems were interconnected by tubes and the water was circulated by means of a pump to promote a uniform development.

The present paper deals with four model ecosystems treated with a single dose of the insecticide and with four corresponding controls. In both experiments the insecticide was applied at the water surface intending a nominal chlorpyrifos concentration of 35 µg/L.

10.4.1 Fate of the pesticide

In Figure 10.1 the stratification of the insecticide chlorpyrifos in the overlying water of the indoor freshwater model ecosystems with and without macrophytes is presented. When spraying the insecticide on the water surface (simulating aerial drift) it appears that the mixing rate of the insecticide in the macrophyte-dominated model ecosystems is relatively slow compared to open water systems. Particularly in the water layer near the bottom surface (45 cm) chlorpyrifos concentrations were considerably lower during the first days post insecticide application than in the higher strata. Apparently the presence of a high biomass of vascular plants (ca. 280 g dry weight per m^2) hampers water movements and thus causes a prolonged stratification of the insecticide in the water column. It has to be mentioned, however, that in the climate room sheltered conditions are simulated and that here no turbulence is caused by rain showers and wind. Nevertheless, it is obvious that organisms that predominantly dwelled near the bottom suffered lower concentrations of the pesticide than organisms living near the water surface, particularly in macrophyte-dominated ecosystems.

In Figure 10.2 the dissipation of chlorpyrifos from the overlying water in both types of ecosystem is presented. It appears that in the water column the

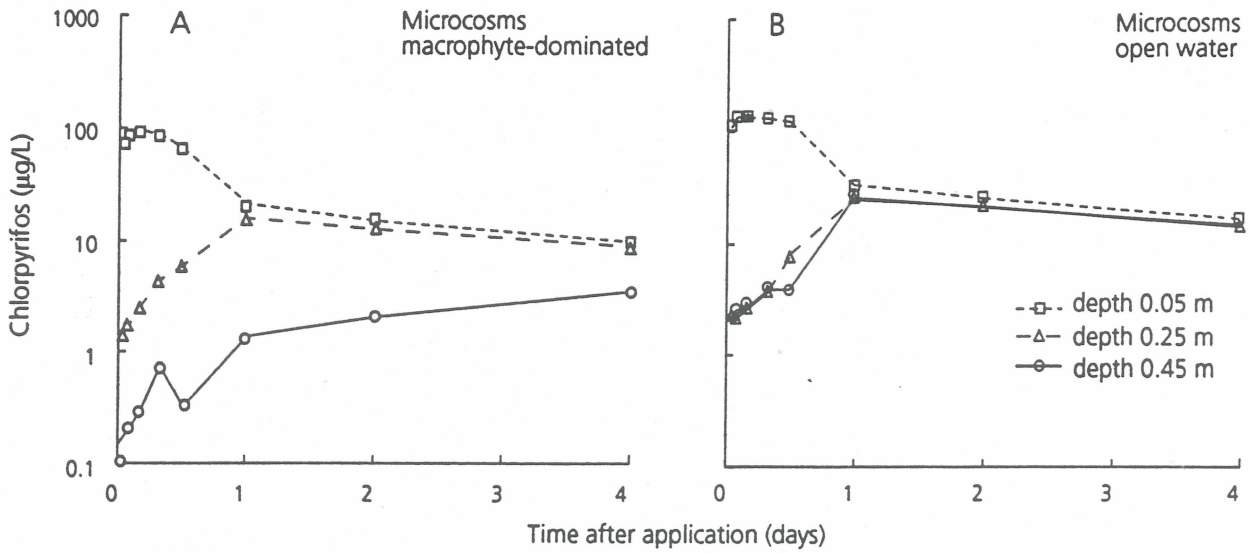

Figure 10.1: Dynamics of mean chlorpyrifos concentration at depths of 5, 25 and 45 cm in the water column of macrophyte-dominated (A) and open water (B) model systems during the first four days after the application of a dose intending a nominal concentration of 35 µg/L. The insecticide was sprayed onto the water surface.

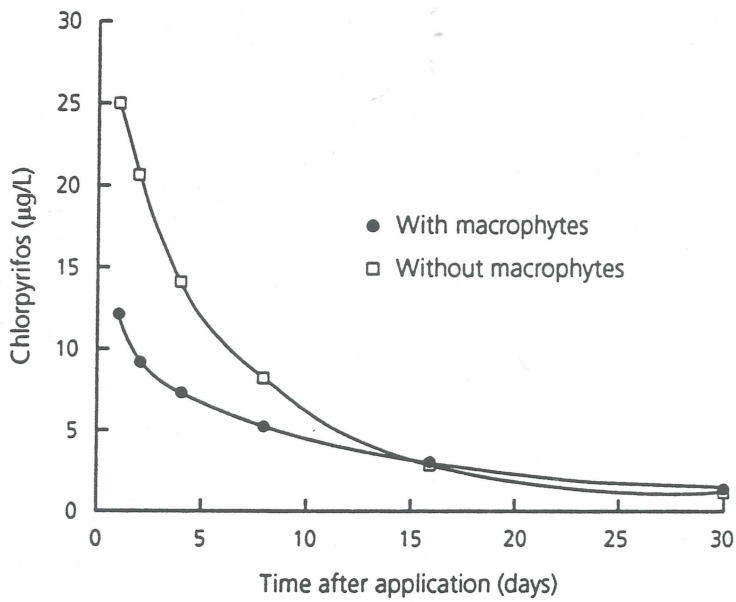

Figure 10.2: Dynamics of mean chlorpyrifos concentration (µg/L) in the water column in the macrophyte-dominated and open water microcosms treated with a similar dose (nominal chlorpyrifos concentration 35 µg/L).

rate of disappearance of the insecticide is relatively fast, particularly in the macrophyte-dominated model ecosystems during the initial phase post insecticide application. The faster disappearance of chlorpyrifos in the presence of macrophytes for a large part can be explained by sorption of the insecticide to macrophytes.

In both the macrophyte-dominated and open water model ecosystems, an average of more than 50% of the dose of chlorpyrifos applied had apparently

already disappeared after eight days (Figure 10.3). Nevertheless, considerable differences in the partitioning of the insecticide were observed between macrophyte-dominated and open water systems. In the open water systems, an average of 24.7% of the dose applied was found back in the sediment and 23.1% in the overlying water on day 8 after the treatment. In macrophyte-dominated systems, the amounts of chlorpyrifos stored in the water (15.2%), and particularly that in the sediment compartment (3.9%), were much lower at that time. In these systems, however, a large share of the dose applied was found associated with *Elodea nuttallii* (26.5%) on day 8. Since the rate of disappearance of chlorpyrifos from the sediment compartment was relatively slow compared to that from the macrophyte compartment, a higher proportion of the dose applied was found in open water systems (6.9%) than in macrophyte-dominated systems (1.9%) on day 118 after insecticide application.

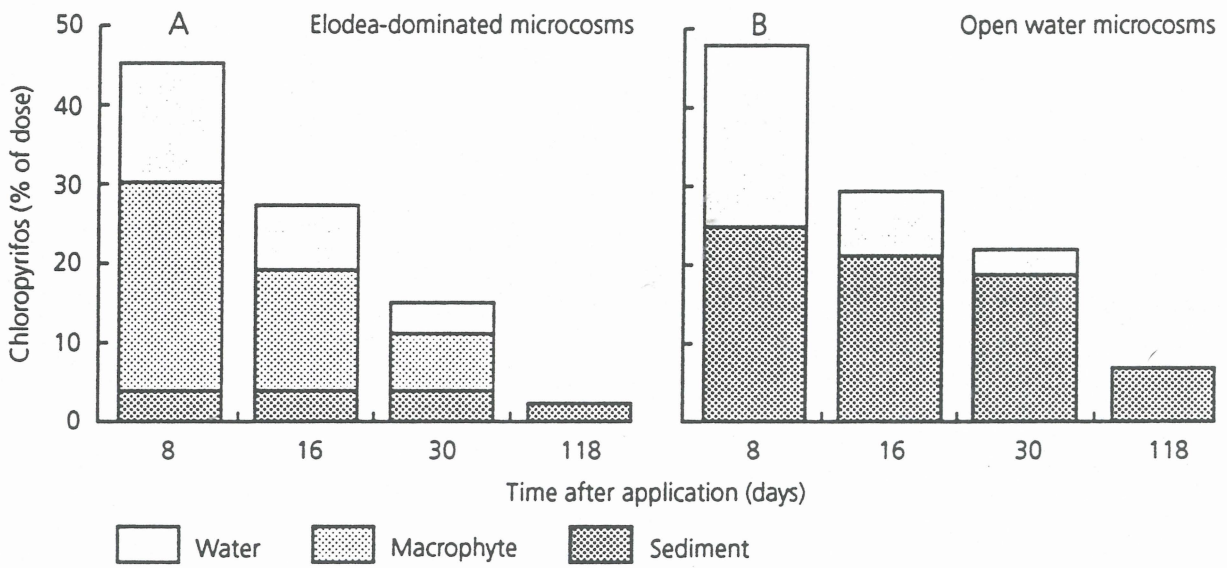

Figure 10.3: Partitioning of chlorpyrifos in the water, macrophyte and sediment compartments of *Elodea*-dominated and open water microcosms expressed as percentage of the applied dose.

The data presented above clearly demonstrate that the presence of macrophytes cannot be ignored when assessing the fate of pesticides in aquatic ecosystems. Furthermore, it is shown that model ecosystem experiments may provide valuable information on the overall fate of a pesticide (particularly its stratification, partitioning and dissipation) in a whole system. Insight in the importance of individual transport processes and degradation pathways of pesticides, however, can only be obtained in conjunction with other laboratory tests.

10.4.2 Primary effects

Primary effects of a pesticide in an ecosystem are the toxicological effects (acute or chronic) that negatively affect growth, survival and/or reproduction of organisms. In view of the large body of toxicity data of chlorpyrifos in the literature we postulated that at the exposure levels in our study, it would be the group of Arthropoda that predominantly suffer primary effects of the active ingredient chlorpyrifos. In our study, during the first weeks after pesticide application mortality occurred primarily among Cladocera, Copepoda, Amphipoda and Isopoda (all Crustacea) and Insecta, suggesting a more or less acute toxic effect of chlorpyrifos (Table 10.1).

Most insect taxa were present in low numbers. Only larvae of the phantom midge *Chaoborus obscuripes* were abundant and quantitatively dominated the insect community in both types of model ecosystem. Figure 10.4

Table 10.1: Responses of Arthropoda to chlorpyrifos in microcosms, in situ cage experiments, and single species tests.
A: Comparison of the responses of various populations of arthropods to a single dose of chlorpyrifos (nominal 35 µg/L) between *Elodea*-dominated and open water microcosms. A decrease or increase in population density that consisted for more than one sampling date is indicated by the symbols – and +, respectively. Between brackets the chlorpyrifos concentration is given below which a recovery in population density (relative to controls) was observed (n.p. = not present; no rec. = no recovery).
B: Chlorpyrifos concentration in the water of microcosms at times when effects (mortality, immobility) on arthropods in 48-h cage experiments could no longer be demonstrated between treated and control microcosms (n.a. = not available).
C: EC_{10} (48-h) values of single species toxicity tests as reported by Van Wijngaarden et al. [13].

Arthropods	A macrophyte-dominated	A open water	B in situ cage experiments	C EC_{10} (48-h)
Insecta				
Chaoborus obscuripes	- (no rec.)	- (no rec.)	0.2-0.5 µg/L	0.6 µg/L
Cloeon dipterum	- (no rec.)	n.p.	0.2 µg/L	0.3 µg/L
Cladocera				
Alona quadrangularis	- (0.1 µg/L)	n.p.	n.a.	n.a.
Bosmina coregoni	- (0.1 µg/L)	n.p.	n.a.	n.a.
Simocephalus vetulus	- (0.2 µg/L)	- (0.2 µg/L)	n.a.	0.3 µg/L
Daphnia pulex	- (0.2 µg/L)	- (0.2 µg/L)	n.a.	0.1 µg/L
Daphnia longispina	n.p.	- (0.1 µg/L)	n.a.	0.2 µg/L
Copepoda	- (0.2 µg/L)	- (0.5 µg/L)	n.a.	n.a.
Amphipoda				
Gammarus pulex	- (no rec.)	- (no rec.)	0.2 µg/L	0.03 µg/L
Isopoda				
Asellus aquaticus	- (rec.trend)	- (no rec.)	1.3 µg/L	2.0 µg/L
Proasellus coxalis	n.p.	+	n.a.	>20 µg/L

* = NOEC as reported by Van der Noeven [16].

illustrates the mortality of *Chaoborus* during the first three days after insecticide application in the open water microcosms. After a few hours the first animals were found dead at the water surface of the treated microcosms and about 25 hours post application numerous dead individuals of *Chaoborus* were found. A massive mortality was observed when the insecticide was well mixed in the water column so that sites with lower insecticide concentrations, that may have served as refuge for *Chaoborus*, were no longer present.

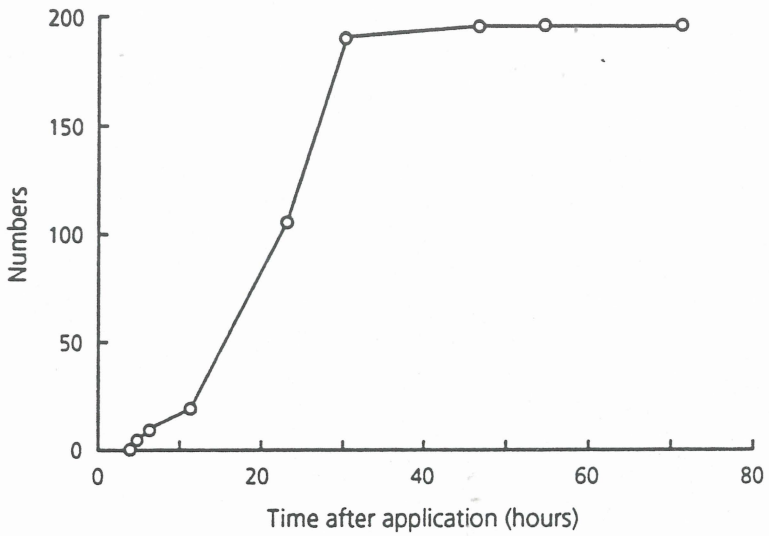

Figure 10.4: Mean cumulative number of *Chaoborus obscuripes* individuals that were found dead at the water surface of treated open water systems (n = 4) during the first days post insecticide application (nominal chlorpyrifos concentration 35 µg/L).

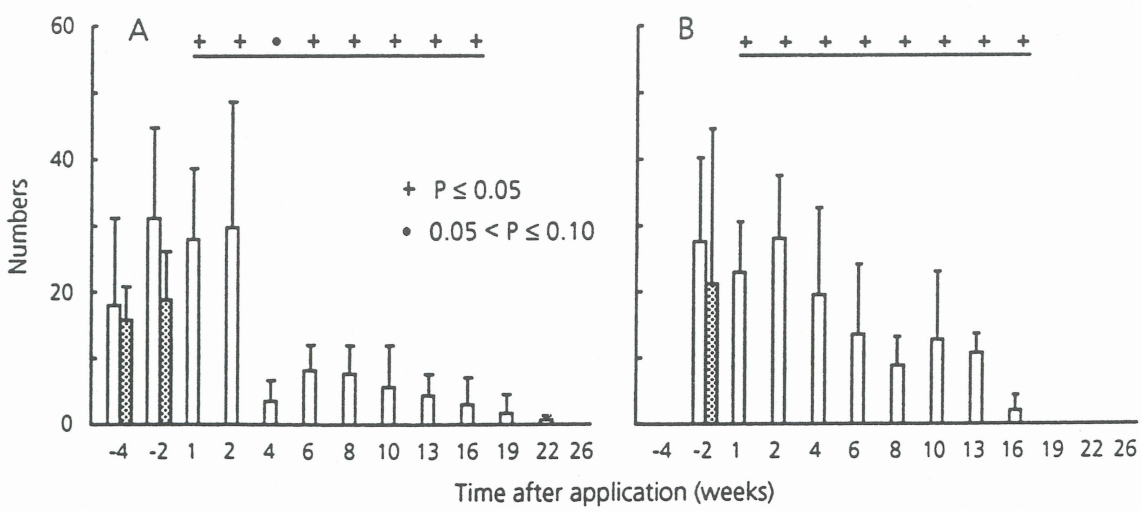

Figure 10.5: Dynamics in numbers of *Chaoborus obscuripes* (mean ± s.d.; n = 4) as sampled with artificial substrates in *Elodea*-dominated (A) and open water (B) microcosms that were treated with chlorpyrifos, intending a nominal concentration of 35 µg/L (shaded bars), and in the corresponding controls (open bars). The period in which statistical differences (Mann-Whitney U test) could be demonstrated between treatments is indicated at the top of each graph. A cross (+) indicates that treated systems differ significantly ($p \leq 0.05$) from corresponding controls; a dot (•) indicates a trend of difference ($0.10 \geq p > 0.05$). If these differences occurred on successive sampling dates this is indicated by underlining.

After the extermination of nearly all individuals of *Chaoborus* within one week after insecticide application no recovery of this species was observed (Figure 10.5). In the control systems the abundance of *Chaoborus* also declined steadily, mainly due to emergence. The fact that no recovery of *Chaoborus* was observed can be attributed to the lack of resistant aquatic stages in the life cycle of this insect, and the lack of opportunities to recolonize the model ecosystems in the climate room. Before they could mate and deposit eggs the emerged midges were usually killed by the heat of the lamps that provided artificial daylight.

In order to gain insight in the potential recovery of Arthropoda that were unable to recolonize our systems in situ cage experiments were performed in the microcosms with several Insecta and Crustacea (cf. Table 10.1) on several periods after insecticide application. These cage experiments also served to compare effects at a certain chlorpyrifos level in the microcosms with those found in single species toxicity tests. The results of the cage experiments performed with *Chaoborus* are shown in Figure 10.6. The results of the cage experiments suggested that a potential recovery of *Chaoborus* in the microcosms was possible when chlorpyrifos concentrations dropped below 0.2-0.5 µg/L. In general, the "recovery concentrations" obtained with these 48-h in situ cage experiments were in fairly good agreement with EC_{10} values of 48-h single species tests (cf. Table 10.1).

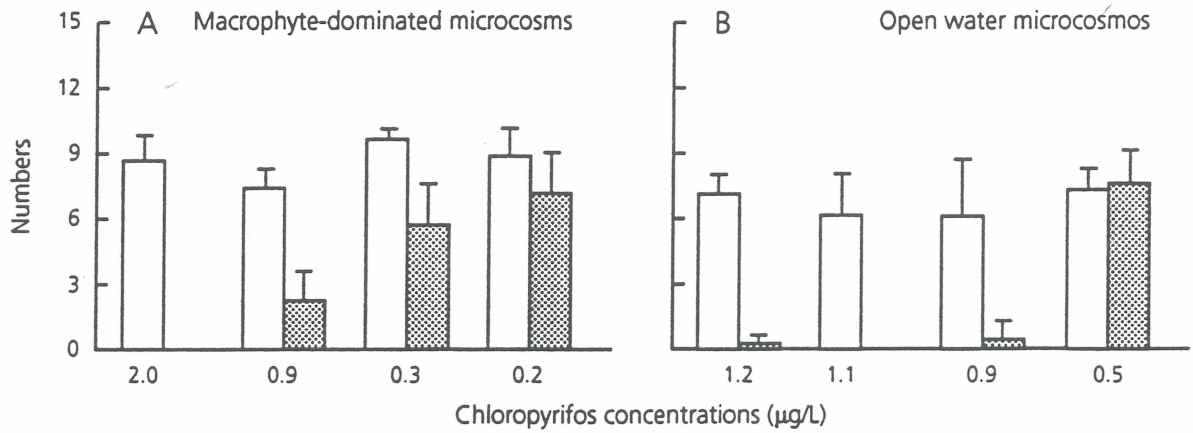

Figure 10.6: Final numbers of visually unaffected individuals of ten caged specimens of *Chaoborus obscuripes* (mean ± s.d.; n = 4) that on several dates post application were incubated for 48 h at mid-depth of the water column of *Elodea*-dominated and open water microcosms treated with chlorpyrifos (shaded bars) compared with the corresponding controls (open bars). The mean chlorpyrifos concentrations that were measured in the insecticide-treated microcosms during the different cage experiments are reported below the bars (X-axis).

In our indoor microcosms several Arthropoda were not completely eliminated because they showed a recovery in population density. Particularly microcrustaceans (Cladocera, Copepoda) showed a recovery in population size since these animals have resistant life stages (resting eggs) that remain viable for a

long time in the sediment. As an example, effects of chlorpyrifos application on the density of Cladocera in both types of model ecosystems are given in Figure 10.7. In open water systems a very rapid decline (within the first few days) in numbers of cladocerans was observed, while in the macrophyte-dominated systems significant reductions could be demonstrated not earlier than week 4. The delayed effect in the presence of macrophytes can be explained, at least in part, by the relatively low chlorpyrifos concentrations observed in the lower part of the water column due to a longer period of stratification of the insecticide. Nevertheless, in both types of microcosm the reduction of juvenile and adult Cladocera was complete, in that not a single individual was observed for several weeks. Although effects on the total Cladocera population were observed much later in the presence of macrophytes, a recovery occurred more or less at the same time interval after the application in both open water and macrophyte-dominated systems. In open water microcosms several characteristic species of Cladocera had recovered between weeks 12 and 15 post application and in macrophyte-dominated systems between weeks 13 and 16. At these moments, chlorpyrifos concentrations in the water had dropped below 0.2-0.1 µg/L (cf. Table 10.1). The "recovery concentrations" are in accordance with available results of laboratory protocol tests performed with *Daphnia pulex*, *Daphnia longispina* and *Simocephalus vetulus* (cf. Table 10.1).

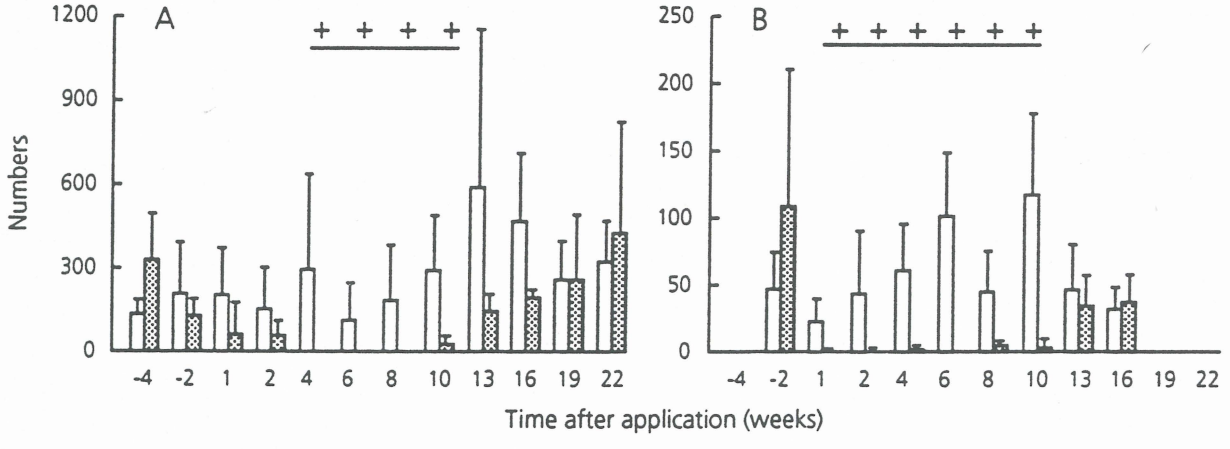

Figure 10.7: Dynamics in numbers of Cladocera (mean ± s.d.; n = 4) in *Elodea*-dominated systems (A) and in open water model ecosystems (B) treated with chlorpyrifos, intending a nominal concentration of 35 µg/L (shaded bars), compared with corresponding controls (open bars). The period in which statistical differences (Mann-Whitney U test) could be demonstrated between treatments is indicated at the top of each graph. A cross (+) indicates that treated systems differ significantly ($p \leq 0.05$) from corresponding controls; a dot (•) indicates a trend of difference ($0.10 \geq p > 0.05$). If these differences occurred on successive sampling dates this is indicated by underlining.

Both the results of the in situ cage experiments and of the recovery data of the Cladocera in the microcosms indicate that acute toxicity data obtained in single species tests can be extrapolated to a population of the same species in ecologically more complex systems, at least when information is available

on the exposure of the population in these systems. The extrapolation of toxicity data obtained for one species to populations of taxonomically related species, however, should done with caution. This is clearly demonstrated by the differences in response of two isopod species *(Asellus aquaticus* and *Proasellus coxalis)* in the open water microcosms (cf. Table 10.1). In these systems the *Asellus* population was eliminated while *Proasellus* increased in population size. The fact that no negative effect on the population size of *Proasellus* occurred can be explained by the low sensitivity of this species to chlorpyrifos ($EC_{10} > 20$ µg/L) and that a concentration higher than 20 µg/L was hardly reached near the sediment surface of the open water microcosms (cf. Figure 10.1).

10.4.3 Secondary effects

Secondary effects of a pesticide in an ecosystem are the effects that result from a reduction or elimination of biological populations due to primary effects [17]. In other words, a decrease in activity or reduction in population size of pesticide-susceptible species may result in shifts in interactions between species not directly affected by the pesticide and in a disturbance of ecosystem processes. Responses of non-arthropod invertebrates and algae observed in the microcosms suggest that these taxa did not suffer acute toxic effects since there was hardly any significant decrease in the population densities of these organisms within the first four weeks after insecticide application. Furthermore, we could not find any evidence in the literature of direct toxic effects of chlorpyrifos at concentrations less than 35 µg/L on the species other than Arthropoda that inhabited our systems. For these reasons we consider the responses of non-arthropod organisms that resulted from chlorpyrifos application as secondary effects.

10.4.3.1 Secondary effects in macrophyte-dominated systems

In the microcosms treated with chlorpyrifos an increase in algal populations, particularly of the bluegreen alga *Oscillatoria* and of the green algae *Oedogonium* and *Characium,* was observed in the periphytic community associated with *Elodea nuttallii* (Table 10.2). Planktonic algae, however, did not increase, which is in accordance with the fact that in macrophyte-dominated systems phytoplankton usually is of minor importance. The increase in periphyton in microcosms treated with chlorpyrifos can most probably be explained by a reduction in grazing, and possibly also by a decrease in physical disturbance, due to disappearance or reduction of Insecta and Crustacea (particularly Cladocera). Furthermore, the sheltered conditions in the climate room were most probably favourable for *Oscillatoria* growth, since it was

found to be loosely attached to the macrophytes in the upper layer of the water column.

At the end of the experiment dry weight values of *Elodea* were much lower in treated systems (mean ± s.d. = 218 ± 77 g) than in controls (315 ± 47 g). Furthermore, at that time, the *Elodea* vegetation in three of the

Table 10.2: Comparison of the secondary effects of a single dose of chlorpyrifos (nominal 35 µg/L) on various plant and animal populations and on community metabolism between *Elodea*-dominated and open water model ecosystems. A zero (0) indicates no consistent effect; a decrease or increase in population density (relative to controls) that consisted for more than one sampling date is indicated by the symbols – and +, respectively (n.p. = not present).

	macrophyte-dominated	open water
Community Structure		
Phytoplankton		
chlorophyll-a	0	0
small taxa (< 35 µm)	0	+
large taxa (> 35 µm)		
Volvox aureus	0	–
Periphyton on glass slides		
chlorophyll-a	0	0
Periphyton on *Elodea nuttallii*		
chlorophyll-a	+	n.p.
Macrophytes		
dry weight biomass *Elodea*	–	n.p.
Zooplankton		
Testacea	0	0
Rotatoria	0	+
Polyarthra	0	+
Asplanchna	0	+
Macro-invertebrates		
Turbellaria	–	0
Hirudinea	0	–
Oligochaeta	0	–
Naididae	0	0
Tubificidae	–	–
Mollusca	+	0
Bithynia tentaculata	+	0
Potamopyrgus jenkinsi	0	–
Sphaeriidae	0	+
Isopoda		
Proasellus coxalis	n.p.	+
Community Metabolism		
O_2: daily minimum	–	0
daily maximum	–	0
pH: daily minimum	–	0
daily maximum	–	0
Alkalinity: daily minimum	+	0
daily maximum	+	0

four systems treated was no longer rooted but floated free at the water surface while in the control microcosms the majority of *Elodea* plants still rooted in the sediment. A relatively low and free-floating biomass of *Elodea* was observed in particular in those treated replicate systems in which a more luxurious growth of *Elodea*-associated periphyton had occurred (Figure 10.8). Most probably the reduction in biomass of *Elodea* in the systems treated is the result of the increased shading and or the hampering of molecular transport between macrophytes and ambient water due to the dense growth of periphytic algae in the upper layer of the water column.

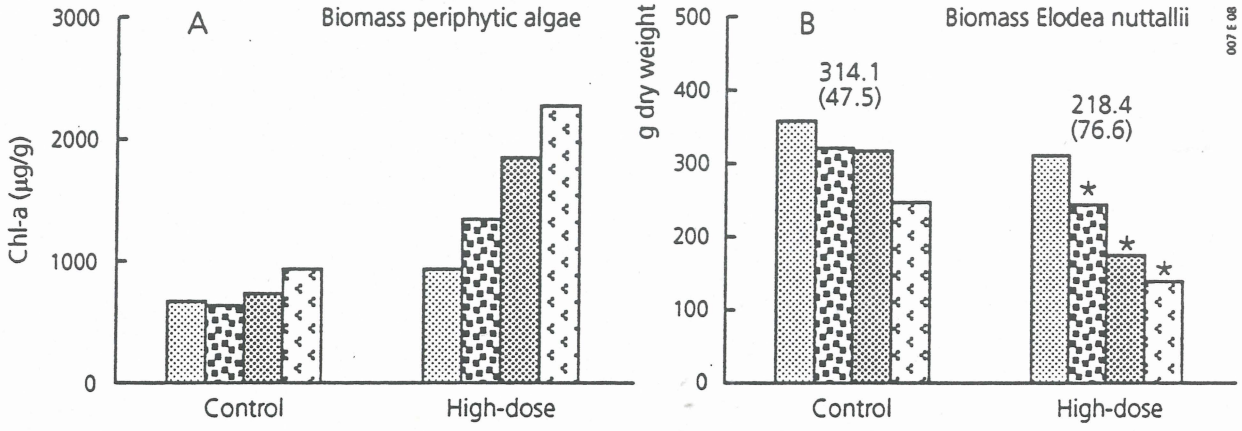

Figure 10.8A: Mean amount of loosely associated periphyton chlorophyll-a per g dry weight *Elodea* as sampled in each control and insecticide treated (nominal 35 µg/L) macrophyte-dominated microcosm during the post-treatment period.
B: Biomass of *Elodea nuttallii* harvested at the end of the experiment in each macrophyte-dominated microcosm. An asterisk indicates that most of the *Elodea* vegetation was no longer rooted at the time of harvesting, but floated free at the water surface. Mean biomass values and standard deviations are presented above the bars.

The data presented above indicate that a decrease in herbivorous Arthropoda (primary effect) may be followed by changes in populations of algae on which these herbivores graze, and that the algae in turn interact with other species. This interaction may be negative for the aquatic vascular plant *Elodea nuttallii,* or positive for Gastropoda as reported in Table 10.2. The increase of particularly green algae in the periphyton, an important source of food for snails, most probably resulted in an increase in Gastropoda, especially *Bithynia tentaculata.*

A reduction in numbers of Arthropoda in ecosystems may not only affect primary producers and animals associated with the grazer food chain, but also carnivores. In the treated systems a decline in numbers of Turbellaria was observed (cf. Table 10.2). This decline in flatworms can be explained by the disappearance of essential prey animals (Insecta, Crustacea) followed by an increased competition with other carnivores (particularly Hirudinea). The species of Turbellaria and Hirudinea present in the model ecosystems have the same prey animals on their menu, at least in part. Since no decline in the num-

bers of Hirudinea was observed it is possible that they succeeded at the expense of the Turbellaria.

Shifts in ecosystem structure may also have their impact on ecosystem function, at least temporarily. In aquatic ecosystems shifts in populations of primary producers (algae, vascular plants) may result in changes in community metabolism and consequently in alterations in the levels (and daily fluctuations) of oxygen and inorganic carbon in the overlying water. In the present study the increase in periphytic algae in the systems treated was accompanied by a decrease in oxygen concentrations and pH values and an increase in alkalinity levels in the overlying water (cf. Table 10.2). These changes indicate that less photosynthesis and/or more respiration took place. This can be explained by *Elodea nuttallii* being shaded by periphytic algae in the upper layer of the water column, resulting in less photosynthesis and more respiration of the macrophyte biomass in deeper water layers. This alteration in oxygen metabolism might have caused the decline of the sediment dwelling Tubificidae since in the systems treated deoxygenation of the water near the sediment could be demonstrated. The Tubificidae, however, may also have suffered from increased predation by Turbellaria and Hirudinea.

10.4.3.2 Secondary effects in open water systems

In contrast to macrophyte-dominated microcosms, phytoplankters were quantitatively important in microcosms without macrophytes. In these systems some remarkable effects of insecticide application on the species composition of the phytoplankton were observed in that small taxa (e.g., *Chlamydomonas, Cryptomonas, Chroomonas)* increased in numbers, while the abundance of the large colony forming species *Volvox aureus* decreased (Figure 10.9). In the control open water microcosms the "inedible" *Volvox aureus* was apparently favoured in that Cladocera selectively grazed the smaller phytoplankters. *Volvox* lost this advantage when micro-crustaceans were killed by the insecticide. Despite the shifts in species composition a significant effect on phytoplankton biomass and chlorophyll-a concentrations in the water could not, however, be demonstrated (cf. Table 10.2). As a consequence no consistent effects on oxygen, pH and alkalinity levels were found in the water column of the open water microcosms (cf. Table 10.2).

Of the zooplankton in the treated open water microcosms an increase in population size of some rotifer populations was observed (cf. Table 10.2). The increase in numbers of the herbivore *Polyarthra* might be explained by the release from competition with Cladocera and/or the disappearance of Arthropoda that prey on rotifers (e.g., some copepods and *Chaoborus).* The increased numbers of the carnivore *Asplanchi* might be the result of increased food availability in the form of small rotifers (e.g., *Polyarthra)* on which they prey.

Of the macro-invertebrates in the treated open water microcosms the populations of Hirudinea, Tubificidae and the snail *Potamopyrgus jenkinsi*

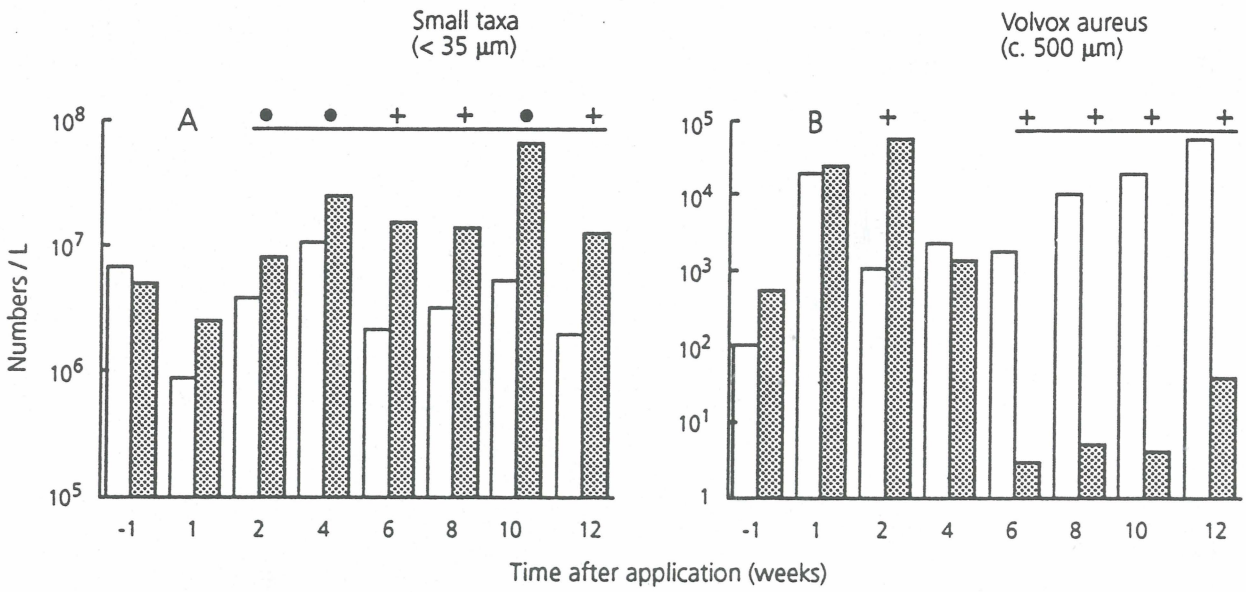

Figure 10.9: Density dynamics of small (<35 µm) phytoplankters (A) and of the large planktonic alga *Volvox aureus* (B) in chlorpyrifos treated (nominal 35 µg/L) and control open water microcosms. The period in which statistical differences (Mann-Whitney U test) could be demonstrated between treatments is indicated at the top of each graph. A cross (+) indicates that treated systems differ significantly ($p \leq 0.05$) from corresponding controls; a dot (•) indicates a trend of difference ($0.10 \geq p > 0.05$). If these differences occurred on successive sampling dates this is indicated by underlining.

showed a (temporary) decline in population size, while the Sphaeriidae (bivalves) and the isopod *Proasellus coxalis* increased in numbers. The decline in the carnivorous Hirudinea can be explained by the disappearance of essential prey animals (Insecta and Crustacea) and an increased competition with the Turbellaria for the remaining food items. In turn, the decrease in numbers of Tubificidae and *Potamopyrgus* is most probably due to increased predation by these carnivores since representatives of Hirudinea and Turbellaria not only have arthropods on their menu but also oligochaetes and snails. An increase in food availability in the form of small phytoplankters might have caused the population increase of filter feeding Sphaeriidae. The increase of the isopod *Proasellus* can be explained by its relatively high tolerance to chlorpyrifos (cf. Table 10.1) and the elimination of competing detritivorous Arthropoda *(Asellus aquaticus, Gammarus pulex)* which were more susceptible to this insecticide.

10.4.3.3 Predictability of secondary effects

In *Elodea*-dominated and macrophyte-free microcosms, populations of nearby all trophic levels were indirectly affected via the loss of populations of Arthropoda as a direct result of insecticide application. However, the taxa in which secondary effects were observed differed considerably between the two types of microcosms. Furthermore, conspicuous secondary effects of insecti-

cide application on community metabolism were observed in macrophyte-dominated systems only (cf. Table 10.2). Although single species tests were successful in predicting the primary effects of chlorpyrifos in our microcosms the secondary effects observed could not be predicted with these laboratory protocol tests alone. Types of secondary effect which probably will be the most easy to predict are those that result from release of competition or that involve two adjacent trophic levels only. Types of secondary effect that involve more than two trophic levels certainly are of importance, but these cascading effects are harder to detect and not easy to predict. Although we might be able to explain many of the observed responses of organisms in stressed model ecosystems after we have finished the experiments, we usually do not have enough insight in the system structure and trophic dynamics to allow an a priori prediction of the secondary effects that will occur. More insight into those ecosystem properties that strongly affect the response of the system after pesticide contamination may be obtained when studying the fate and biological effects of the same chemical in different types of ecosystem. Model ecosystems might be very convenient for this purpose.

10.5 Summary

The present paper deals with freshwater model ecosystems as a tool to assess the potential hazards of pesticides in aquatic ecosystems. In the first part of the paper the advantages and drawbacks of model ecosystems are discussed. It is concluded that model ecosystems are capable of providing valuable data for hazard assessment of pesticides, particularly:

- to assess factors that determine the fate of pollutants,
- to validate the significance of single species toxicity tests,
- to gain insight in secondary (indirect) effects,
- to assess the (potential) recovery of populations of species affected by pesticide contamination.

The second part of the paper intends to give some examples of the types of information that can be obtained with model ecosystems. Results on the fate and effects of chlorpyrifos in macrophyte-dominated and macrophyte-free freshwater model ecosystems are presented.

It appeared that macrophytes may affect the fate of chlorpyrifos considerably by causing a prolonged stratification of the chemical in the water column (at least in indoor microcosms), and by adsorbing a large proportion of the dose applied. In both types of model ecosystems it were populations of Insecta and Crustacea that suffered acute toxicity of chlorpyrifos. The direct effects of the insecticide could be well be predicted with results of single species tests,

at least when information was available on the exposure concentration of the insecticide. The indirect effects observed, however, differed considerably between the two types of model ecosystem, indicating that the system's structure should be taken into account when predicting ecological effects.

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Part IV Summary and Conclusions

The results of the interdisciplinary research group demonstrated in an impressive manner the progress which has been achieved. At the beginning (1988) immunochemical detection techniques were faced with great suspicion by the traditional analysts. It was feared that immunoassays would substitute the well-established techniques, like gas chromatography or HPLC.

In the meantime (1993) it became evident that immunoassays can be an ideal supplement of the environmental analytical tools in an up-to-date laboratory. Not only superior sensitivity helps to avoid artefacts during enrichment steps. Preconcentration is there no longer necessary, as this could be convincingly demonstrated for various triazine herbicides. Most important is also the inherent microchemical capability: within smallest volumes even femtograms of pesticides can be localized.

There is no question: without the advent of handy and inexpensive immunoassays our knowledge on the distribution and fate of herbicides would be rather scare. In the meantime we can follow quite exactly the traces of atrazine from the dispersion via clouds, wet and dry deposition, along the migration pathway through the top soil layer down to the unsaturated aquifer, finally ending up in metabolization or fixation on colloids or plant material.

The work with immunological techniques has also considerably stimulated side-disciplines of Analytical Chemistry. The development of chemical sensors has gained extreme power by the implementation of antibodies as recognition molecules. In combination with modern opto-electronic equipment we can expect a new generation of extremely efficient monitoring tools.

On the other hand simple dip-sticks as a first screening will also find their place at the front, where people are interested in a first information on a contamination level. Also the use of antibodies as "more-selective" adsorbers is quite promising. Coupling of immuno-adsorbers with HPLC or TLC would open the door to fields where we are slaves of traditional instrumental Analytical Chemistry up to now.

The creation of antibodies of highest selectivity and affinity is the key step for new developments. Whether genetic engineering or "template" antibodies will be the answer is not quite clear. But there is no doubt that only the unlimited production of reliable antibodies will take away the reservation which remained with many colleagues. Validation and certification of each newly developed assay must be the argument for a fruitful co-existence.

It was an adventurous and sometime troublesome journey during the last five years, but we are sure the results and experience which we have gained are a real counterbalance.