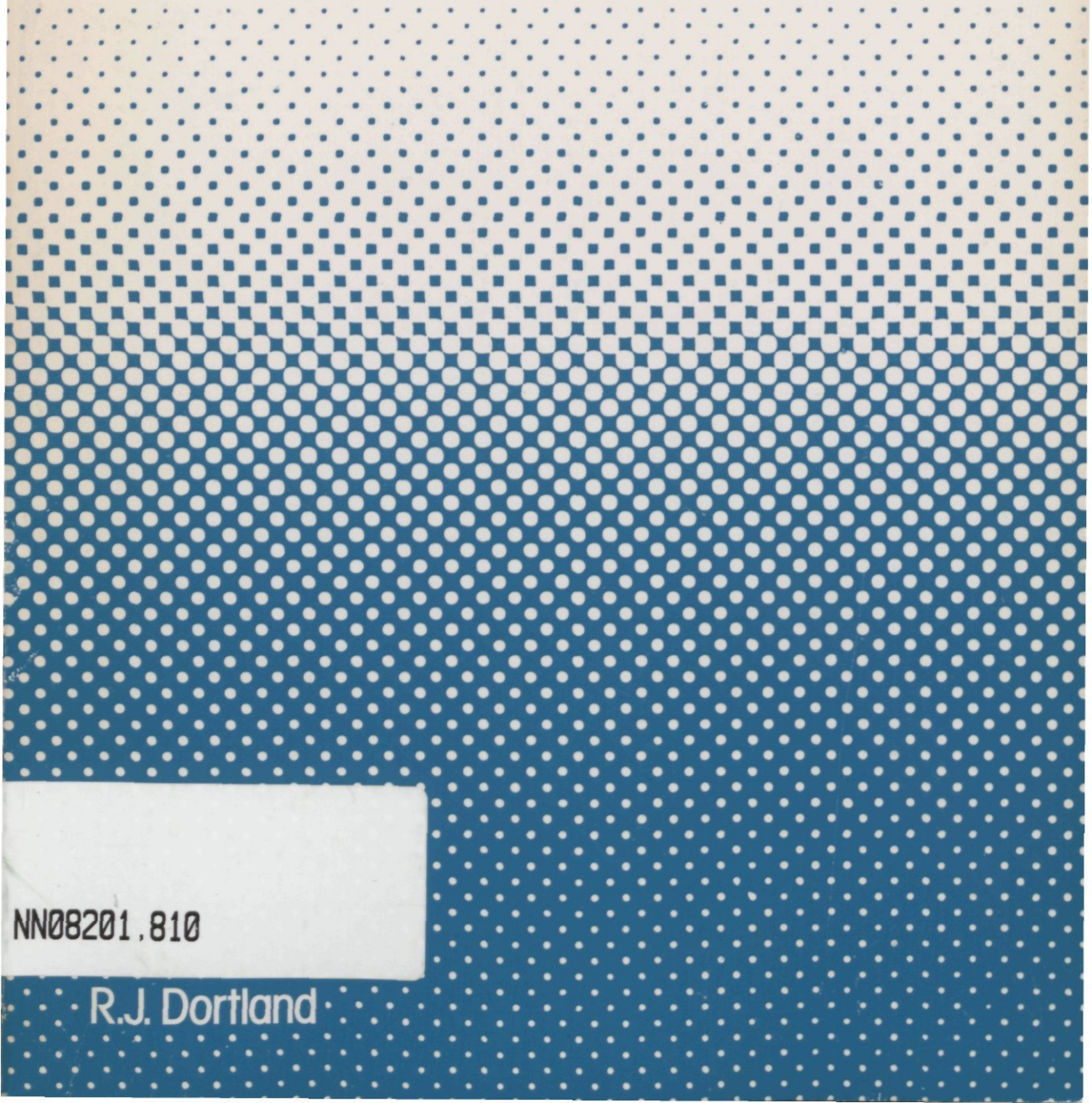
# Toxicological evaluation of parathion and azinphosmethyl in freshwater model ecosystems





R.J. Dortland

Toxicological evaluation of parathion and azinphosmethyl in freshwater model ecosystems

Promotor: dr. J.H. Koeman, hoogleraar in de toxicologie.

R.J. Dortland

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op vrijdag 20 juni 1980
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen


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# Astract

Dortland, R.J., 1980. Toxicological evaluation of parathion and azinphosmethyl in freshwater model ecosystems. Agric. Res. Rep. (Versl. landbouwk. Onderz.) 898, ISBN 90 220 0732 4, (viii) + 112 p., 33 figs, 42 tables, 131 refs, appendices, Eng. and Dutch summaries.
Also: Doctoral thesis, Wageningen.

A study was made of the possible hazards of long-term exposure of freshwater ecosystems to low (< 1 mg $m^{-3}$) concentrations of organophosphorus insecticides. Range-finding, acute and sub-acute (3 weeks) laboratory toxicity trials were carried out with parathion, azinphosmethyl, diazinon, malathion and parathion-methyl. The rates of dissipation of these compounds from the water phase of aquaria with and without plants and sediments were studied. The main test species were Daphnia magna, Asellus aquaticus, Chaoborus crystallinus and Cloeon dipterum. D. magna was the most sensitive species, 3-week no-effect concentrations ranged from 0.1 to 1.2 mg $m^{-3}$. Parathion was the most toxic compound tested. Parathion and azinphosmethyl were tested in 3 x 1 x 1 m outdoor model ecosystems in concentrations < 1 mg $m^{-3}$ over periods of more than 3 months. With a waterproof partition the model ecosystems were divided into two subsystems. Without insecticide treatment these subsystems developed similarly. Insecticide treatment of one subsystem resulted in developments dissimilar to those of the control. Parathion strongly reduced populations of Daphia spp (0.5 and 1 mg $m^{-3}$), Simocephalus vetulus (1 mg $m^{-3}$), Chydorus sphaericus (1 mg $m^{-3}$) and Chaoborus crystallinus (1 mg $m^{-3}$). No effects were observed at 0.2 mg $m^{-3}$. Azinphosmethyl at 1 mg $m^{-3}$ reduced populations of S. vetulus and Daphnia spp. No detrimental effects were observed on Cyclopoida and Ostracoda.

Free descriptors: hazard assessment, toxicity, $EC_{50}$, no-effect concentration, reproduction, half-life, diazinon, malathion, parathion-methyl, Daphnia magna, Asellus aquaticus, Chaoborus crystallinus, Cloeon dipterum, Cyclopoida, Ostracoda, aquatic invertebrates.

This thesis will also be published as Agricultural Research Reports 898

Stellingen

1. De in de onderhavige studie gebruikte modelecosystemen zijn goed bruikbaar voor de toetsing van no-effect-concentraties van toxische stoffen in zoetwater.

2. De voorlopige grenswaarde voor de concentratie aan cholinesterase-remmende stoffen in Nederlandse oppervlaktewateren dient te worden verlaagd van 1 $mg.m^{-3}$ naar 0,1 $mg.m^{-3}$ paraoxon-equivalenten.

3. Voor organofosforinsekticiden kunnen no-effect-concentraties voor veldsituaties worden afgeleid van 48-uur $EC_{50}$-waarden voor Daphnia magna Straus door deze waarden te vermenigvuldigen met een factor 0,1.

4. De in de Kwartaalverslagen van Rijkswaterstaat als paraoxonequivalenten vermelde concentraties aan cholinesterase-remmende stoffen in het Nederlandse oppervlaktewater zijn, als gevolg van de gebruikte bepalingsmethodiek, van beperkte waarde als indicator voor mogelijke ecologische schade.

5. In tegenstelling tot de bewering van Parker et al., is het gaan drijven van daphnia's geen symptoom van vergiftiging door cholinesterase-remmende stoffen.

B.L. Parker, J.E. Dewey & C.A. Back, 1970. Carbamate bioassay using Daphnia magna. J.Econ.Entom. 63(3): 710-714.

6. Bij het industrieel-toxicologische onderzoek van werknemers dienen slechts die expositie- en functietests te worden gehanteerd die relevant zijn met betrekking tot de blootstelling.

7. Vanuit een oogpunt van natuurbehoud is aanwezigheid van milieutoxicologische kennis binnen de teams die met tsetsevliegbestrijding zijn belast van groot belang.

8. De grote invloed van de produkt- en bedrijfsschappen op de formulering van de Besluiten krachtend de Warenwet is, vanuit het standpunt van de consument bezien, volstrekt strijdig met de in de Toelichting op deze wet als één der hoofddoelen genoemde 'bevordering van de eerlijkheid in de handel'.

9. De suggestie van Cunningham om van patiënten met lymfomen de 'dietary histories' te onderzoeken lijkt zinvol.

A.S. Cunningham, 1976. Lymphomas and animal-protein consumption. The Lancet: 1184-1186.

10. Het grootst opgezette onderzoek van TNO ter ontwikkeling van een nieuwe broodsoort die 'de smakelijkheid van witbrood moet paren aan de gezondheid van vezelrijk volkorenbrood' is overbodig.

R. Kroes, 1980. Voeding en gezondheid. Innovatie 38: 1-3.

11. De bewering dat de hierarchie in het leger louter functioneel is, wordt gelogenstraft door de vele privileges die de hogeren in rang genieten.

12. Dat heden ten dage kinderen in hun spel de komst van een politiewagen aankondigen met het Amerikaanse pioew-pioew-pioew in plaats van tatietata-tatietata, geeft te denken.

R.J. Dortland
Toxicological evaluation of parathion and azinphosmethyl in freshwater model ecosystems
Wageningen, 20 juni 1980

*Aan mijn ouders*
*Ter herinnering aan Soorti*

# Woord vooraf

Aan de totstandkoming van dit proefschrift hebben vele personen een bijdrage geleverd.

Prof.dr. J.H. Koeman bedank ik voor het in mij gestelde vertrouwen en voor de intensieve begeleiding bij het bewerken van het manuscript.

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Van het instituut voor Onderzoek van Bestrijdingsmiddelen dank ik prof.dr. F.J. Oppenoorth als mede-opsteller van het projectvoorstel, drs. H.R. Smissaert voor zijn waardevolle, ongezouten kritiek en dr.ir. H. de Heer voor zijn hulp bij de gas-chromatografie en het inrichten van de "proefsloten". Laatstgenoemde en dr.ir. M. Leistra hebben bovendien onder welhaast tropische omstandigheden meegeholpen met het ingraven van proefsloten.

Voorts wil ik bedanken:

- de vakgroep Erfelijkheidsleer voor het ter beschikking stellen van het terrein voor de proefsloten.
- dr. W.F. ten Berge (toen nog werkzaam bij Akzo Research) voor de hulp bij het op-zetten van de watervlooienkweek
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- de heer C. van Ginkel voor het snel en grondig reinigen van vele karrevrachten glaswerk
- de heer C. Rijpma voor het uitstekende tekenwerk

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Voorts dank ik alle medewerkers van de vakgroep Toxicologie voor de uiterst plezierige en collegiale werksfeer.

Tenslotte dank ik Joke, Kasper en Sterre voor de tijd die ik - tegen onze beginselen in - van hen mocht roven om dit boekje eindelijk af te maken.

# Curriculum vitae

Rob Dortland werd op 14 maart 1948 te Gouda geboren. Na in 1966 zijn einddiploma Gymnasium-beta te hebben behaald aan het Casimirlyceum te Amstelveen, begon hij in datzelfde jaar zijn studie aan de Landbouwhogeschool te Wageningen. September 1973 behaalde hij zijn ingenieursdiploma (met lof) met als hoofdvak de kennis van de levensmiddelen en als bijvakken toxicologie (verzwaard) en pedagogiek en algemene didactiek. Het bijvak toxicologie werd bewerkt op het Instituut voor Veterinaire Farmacologie en Toxicologie van de Rijks Universiteit te Utrecht.

Na zijn militaire dienst kwam hij als promotie-assistent in dienst van de Landbouwhogeschool, bij de vakgroep Toxicologie.

Sinds oktober 1979 is hij werkzaam bij de Directie Noordzee van Rijkswaterstaat, alwaar hij zich bezighoudt met advisering en het initiëren van onderzoek met betrekking tot de milieu-toxicologische aspecten van het beheer over de Noordzee.

# Contents

*But, thank God, they are not as intelligent as we who kill them; although they are more noble and more able.*

ERNEST HEMINGWAY
The old man and the sea

# 1 Introduction

A number of organophosphorus pesticides are continuously present as contaminants in surface waters in the Netherlands. By gas liquid chromatography, thin-layer chromatography and sometimes by mass-spectroscopy the organophosphorus compounds azinphosmethyl, chlorfenvinphos, diazinon, dimethoate, malathion and parathion as well as the carbamate insecticide carbaryl have been detected in the River Rhine and its distributaries and in other surface waters (Greve et al., 1972; Wegman et al., 1978; Pons, 1972; Blauw, 1975). Similar findings have been reported from other European countries like Great Britain, Italy and Western Germany (Lowden et al., 1969; Del Vecchio et al., 1970; Sörensen, 1973). Some figures for the River Rhine are given in Table 1.

The source and origin of these insecticides are not always known. They may partially originate from agriculture and horticulture but direct disposal from factories also may contribute to their general occurence. (Fritschi et al., 1978; Coppage et al., 1976).

The levels reported can be considered as relatively high, because they are of the same order as the levels found to be toxic to several aquatic organisms under laboratory conditions (Table 2). However, it is difficult to assess how hazardous these compounds may be for the aquatic ecosystems because toxicity data obtained under laboratory conditions often cannot be extrapolated directly to field situations. The toxicity of chemicals in water is strongly influenced by various environmental conditions such as pH, hardness, the presence of suspended matter and micro-organisms, which generally are not or cannot be simulated adequately in laboratory models.

Table 1. Average mass concentration of cholinesterase inhibitors in Rhine water sampled near Lobith. Concentration expressed in mg paraoxon equivalent per $m^3$ water. (After Rijkswaterstaat, 1973-1978).

| Quarter | Mass concentration (mg $m^{-3}$) in different years | | | | | |
|---|---|---|---|---|---|---|
| | 1973 | 1974 | 1975 | 1976 | 1977 | 1978 |
| 1st | 5.73 | 0.92 | 4.5 | 31.0 | 5.9 | 1.54 |
| 2nd | 1.52 | 0.95 | 2.9 | 21.0 | 5.4 | 2.01 |
| 3rd | 2.42 | 2.74 | 10.1 | 2.2 | 3.8 | 0.65 |
| 4th | 3.27 | 1.64 | 12.1 | 3.8 | 1.9 | 2.74 |
| Annual average | 3.24 | 1.56 | 7.4 | 14.5 | 4.3 | 1.73 |

Table 2. Acute toxicity of some cholinesterase inhibitors to freshwater organisms. Source D. pulex is Sanders & Cope (1966). Other data are derived from references in Pimental (1972). Effect refers to immobilization (D. pulex) or death (other species).

| Species tested | Substance | $EC_{50}$ (mg $m^{-3}$) | Exposure time (h) |
|---|---|---|---|
| Crustaceans: | | | |
| Daphnia pulex | DDVP | 0.066 | 48 |
| | Dipterex | 0.18 | 48 |
| | Parathion | 0.60 | 48 |
| | Diazinon | 0.90 | 48 |
| | Malathion | 1.8 | 48 |
| | Phosphamidon | 8.8 | 48 |
| | Carbaryl | 6 | 48 |
| Gammarus lacustris | Azinphosmethyl | 0.3 | 48 |
| | Malathion | 1.8 | 48 |
| | Parathion | 6 | 48 |
| | Diazinon | 500 | 48 |
| | Carbaryl | 22 | 48 |
| Insects: | | | |
| Pteronarcys californica | Malathion | 6 | 48 |
| | Azinphosmethyl | 8 | 48 |
| | Parathion | 11 | 48 |
| | Diazinon | 60 | 48 |
| | Carbaryl | 15 | 48 |
| Fishes: | | | |
| Perca flavescens | Azinphosmethyl | 13 | 96 |
| | Malathion | 263 | 96 |
| | Carbaryl | 745 | 96 |
| Salmo gairderii | Azinphosmethyl | 14 | 96 |
| | Malathion | 170 | 96 |
| | Parathion | 275 | 96 |
| | Carbaryl | 438 | 96 |

*The aim of the investigations* was to assess the hazards of long-term exposure of aquatic ecosystems to organophosphorus insecticides, with special emphasis on the most susceptible groups of aquatic organisms like crustaceans and insects. A proper hazard assessment cannot be based on laboratory toxicity data alone. Therefore an attempt was made to develop a test model incorporating some of the major environmental variables to narrow down the risk margin in the extrapolation of laboratory data to field situation and to enable a more realistic hazard assessment.

*Research strategy* Five organophosphorus insecticides were chosen because they were present in surface water and used in agriculture and horticulture in the Netherlands. These five compounds were parathion, azinphosmethyl, diazinon, malathion and parathion-methyl. Their formulas and physico-chemical properties are illustrated in Tables 15 and 16. Of these insecticides parathion and azinphosmethyl were studied in most detail. The research programme consisted of three parts:

- Toxicity testing in the laboratory: determination of acute and chronic toxicity of every compound to four species of aquatic invertebrates. These species were Daphnia magna Straus (Cladocera, Crustacea), Asellus aquaticus L. (Isopoda, Crustacea), Chaoborus crystallinus (De Geer) (Diptera, Insecta) and Cloeon dipterum L. (Ephemeroptera, Insecta).
- A laboratory model ecosystem study to measure the relative rate of dissipation of these compounds from the water phase.
- Outdoor model ecosystem studies to investigate the effects of long-term low-level ($< 1$ mg $m^{-3}$) exposure on the aquatic invertebrate population in a situation which closely resembles natural circumstances.

These three parts are dealt with in the Chapters 4, 5 and 6 respectively, whereas the analytical-chemical methods for the measurements of the insecticides used in these three studies are described in Chapter 3.

# 2 Toxicology of organophosphorus insecticides in freshwater ecosystems

## 2.1 DISPERSION BEHAVIOUR

### *2.1.1 Volatilization*

Data on the volatilization of organophosphorus compounds have not been found in the literature considered in the present study. As these compounds have a high molecular mass and low vapour pressures, a low rate of volatilization would be expected. De Heer (1979) calculated the volatilization half-lives for azinphosmethyl and dimethoate to be 413 and $0.17 \times 10^6$ days respectively.

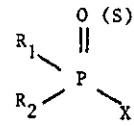
These calculations were based on assumptions of pure solutions and a rapid diffusion of the substance to the water surface. It therefore seems realistic to consider these data as minimum half-lives. If these two compounds are representative for the group of organophosphorus insecticides as a whole, volatilization from water bodies can be considered as being of minor importance.

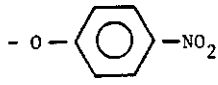
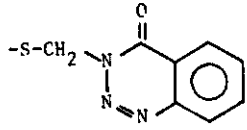
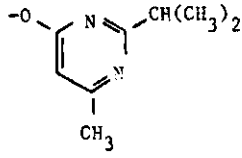
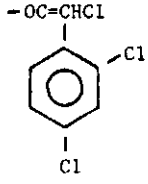
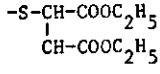
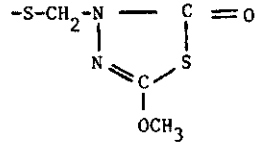
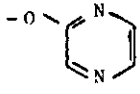
### *2.1.2 Distribution*

An important prerequisite for a proper hazard assessment is to consider the distribution of the compound over various compartments of an ecosystem in order to learn first, which organisms are liable to become exposed and secondly, how long they are exposed and to what concentrations. In general the distribution of chemical substances in the environment depends on water solubility, sorption characteristics, volatilities etc. Within this group of organophosphorus compounds there is a large variation in these properties which can be attributed mainly to the variety of substituted groups in the X-position. In Table 3 some examples are given of groups substituted in this position and the aqueous solubilities of the corresponding compounds. Although some compounds have high water solubilities, the majority belongs to the group that are not readily soluble in water. The organophosphorus insecticides used in this study also belong to this group. The physico-chemical parameter, which together with the aqueous solubility is of main importance for the ultimate distribution of a chemical over the aquatic ecosystem, is the n-octanol/water partition coefficient. This coefficient was first used to predict the uptake of a chemical by organisms (Kenaga, 1972) and later for the assessment of the potential for biomagnification (Lu & Metcalf, 1975; Metcalf, 1973; Neeley et al., 1974) and lipophilic storage (Davies et al., 1975). The partitioning coefficient might also be used to predict the

Table 3. Structural formulas of the X-group of selected organophosphorus insecticides and the aqueous solubilities of these compounds.

General formula of an organophosphorus insecticide:



| Compound | Structural formula of the X-group | Solubility in water mg $m^{-3}$ |
|---|---|---|
| Parathion |  | 24 |
| Disulfoton | $-S-CH_2-CH_2-S-C_2H_5$ | 25 |
| Azinphosmethyl |  | 33 |
| Diazinon |  | 40 |
| Phorate | $-S-CH_2-S-C_2H_5$ | 50 |
| Chlorfenvinphos |  | 145 |
| Malathion |  | 145 |
| Methidathion |  | 240 |
| Thionazin |  | 1140 |
| Dimethoate | $-S-CH_2-CO-NH-CH_3$ | 2500 |

distribution over the different compartments of an ecosystem (Neeley, 1979). In relation to the distribution of organophosphorus compounds in aquatic ecosystems the results of many field studies show a rapid dissipation of these compounds from the water phase to the bottom layer. Mulla et al., (1966) reported a decrease in parathion concentration in ponds from 400-510 mg $m^{-3}$ to 10 mg $m^{-3}$ after 8 days and to 3 mg $m^{-3}$ after a fortnight. Concentrations in mud were up to 6 x $10^{-4}$ mg $kg^{-1}$, lasting 22 days. Similar data were obtained for parathion applied to a cranberry bog (Miller et al., 1967). These authors suggested that the high concentrations in water found immediately after application were caused by stratification of the pesticide and that the rapid decrease was a result of mixing and dilution. Observations after applications of [$^{32}P$] labelled malathion to paddy rice field (depth of the irrigated water 10 cm) support this hypothesis. After spraying a strong radioactivity was first detected in the surface layer, gradually diffusing into deeper layers of water and followed by adsorption to mud. More than 95 per cent of the radioactivity disappeared from the irrigated waters within 2 days (Sato & Kubo, 1964). The same authors reported a complete disappearance from irrigation waters (depth 5 cm) within 7 days after an initial concentration of 1.7 g $m^{-3}$ parathion. Similar results were reported for chlorfenvinphos sprayed on a 1.5 m deep pond at a dose rate of 74 kg active ingredient/ha. Here the concentration in water decreased from 6.1 g $m^{-3}$ to less than 1 g $m^{-3}$ within 19 hours and to 0.12 g $m^{-3}$ after about 34 days. Residues in mud increased from 0.25 mg $kg^{-1}$ after 5 hours to 0.32 mg $kg^{-1}$ after 115 hours, decreasing to 0.15 mg $kg^{-1}$ on Day 34 (Beynon et al., 1971). Again the initial rapid decrease of the concentration in water was probably the result of mixing and dilution. However, a substantial amount of insecticide was probably deposited through sedimentation of suspended particles, especially as the water appeared turbid before spraying but was clear 13 hours later. The authors suggested either a rapid precipitation of the suspended particles due to adsorption of the pesticide to the particles and subsequent sedimentation or a destruction of planktonic animals. Rapid adsorption to plants and bottom material was also reported for Dursban (Smith et al., 1966) and adsorption to plants and algae was mentioned for parathion (King, 1969; Mulla et al., 1966) and fenitrothion (Moody et al., 1978).

So far no biological magnification of these compounds in fish has been reported. Their chemical properties, such as susceptibility to degradation by physical, chemical and biological means to polar products readily account for this. The persistence in fish is low. Macek (1970) presented a table in which the half-life times of five organophosphates in fish are summarized. In this table malathion has a half-life of less than one day, while the persistence of Dursban, azinphosmethyl, parathion and diazinon is less than one week.

In general it can be said that, apart from parathion and some other compounds, little information is available on the behaviour and toxicological impact of organophosphorus insecticides in aquatic environments.

## 2.2 TRANSFORMATION

Chemical hydrolysis, microbial degradation and photolysis can be regarded as the most important routes of transformation for the organophosphorus insecticides.

### *2.2.1 Chemical hydrolysis*

Hydrolytic cleavage is the transformation pathway which has been studied most intensively and under very diverse conditions, especially with regards to temperature, pH and type of water. The temperature ranges from 10 to 70 °C, and the medium varies from distilled water and buffer solution to raw river water. Some investigators made their observations in acidic solutions, while others used alkaline conditions for their experiments. Data on the compounds used in this study are compiled in Table 4.

The rapid disappearance of parathion in river water can be attributed to a biological reaction. The authors stated that since suspended matter was present microbial breakdown may have contributed to the disappearance of the mother compound. For malathion and parathionmethyl the half-lives are even shorter. De Heer (1979) found

Table 4. Half-lives ($t_{\frac{1}{2}}$) of organophosphorus insecticides in aqueous solutions of neutral and alkaline pH.
References: (1) Eichelberg & Lichtenberg (1971); (2) Faust & Gomaa (1972); (3) De Heer (1978); (4) Heuer et al. (1974); (5) Quentin (1969); (6) Wolfe et al. (1977); (7) Yu & Sandborn (1974).

| Insecticide | Type of water | Temp. (°C) | pH | Half-life in days | References |
|---|---|---|---|---|---|
| parathion | river water | . | 7.3-8.0 | 7[2] | 1 |
| | buffer solution | 20 | 7.4 | 108 | 2 |
| | tap water | 20 | 7.5 | 56.5 | 5 |
| | buffer solution | 20 | 9.0 | 22.8 | 2 |
| | standard ref.w. | 26.7 | 7.9 | 15 - 16[1] | 7 |
| azinphosmethyl | surface water | 10 | 7.4 | 624[1] | 3 |
| | surface water (outdoor tanks) | 10-30 | 7.5-11.0 | 0.63-7.3 | 3 |
| | buffer solution | 6 | 8.6 | 36.4 | 4 |
| | buffer solution | 25 | 8.6 | 27.9[1] | 4 |
| | buffer solution | 20 | 8.7 | 6.1[1] | 3 |
| | buffer solution | 20 | 8.8 | 12.8[1] | 3 |
| diazinon | buffer solution | 20 | 7.4 | 185 | 2 |
| | buffer solution | 20 | 9.0 | 136[2] | 2 |
| malathion | river water | . | 7.3-8.0 | 7[2] (25% left after one week) | 1 |
| | buffer solution | 27 | 8.0 | 1.5[1] | 6 |
| parathionmethyl | river water | . | 7.3-8.0 | 7[2] (25% left after one week) | 1 |

1 storage in the dark
2 storage under sunlight and fluorescent ligth conditions.

the conversion of azinphosmethyl in outdoor tanks to be much faster than in the laboratory in the dark and ascribed this to the effect of light. However, the differences in temperature and pH, and their effect on hydrolytic conversion rates may have been more important. The pH in these outdoor tanks ranged from 7.5 to 11.0 over a temperature range of 10-30 $^{o}$C, while in the laboratory this range was only 6.4-8.1 at a fixed temperature of 10 $^{o}$C.

The slow conversion rate of diazinon in water of neutral or moderately alkaline pH might explain the concentration of diazinon, found in drainage ditches in the Great Lake Region of Canada, being higher than those of ethion and parathion, as found by Harris & Miles (1975). Malathion is the compound which at pH>7 is most rapidly hydrolysed, but it is extremely stable at pH<7. (Spiller, 1961; Weiss & Gakstätter, 1964).

Although there are no studies on the hydrolysis of parathionmethyl under laboratory conditions, it can be predicted that this compound will hydrolyse more rapidly than its ethyl analogue because of the greater electrophilic character of the methyl group (Ruzicka et al., 1967). The higher rate of conversion of parathion-methyl found in river water compared with that of parathion, is in line with this prediction.

From the different studies on hydrolysis some general conclusions can be drawn:
- Organophosphorus insecticides are fairly stable in neutral and acidic conditions (Faust & Gomaa, 1972; Grahl, 1973; Weiss & Gakstätter, 1974). An exception is diazinon which is also hydrolysed under acidic conditions (Gomaa et al., 1969). The more alkaline the aqueous solution, the more rapid is the hydrolysis. Malathion for instance shows instantaneous hydrolysis at pH 12 (Spiller, 1961).
- Temperature increase greatly accelerates the hydrolytic cleavage (Ruzicka et al., 1967). From the activation energies involved, Faust & Gomaa (1972) concluded that at lower temperatures hydrolysis may slow down to such a low rate that the compound becomes fairly persistent. This could also partly explain the sometimes high concentrations of cholinesterase inhibitors found in the Rhine during wintertime.
- The phophorothionates and thiolothionates are more stable than the corresponding oxygen analogues. Parathion is an exception since this compound hydrolyses more rapidly than paraoxon under acidic or slightly alkaline conditions (Faust & Gomaa, 1972).
- Those organophosphorus insecticides in which the alkyl (R) group (Table 3) is a methyl group, hydrolyse more rapidly than their ethyl analogues due to the greater electrophilic character of the methyl group (Ruzicka et al., 1967).
- For certain organophosphorus compounds the process of hydrolysis seems to be enhanced by adsorption. Pionke & Chester (1973) gave examples of diazinon and malathion which appeared to degrade more rapidly in association with colloids or suspended solids. On the other hand they also mentioned a prolonged persistence of Dursban due to adsorption on organic solids.

### 2.2.2 *Microbial degradation*

Many studies have been made on the microbial degradation of organophosphorus compounds. Most of these refer to soil systems. A good review on this breakdown in soil was prepared by Tu & Miles (1976). They listed bacteria, actinomycetes and fungi capable of degrading one or more insecticides.

Less is known about microbial degradation in water, but as in soil, warm temperatures and the presence of organic matter will generally promote bacterial activity and, consequently, the metabolism of pesticides.

For instance, Yasuno et al. (1975) explained the rapid inactivation of parathion used as a larvicide in polluted water (in contrast to pure water) by bacterial degradation. An important phenomenon in the microbial breakdown of pesticides is the adaptation of the microflora to these compounds. Sethunathan (1973) found that in flooded rice fields repeated application of diazinon markedly increased the degrading capacity compared with a single application.

Arthrobacter sp., Corynebacterium sp. and a facultative anaerobic Flavobacterium were isolated and found capable of metabolizing diazinon.

A similar adaptation was found after parathion treatment. Bacteria capable of hydrolysing parathion and degrading its hydrolysis product p-nitrophenol were isolated from parathion-amended flooded soils. They were identified as Pseudomonas sp. and Bacillus sp. (Siddaramappa et al., 1973). This finding is in contrast to several studies in which nitrogroup reduction is regarded as the major pathway of parathion degradation by micro-organisms in both soil, water and pure cultures (e.g. Graetz et al., 1970; Zuckerman, 1970). A synergistic action was reported by Gunner & Zuckerman (1968) for Arthrobacter sp. and Streptomyces sp. in the degradation of diazinon. It is conceivable that similar adaptation processes as the ones referred to above can develop in water bodies that are repeatedly or continuously contaminated with organophosphorus insecticides, such as the Rhine and its distributaries in the Netherlands.

The scientists engaged in this type of research do not agree on whether or not aerobic or anaerobic conditions favour microbial degradation. Sethunatan (1973) suggested that alternative oxidizing and reducing conditions may provide more suitable conditions for a complete destruction of pesticides than oxidation or reduction alone, especially for compounds possessing a ring moiety, since ring cleavage reactions require oxygen. This view is supported by the finding of Graetz et al. (1979) that under anaerobic conditions parathion was converted to aminoparathion, but that no further degradation took place.

A similar alternation of aerobiosis and anaerobiosis can be found in organically polluted waters with a large algal biomass and perhaps on the surface of higher water plants in alternating periods of photosynthesis and respiration. Anaerobic conditions can also be found in the bottom layers of water bodies. In the water phase the aerobic conditions will generally prevail. Therefore in most aquatic ecosystems conditions are present that enable microbial degradation of organophosphorus insecticides.

### 2.2.3 *Photolysis*

Photolysis of organophosphorus insecticides by ultra violet light has been studied by several investigators (Cook, 1954, 1955; Cook & Oltes, 1959; Mitchel et al., 1968; Liang & Lichtenstein, 1972; Heuer et al., 1974).

From these studies the conclusion can be drawn that by this mechanism organophosphorus compounds can be converted into transformation products. Most studies were made under artificial conditions and only the studies by Liang & Lichtenstein and Heuer et al. refer to the photolytic conversion in aqueous solution of an organophosphorus insecticide (azinphosmethyl). Liang & Lichtenstein (1972) found a rapid conversion of azinphosmethyl in aqueous solutions into seven breakdown products. But this finding does not guarantee a similar rapidity under natural conditions, since sunlight passing down through a water body decreases in intensity and marked changes in its spectral distribution occur. Therefore there are insufficient data to conclude that photolysis of organophosphorus compounds is of importance in aquatic ecosystems.

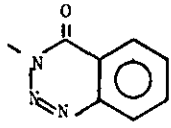
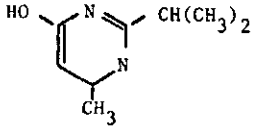
### 2.2.4 *Transformation products*

Many transformation products of organophosphorus insecticides have been identified, especially by studies with model ecosystems of the Metcalf-type (see for instance references in Metcalf, 1977). The transformation products of the compounds used in the present study are listed in Table 5. Among the major tranformation pro-

Table 5. Metabolites of four organophosphorus insecticides used in this study. For parathion-methyl no literature data were found, but its breakdown is probably analogous to that of parathion.
Sources: parathion: Sethunathan et al. (1977); Yu & Sandborn (1974); azinphosmethyl: Liang & Lichtenstein (1972); diazinon: Sethunathan & Pathak (1972), Gomaa et al. (1969); malathion: Wolfe et al. (1977).

| | | |
|---|---|---|
| Parathion | (a) Diethylphosphorothioic acid | $(C_2H_5O)_2\overset{H}{\underset{S}{P}}\text{-OH}$ |
| | (b) Diethylphosphoroic acid | $(C_2H_5O)_2\overset{H}{\underset{O}{P}}\text{-OH}$ |
| | Aminoparathion | $(C_2H_5O)_2\overset{H}{\underset{S}{P}}\text{-O-}C_6H_4\text{-}NH_2$ |
| | Paraoxon | $(C_2H_5O)_2\overset{H}{\underset{O}{P}}\text{-O-}C_6H_4\text{-}NO_2$ |
| | p-Nitrophenol | $HO\text{-}C_6H_4\text{-}NO_2$ |
| | p-Aminophenol | $HO\text{-}C_6H_4\text{-}NH_2$ |

Table 5. Continued.

| | | |
|---|---|---|
| Azinphosmethyl | (c) Dimethylphosphorodithioic acid | $(CH_3O)_2\overset{\overset{S}{\|}}{P}-SH$ |
| | (d) Dimethylphosphorothionic acid | $(CH_3O)_2\overset{\overset{O}{\|}}{P}-SH$ |
| | Dimethyl benzazimidesulfide | $R-CH_2-S-CH_2-R$ |
| | Dimethyl benzazimidedisulfide | $R-CH_2-S-S-CH_2-R$ |
| | N-methyl benzazimide | $CH_3-R$ |
| | benzazimide | $H-R$ |
| | azinphosmethyloxon | $(CH_3O)_2\overset{\overset{S}{\|}}{P}-CH_2-R$ |
| | R= |  |
| Diazinon | (a) + (b) | |
| | 2-Isopropyl-4-methyl-6-hydroxypirimidine (IMPH) |  |
| Malathion | (c) + (d) | |
| | Malathion β mono acid | $(CH_3O)-\overset{\overset{S}{\|}}{P}-S-\underset{\underset{CH_2-COOH}{\|}}{CH}-COOC_2H_5$ |
| | Diethylfumarate | $C_2H_5OOC\overset{\overset{CH-COOC_2H_5}{\|}}{C}H$ |
| | Malathion α mono acid | $(CH_3O)_2-\overset{\overset{S}{\|}}{P}-S-\underset{\underset{CH_2-COOC_2H_5}{\|}}{CH}-COOH$ |
| | Malaoxon | $(CH_3O)_2-P-S-\underset{\underset{CH_2-COOC_2H_5}{\|}}{CH}-COO_2H_5$ |
| | Diethylthiosuccinate | $HS-\underset{\underset{CH_2-COOC_2H_5}{\|}}{CH}-COOC_2H_5$ |

ducts of these insecticides are, of course, the diethyl and dimethyl phosphorodithioic, phosphorothioic and phosphoric acids.

Oxygen analogues have been found only for parathion and azinphosmethyl. In a model ecosystem study Yu & Sandborn (1974) detected 0.30, 0.47 and 1.4 mg $m^{-3}$ of parathion, paraoxon and p-nitrophenol respectively in the water phase after a period of 33 days. The high concentration of paraoxon is surprising as no other authors have reported the occurrence of this oxygen analogue in aquatic systems. Liang & Lichtenstein (1972) found substantial amounts of the oxygen analogue of azinphosmethyl under laboratory conditions. The oxygen analogues generally hydrolyse more

rapidly than compounds containing sulphur (Ruzicka et al., 1967).

For malathion six more metabolites were mentioned by Bender (1969) than are listed in Table 5. Only those are listed which, according to Wolfe et al. (1977) and Paris et al. (1975) can be considered as important.

## 2.3 TOXICITY TO AQUATIC ORGANISMS

### *2.3.1 Mode of action*

It seems well established that the insecticidal action of organophosphorus insecticides is primarily based on the inhibition of cholinesterases, present in the nervous system. The oxygen analogues of the thio-compounds react with the enzyme and block the hydrolysis of the normal substrate, acetylcholine (Corbett, 1974). The high reactivity of these compounds for the enzymes is coupled with a low dephosphorylation rate. This may result in an accumulation of acetylcholine, causing excessive stimulation, followed by a total block of impulse transmission. The symptoms of poisoning can be explained on the basis of this mode of action. Poisoned organisms initially show hyperactivity, followed by convulsive and uncoordinated movements, ending in paralysis and death.

Although the organophosphorus insecticides are mainly used as insecticides, they are also effective against mites, ticks, parasitic nematodes in animals and plants and molluscs (Corbett, 1974). They are also toxic to vertebrates, due to the same mechanism as in insects. In mammals acetylcholine is not involved only in the transfer of impulses across synaptic junctions in the central and autonomic nervous-system, but also in neuro-muscle transmission. The major cause of death in warm-blooded vertebrates when fatally poisoned with organophosphorus compounds is asphyxia, resulting from respiratory failure (Murphy, 1975).

Acetylcholinesterase is not the only enzyme inhibited by these insecticides. Various other esterases are inhibited by organophosphorus compounds, such as other cholinesterases, aliphatic (ali-)esterases and aromatic esterases. One group of these enzymes is the so-called pseudo-cholinesterases which occur in blood and other tissues of man and other mammals. The degree of inhibition of this enzyme is considered to be an indication of a certain degree of exposure.

In fish measurement of acetylcholinesterase inhibition in the brain can be used as a bio-assay technique to measure levels of cholinesterase inhibitors in water (Weiss & Gakstätter, 1974). Data on the inhibition of esterases in aquatic invertebrates are not readily available. From the poisoning symptoms described for daphnids (Section 6.4.1) and Asellus aquaticus (Section 6.4.2) the conclusion can be drawn that the mode of action in crustaceans is similar to that in insects. Other esterases are inhibited as well. The enzyme aliesterase in Daphnia magna for instance is strongly inhibited by in vivo exposure to organophosphates in concentrations less than 1 mg $m^{-3}$ (Dortland, 1978). As the biological meaning of other esterases than acetyl-

cholinesterase is not known, it is not possible to judge the toxicological consequences of their inhibition.

### *2.3.2 Toxicity to fish*

Comparative studies on the toxicity of different pesticides on estuarine (Eisler, 1970) and freshwater fish (Macek & McAllister, 1970) show that the organophosphorus insecticides are generally less toxic to fish than the organochlorines. In the latter study an assessment was made of the toxicity of three organochlorine, four organophosphorus and two carbamate insecticides on twelve species of freshwater fish, belonging to five different families. The 96-hour $TL_{50}$ values for the organochlorines ranged from 2 to 131 mg $m^{-3}$, while for the other two groups of insecticides these values varied from over 100 to more than 10 000 mg $m^{-3}$. The only exception was azinphosmethyl of which values smaller than 10 mg $m^{-3}$ were found (Table 6). This table shows that there is a marked species difference among the fish in susceptibility to

Table 6. Acute toxicity (in mg $m^{-3}$) of parathion, azinphosmethyl, diazinon, malathion and parathion-methyl to eight species of freshwater fish. Main source Macek & McAllister (1970). Other sources are references in (1) Pimentel (1972) and in (2) U.S. Environmental Protection Agency (1973).
Unless stated otherwise the data refer to 96-hour $LC_{50}$ (mg $m^{-3}$).

| Species | Temperature | Parathion | Azinphos-methyl | Diazinon | Malathion | Parathion-methyl |
|---|---|---|---|---|---|---|
| Pimephalus promelas (fathead minnow) | 18 °C | 1410 (2) | 235[1] | . | 8650 | 8900 |
| Lepomis macrochiris (bluegill) | 18 °C | 65 (2) | 22 | 52 (1) (24 h 24 °C) | 103 | 5720 |
| Micropterus salmoides (largemouth bass) | 18 °C | 190 (2) | 5 | | 285 | 5220 |
| Salmo trutta (brown trout) | 13 °C | . | 4 | . | 200 | 4740 |
| Salmo gairdnerii (rainbow trout) | 13 °C | . | 14 | 380 (1) (24 h 13 °C) | 170 | 2750 |
| Cyprinus carpio (carp) | 18 °C | )[2] | 695 | | 6590 | 7130 |
| Ictaluris punctatus (channel catfish) | 18 °C | . | 3290 | . | 8970 | 5710 |
| Perca flavescens (yellow perch) | 18 °C | . | 13 | . | 263 | 3060 |

1. Adelman et al. (1976) found 0.51 mg $m^{-3}$ as no effect level in a fecundity test.
2. Pimentel (1972) gives a no-effect level (96 h) of 500 mg $m^{-3}$; temperature not mentioned.

organophosphorus insecticides. Compared with other freshwater organisms like crustaceans and aquatic insects fish can be considered as relatively tolerant to this class of insecticides. A sub-lethal effect of an organophosphorus insecticide in fish was reported for malathion. Eaton (1970) described the appearance of spinal deformities in the bluegill (Lepomis macrochirus) after several months exposure to 7.4 mg $m^{-3}$. This crippling effect did not occur after eleven months exposure to 3.6 mg $m^{-3}$. The toxicological implications of low degrees of inhibition of brain-acetylcholinesterase in fish, as reported by Weiss (1959) are not clear.

*2.3.3 Toxicity to crustaceans*

With 25 000 species the crustaceans form a large and varied group of invertebrate organisms. Among the larger species are crabs, lobsters and shrimps but far more abundant are the smaller species. A classification of crustaceans which occur in freshwater in the Netherlands is given in Appendix A. Many of these represent important food organisms for fish, both marine and freshwater and therefore form an essential link in the food chain of aquatic life (Crosby & Tucker, 1966). This probably is one of the reasons why representatives of this class have frequently been used to determine the toxicity of various substances for aquatic life. The family Daphniidae with the well-known genera Daphnia and Simocephalus has been studied most frequently. Especially Daphnia spp. are predominant zooplankters which are very susceptible to toxic substances like pesticides. (Sanders & Cope, 1966). Generally the suborder Cladocera, of which the Daphniidae are a family, are most sensitive to organophosphorus insecticides. Other crustaceans like Copepoda (Muirhead-Thompson, 1971) and Ostracoda (Muirhead-Thompson, 1971; Khudairi & Ruber, 1974) are less sensitive to organophosphorus insecticides. The toxicity for Amphipoda closely resembles that for the Cladocera. A comparative study by Sanders & Cope (1966) with Daphnia pulex and Simocephalus serrulatus showed that daphnids are more sensitive to these insecticides than they are to the organochlorine insecticides and herbicides (Table 7). Exceptions are DDT, TDE and methoxychlor whose toxicity to daphnids is comparable with that of the organophosphorus compounds.

A similar trend can be observed from the toxicity data for American amphipods (Table 8). The gammarids are more sensitive to organophosphates than to organochlorines, with the exception of DDT, TDE, methoxychlor and aldrin. Such a clear difference was not found by Lüdemann & Neumann (1962) for the European species Carinogammarus roeselii. However, this difference might have been obscured by the short time of exposure (24 hours) and the relatively large concentration intervals that were used in this study. Therefore no accurate assessment of the LC50 could be made. For G. pseudolimnaeus subacute toxicity data were established in a 30 day testing period. No effect concentrations amounted to 0.20, 0.10 and 0.008 mg $m^{-3}$ for diazinon, azinphosmethyl and malathion respectively, indicating that even very low levels may be hazardous (U.S. Environmental Protection Agency, 1973). The data given by Lüdemann &

Table 7. Acute toxicity of selected organophosphorus and organochlorine insecticides for two species of Cladocera (95% confidence limits in parentheses). Figures represent 48-hour $EC_{50}$ immobilization values at 15.6 °C (mg $m^{-3}$). Source: Sanders & Cope (1966).

| Insecticide | Organism | |
|---|---|---|
| | Daphnia pulex | Simocephalus serrulatus |
| Organophosphates: | | |
| Baytex | 0.80 (0.56-1.1) | 0.92 (0.66-1.3) |
| Diazinon | 0.90 (0.67-1.2) | 1.8 (1.4-2.2) |
| Dibrom | 0.35 (0.22-0.78) | 1.1 (1.0-1.3) |
| Dichlorvos | 0.066 (0.049-0.088) | 0.26 (0.16-0.42) |
| Dipterex | 0.18 (0.13-0.25) | 0.70 (0.56-0.87) |
| Guthion[1] | 3.2 (1.8-5.8) | 4.2 (2.9-6.1) |
| Malathion | 1.8 (1.4-2.4) | 3.5 (2.6-4.8) |
| Parathion | 0.60 (0.39-0.79) | 0.37 (0.23-0.57) |
| Phosdrin | 0.16 (0.11-0.22) | 0.43 (0.33-0.57) |
| Phosphamidon | 8.8 (6.2-12.0) | 12.0 (8.0-18.0) |
| Organochlorines: | | |
| Aramite | 160 (110-220) | 180 (120-270) |
| Aldrin | 28 (20-39) | 23 (17-30) |
| Chlordane | 29 (23-36) | 20 (12-32) |
| Chlorobenzilate | 870 (690-1100) | 550 (460-650) |
| DDT | 0.36 (0.28-0.47) | 2.5 (1.9-3.3) |
| DDD (TDE) | 3.2 (2.3-4.4) | 4.5 (3.1-6.6) |
| Dieldrin | 250 (220-290) | 240 (200-280) |
| Endrin | 20 (13-32) | 26 (18-36) |
| Heptachlor | 42 (21-63) | 47 (32-68) |
| Lindane | 460 (390-550) | 520 (450-600) |
| Metoxychlor | 0.78 (0.57-1.1) | 5 (3.8-6.6) |
| Toxaphene | 15 (11-20) | 19 (12-29) |

[1] Guthion = azinphosethyl

Neumann (1962) show, that for both the organochlorine and organophosphorus insecticides the isopod Asellus aquaticus is generally as susceptible as C. roeselii, and also that organochlorines are more toxic to this isopod than organophosphates. Only the last conclusion is in line with the toxicity data for American species (Table 8).

On the whole there are few data referring to exposure periods longer than 96 hours and I found no data at all on effects of these insecticides on reproduction.

*2.3.4 Toxicity to aquatic insects*

Of the aquatic insects, some are truly aquatic in the sense that they live in the water throughout their life: They only leave the water to fly from one water body to another (e.g. Heteroptera, Coleoptera). Others only pass one or more stages of their life in the water. Either they live in the water as larvae (Ephemeroptera, Odonata), as larvae and pupae (Diptera) or as larvae and imagines (certain Coleopte-

Table 8. Acute toxicity of selected organophosphorus and organochlorine insecticides to North-American species of Isopoda and Amphipoda.
Figures represent the 96-hour $LC_{50}$ values (mg $m^{-3}$).
Source: References in U.S. Environmental Protection Agency (1973).

| Insecticide | Organism | | |
|---|---|---|---|
| | Isopoda | Amhipoda | |
| | Asellus brevicaudus | Gammarus lacustris | G. fasciatus |
| Organophosphates: | | | |
| Azinphosmethyl | 21.0 | 0.15 | 0.10 |
| Diazinon | . | 200 | . |
| Dichlorvos | . | 0.50 | 0.40 |
| Dursban | . | 0.11 | 0.32 |
| Fenthion | 1800 | 8.4 | 110 |
| Malathion | 3000 | 1.0 | 0.76 |
| Naled | 230 | 110 | 14 |
| Parathion | 600 | 3.5 | 2.1 |
| Organochlorines: | | | |
| Aldrin | 8 | 9800 | 4300 |
| DDT | 4 | 1.0 | 0.8 |
| Dieldrin | 5 | 460 | 600 |
| Chlordane | . | 26 | 40 |
| Endrin | 1.5 | 3.0 | 0.9 |
| Heptachlor | . | 29 | 40 |
| Lindane | 10 | 48 | 10 |
| Methoxychlor | 3.2 | 0.8 | 1.9 |
| Toxaphene | . | 26 | 6 |

ra). With regard to this group of insects most toxicity studies with insecticides have been made on Diptera. Several of these insecticides were developed especially for application in aquatic environments in order to control biting and non-biting mosquitoes and flies by destruction of their larvae in the water.

In these toxicity studies the Diptera were target organisms, and therefore most data refer to 24 hour LC90 values and no toxicity data for Diptera are listed in the Water Quality Criteria 1972 of the US Environmental Protection Agency (1973), where only non-target organisms are mentioned. When water bodies are unintentionally contaminated with insecticides, however, these larvae do have to be considered as non-target as well, since they form an integrated part of the aquatic ecosystem. It is therefore correct that Lüdemann & Neumann (1962) included Diptera larvae in their studies on toxicity of insecticides to non-target aquatic organisms. They found the 24 hour $LC_{50}$ for Chironomus plumosus to be 12, 29, 105 and 11 mg $m^{-3}$ for malathion, parathion, diazinon and chlorthion respectively. These values were of the same order as those for the organochlorine insecticides they tested. Chaoborus plumicornis was found to be less sensitive to both classes of insecticides.

Mulla et al. (1969) found that 24 hour LC50 values for chironomid larvae ranged

Table 9. Toxicity of selected organophosphorus and organochlorine insecticides to several species of stonefly naiads. Figures represent 96-hour $LC_{50}$ values (mg $m^{-3}$). Source: (1) Sanders and Cope (1968); (2) Jensen and Gaufin (1964).

| Insecticide | Organism | | | | |
|---|---|---|---|---|---|
| | Pteronarcys californica (1) | (2) | Acroneuria pacifica (2) | A. lycorias (1) | Claassenia sabulosa (1) |
| Organophosphates: | | | | | |
| Azinphosmethyl | 1.5 | 22 | 8.5 | . | . |
| Diazinon | 25 | . | . | . | . |
| Dichlorvos | 0.10 | . | . | . | . |
| Dursban | 10 | . | . | . | 0.57 |
| Fenthion | 4.5 | 26.5 | 5.1 | . | . |
| Malathion | 10 | . | . | 10 | 2.8 |
| Naled | 8.0 | . | . | . | . |
| Parathion | | 36 | 30 | | 1.5 |
| Organochlorines: | | | | | |
| Aldrin | 1.3 | 180 | 200 | . | . |
| Chlordane | 15 | . | . | . | . |
| DDT | 7.0 | . | . | . | 3.5 |
| Dieldrin | 0.5 | 39 | 24 | . | 0.58 |
| Endrin | 0.25 | 2.4 | 0.32 | . | 0.76 |
| Heptachlor | 1.1 | . | . | . | 2.8 |
| Lindane | 4.5 | . | . | . | . |
| Metoxychlor | 1.4 | . | . | . | 1.3 |
| Toxaphene | 2.3 | . | . | . | . |

from 0.42 (Dursban, in Chironomus sp.) to 54 mg $m^{-3}$ (fenthion, in Goldichironomus holoprassinus).

For comparison: the 24 hour $LC_{50}$ values of stonefly naiads (Pteronarcys californica) for 19 organophosphorus insecticides ranged from 14 to 2500 mg $m^{-3}$ with the majority in the 14-56 mg $m^{-3}$ range. (Sanders & Cope, 1968). Stoneflies are the most intensively studied freshwater insects in insecticide studies. The result of two comparative studies are summarized in Table 9. The values presented by Jensen & Gaufin (1966) are substantially higher than the corresponding values of Sanders & Cope (1968). The latter authors attribute this to differences in testing methods, temperature, size and age of the naiads, although most of these differences cannot be seen from the article by Jensen and Gaufin. Differences in life history of the naiads as well as the use of emulsifiers in the Jensen & Gaufin tests also may have played a role. Acute and subacute toxicity data for several other species of aquatic insects are summarized in Table 10. The data indicate that in other aquatic insect families, species may be present which are very sensitive to organophosphorus insecticides, especially where exposure is long term.

As for crustaceans, virtually no information exists on long-term effects and reproduction (e.g. pupation, emerging).

Table 10. Acute and sub-acute toxicity of selected organophosphorus and organochlorine insecticides for several species of aquatic insects. First figure represents 48-hour or 96-hour $LC_{50}$, second figure the 30-day $LC_{50}$ (mg $m^{-3}$).
Source: (1) U.S. Environmental Protection Agency (1972), references in (96-hour $LC_{50}$ values), (2) Jensen & Gaufin (1964) (96-hour $LC_{50}$ values), (3) Sanders & Cope (1968) (96-hour $LC_{50}$ values), (4) Cope (1966) (48-hour $LC_{50}$ values).

| Organism | Azinphos-methyl | Diazinon | Malathion | Parathion | Bayer 29493 | Aldrin | Dieldrin | Endrin | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Plecoptera (stoneflies): | | | | | | | | | |
| Pteronarcys dorsata | 12.1 4.9 | . | . | 3.0 0.90 | . | | | | (1) |
| P.californica | 22 1.3 | . | . | . | 26.5 3.6 | 180 2.5<br>1.3 | 39 2.0<br>0.5 | 2.4 1.2<br>0.25 | (2)<br>(3) |
| Acroneuria lycorias | 1.5 | 1.7 1.25 | 1.0 0.3 | . | . | | | | (1) |
| A. pacifica | 8.5 0.24 | . | | | 5.1 0.64 | 200 22 | 24 0.2 | 0.32 0.0035 | (2) |
| Ephemeroptera (mayflies): | | | | | | | | | |
| Ephemerella subvaria | . | . | . | 0.16 0.056 | . | . | . | . | (1) |
| Baetis sp. | . | . | 6.0 | . | . | . | 64 | 5 | (4) |
| Odonata: | | | | | | | | | |
| Ophiogomphus rupinsulensis | 12.0 2.2 | . | . . | 3.25 0.22 | . | . | . | . | (1) |
| Trichoptera: | | | | | | | | | |
| Hydropsyche bettoni | 74 | 3.54 | 0.34 | 0.45 | . | . | . | . | |

### 2.3.5 *Toxicity of transformation products*

In general conversion and degradation of organophosphorus insecticides result in less harmful products. Dipterex however, is converted by mild alkali to the much more toxic DDVP and conversion of phosphorothionates and phosphorothiolothionates to their oxygen analogues generally increases their toxicity. Of the transformation products of the compounds used in the present study (see Table 5) only a few can in themselves be regarded as hazardous. Among these are the oxygen analogues. p-Nitrophenol is a methaemoglobin inducer (Smith, 1974) and may cause a toxicological stress either directly or by the formation of nitrite.

Bender (1969) in studies with the fathead minnow found the known basic hydrolysis product diethylfumarate to be more toxic than the mother compound. He also noted a certain synergistic effect for some metabolites but calculated that only diethylfumarate could be produced in sufficient quantities to produce the synergistic effect in nature. Data pertaining to the toxicity of metabolites of azinphosmethyl and diazinon in aquatic environments are not readily available. The toxicity of metabolites of azinphosmethyl has been indicated by the WHO as an area for the future research (WHO/FAO, 1974). Of the diazinon metabolite IMPH it can only be said that it is rapidly converted to carbon dioxide (Sethunathan & Pathak, 1972).

## 2.4 CONCLUDING REMARKS

The data in the former sections clearly show that crustaceans and aquatic insects are very susceptible to organophosphorus insecticides. Fish are generally less susceptible. Another important observation is that almost all data refer to acute toxicity studies of no more than four days. This implies that possible long-term effects including those on reproduction are largely unknown.

From toxicity data available it cannot be concluded that organophosphorus insecticides are less toxic to aquatic organisms than organochlorines. However, organophosphorus insecticides do have the advantage that they do not show biomagnification to any appreciable extent. Also they appear to be more easily degraded by processes such as chemical hydrolysis and microbial breakdown resulting in shorter exposure times. Especially the generally high pH values in the surface waters in the Netherlands will favour chemical hydrolysis. In this respect it seems that the continuous presence of organophosphorus compounds in these waters has to be attributed to continuous immision rather than to retarded degradation.

According to the toxicity data the concentrations in the surface waters in the Netherlands may represent a toxicological risk for Cladocera and certain aquatic insects. Since adsorption to suspended particles and bottom mud as well as bioconcentration in algae has been reported, the question remains whether or not certain other groups of organisms are running an extra risk. Such groups might be the filterfeeders, filtering suspended particles for food, organisms that scrape sessile algae from

stones, plants and other surfaces (e.g. certain Cladocera, Cyclopoida, Ephemeroptera) and species that feed on decaying organic matter, or burrow in the mud like Tubificidae and certain Chironomidae larvae.

# 3 Methods for the measurement of organophosphorus insecticides

## 3.1 EXTRACTION

Samples of 1 or 1.5 $dm^3$ of water were extracted with aliquots of 150, 50 and 50 $cm^3$ of dichloromethane successively in a 2 $dm^3$ separatory funnel with a Teflon cork and stopcock, by vigorous manual shaking for one minute. The combined extracts were dried with anhydrous sodium sulphate and concentrated in a Kuderna-Danish evaporative concentrator in a waterbath at 60°C. The concentrate was transferred quantitatively into a calibrated 10 $cm^3$ cylinder with glass stopper. Thereafter the dichloromethane was evaporated under nitrogen flow and the sediment was redissolved in acetone up to a total volume of 1 $cm^3$. This solution was used for both the gas-liquid and thin-layer chromatography analysis.

## 3.2 GAS-LIQUID CHROMATOGRAPHY (GLC)

Measurements were carried out on a Tracor 550 gas chromatograph with a flame photometric detector operating in both the phosphorus and sulphur modes (526 nm and 394 nm filters respectively). The phosphorus detection was used for quantitative analysis, while the sulphur response only served as a qualitative check on the insecticides. The gas chromatograph was connected to a Yokogawa 3046 dual pen recorder operated at a speed of 1 cm/min. The column consisted of a 60 cm long, 2.7 mm i.d. coiled glass tube packed with 4% Silicone SE-30 and 6% SP-2401 on Supelcoport 100-120 mesh, obtained from Supelco Inc. (Pleuger Nederland, Amstelveen). Gas-chromatographic conditions are illustrated in Table 11. The samples were injected with microliter syringes at volumes of 5 and 10 $mm^3$.

Linearity of the phosphorus-response was checked with malathion (1-10 ng), parathion (1-10 ng) and diazinon (0-5 ng), respectively. The results indicate a good linear relationship in this range of concentrations (Table 12).

Recoveries of the insecticides from water collected from the outdoor model ecosystems were assessed by analyses of spiked samples of 1 $dm^3$ of water with aliquots of 0.5 and 1 µg of the compounds, administered in acetone. The recoveries were invariably high (Table 13) and therefore the results of the analyses were not corrected. The detection limits for the various insecticides are presented in Table 14.

Table 11. Gas-chromatographic conditions for the measurement of organophosphorus insecticides.
Programme I for any mixture of insecticides
Programme II for parathion alone
Programme III for azinphosmethyl alone
Programme IV additional to Programme I to separate malathion and parathion-methyl.

| | | |
|---|---|---|
| Carrier gas $N_2$ ($cm^3 min^{-1}$) | | 40 (I, II, III)<br>55 (II) |
| Detector gases | | |
| $H_2$ ($cm^3 min^{-1}$) | | 150 |
| $O_2$ ($cm^3 min^{-1}$) | | 15 |
| air ($cm^3 min^{-1}$) | | 30 |
| Temperature ($^oC$ column) | Programme I | 180 (1-2 min)<br>180-250 (2-4 min)<br>250 (4-8 min) |
| | Programme II, IV | 160 (0-8 min) |
| | Programme II | 250 (0-8 min) |
| | injection block | 220 |
| | detector | 220 |

Table 12. Peak height response for amount of insecticide injected (10 $mm^3$ total injection volume), using the phosphorus detector.

| Parathion | | Malathion | | Diazinon | |
|---|---|---|---|---|---|
| injected (ng) | response (cm) | injected (ng) | response (cm) | injected (ng) | response (cm) |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0.20 | 0.31 | 0.21 | 0.40 | 0.20 | 0.64 |
| 0.40 | 0.72 | 0.41 | 0.56 | 0.40 | 1.00 |
| 0.80 | 1.40 | 1.03 | 1.10 | 0.80 | 2.06 |
| 1.60 | 2.72 | 2.06 | 2.00 | 1.00 | 2.56 |
| 3.20 | 5.56 | 5.14 | 4.54 | 2.00 | 4.68 |
| 6.40 | 11.40 | 10.28 | 8.82 | 5.00 | 12.70 |
| 10.00 | 17.21 | | | | |
| correlation coefficient | | correlation coefficient | | correlation coefficient | |
| 0.9999 | | 0.9996 | | 0.9993 | |

Table 13. Recovery percentages after extraction of 1 $dm^3$ spiked water samples obtained from the outdoor model ecosystems.

| Insecticide | Average % recovery | Standard deviation | Min. and max. rec. | Number of samples |
|---|---|---|---|---|
| parathion | 99.5 | 2.6 | 96-105 | 9 |
| azinphosmethyl | 104.2 | 9.5 | 93-120 | 9 |
| diazinon | 105.0 | 7.1 | 100-110 | 2 |
| malathion | 103.0 | 4.3 | 100-106 | 2 |
| parathion-methyl | 96.5 | 5.0 | 93-100 | 2 |

Table 14. Detection limits for five organophosphorus insecticides in acetone solution. Total injection volume 10 $mm^3$. Detection limit at about 2 x noise.

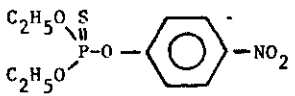
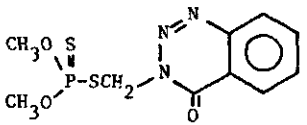
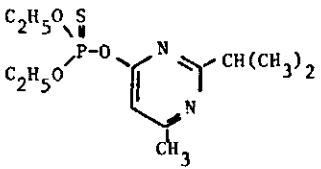
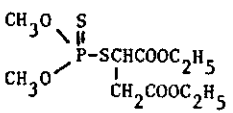
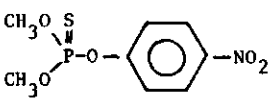
| Insecticide | Detection limit[1] (ng) |
|---|---|
| parathion | 0.4 |
| azinphosmethyl | 0.5 |
| diazinon | 0.2 |
| malathion | 0.3 |
| parathion-methyl | 0.4 |

[1] The detection limit in mg $m^{-3}$ calculated for 1 $dm^3$ of water is found by dividing these figures by 10.

### 3.3 THIN-LAYER CHROMATOGRAPHY (TLC)

Thin-layer chromatography was used for a qualitative confirmation of results obtained with GLC. The method according to Ernst & Schuring (1970) was used, with two minor modifications. The insecticides were oxidized by spraying the plate with 15 $cm^3$ 1% bromine solution instead of using bromine vapour. The solvent system used consisted of 93:7 v/v instead of a 94:6 v/v chloroform-ether mixture. Chromatospots were made by applying 20 $mm^3$ of the extracts in acetone on precoated TCL plates (Silica gel 60F 254 of Merck). Bees were obtained from the Department of Entomology of the Agricultural University and stored at -20°C.

Table 15. Names and formulas of the organophosphorus insecticides used in this present study.
Sources: (1) Merck index (1976); (2) Martin & Worthing (1977)

| Insecticide | Structural formula (1) | Chemical name (1) | Trade names (2) | Molecular formula (1) |
|---|---|---|---|---|
| Parathion |  | 0,0-diethyl O-p-nitrophenyl phosphorothioate | Folidol, Bladan (Bayer)<br>Thiophos (Am,Cyanomid Co)<br>Niron (Monsato chem.Co.)<br>Fosferno (Plant Protection Ltd) | $C_{10}H_{14}NO_5PS$ |
| Azinphosmethyl |  | 0,0-dimethyl ester, S-ester with 3-(mercaptomethyl)-1,2,3-benzotriazin-4 (3H)-one | Guthion<br>Gusathion M | $C_{10}H_{12}N_3O_3PS_2$ |
| Diazinon |  | O-(2-isopropyl-6-methyl-4-pyrimidinyl) ester | Basudin<br>Diazitol<br>Neocidol<br>Nucidol | $C_{12}H_{21}N_2O_3PS$ |
| Malathion |  | S-(1,2-dicarbethoxyethyl) O,O dimethyl-dithiophosphate | Cythion (Am.Cyanomid(CO)<br>Malathion<br>Malathiozol<br>Malathiozoo | $C_{10}H_{19}O_6PS_2$ |
| Parathion-methyl |  | O,O-dimethyl O-p-nitrophenyl phosphorothioate | Dalf<br>Folidol<br>Metacide<br>Bladan M<br>Nitrox 80 | $C_8H_{10}NO_5PS$ |

# 4 Laboratory toxicity tests

## 4.1 INSECTICIDES

The insecticides used in these tests were parathion, azinphosmethyl, diazinon, malathion and parathion-methyl. Their names, formulas and physico-chemical characteristics are tabulated in the Tables 15 and 16. All insecticides were of analytical grade (99% purity) (Pestanal, purchased from Riedl-de Häen, Aktiengesellschaft Seelze-Hannover (GDR)).They were stored at -20 °C in the dark.

## 4.2 TEST SPECIES; ORIGIN, REARING AND MAINTAINING PROCEDURES

Only Daphnia magna was cultured in the laboratory. All other test species were either collected in the field or from the outdoor model ecosystems.

Table 16. Physico-chemical properties of the organophosphorus insecticides used in this study.
Sources: (1) Merck Index (1976), (2) Martin and Worthing (1977), (3) The Chemagro Division Research Staff (1974).

| Insecticide | Molecular mass (1) | Melting point °C (1) | Vapour pressure (2) (mPa) | Solubility (2) |
|---|---|---|---|---|
| parathion | 291.27 | 6 | 5.0 at 20 °C | 24 g $m^{-3}$ in water at 25 °C; soluble in many organic solvents |
| azinphosmethyl | 317.34 | 73 - 74 | <51 at 20 °C | 14 g $m^{-3}$ at 15 °C (3)<br>29 g $m^{-3}$ at 25 °C (3)<br>47 g $m^{-3}$ at 35 °C (3)<br>soluble in most organic solvents, except aliphatics |
| diazinon | 304.36 | 83 - 84 | 186 at 20 °C | 40 g $m^{-3}$ at room temperature; soluble in many organic solvents |
| malathion | 330.36 | 2.9 | 5.3 at 30 °C | 145 g $m^{-3}$ at room temperature; soluble in many organic solvents |
| parathionmethyl | 263.23 | 37 - 38<br>35 - 36 | 1.29 at 20 °C | 55-60 g $m^{-3}$ at 25 °C; soluble in most organic solvents |

The daphnids were cultured in an artificial medium. As medium the Standard Test Medium (STM) according to Frear & Boyd (1976) was used. Initially a modified Flückiger medium (Flückiger & Flück, 1949) was used, but this was abandoned because precipates occurred in the stock solutions. The STM was prepared in 10 $dm^3$ glass bottles with glass-distilled water by adding 100 $cm^3$ 20 kg $NaHCO_3$ $m^{-3}$, 100 $cm^3$ 44.4 kg $CaCl_2.6H_2O$ $m^{-3}$ and 10 $cm^3$ 2.6 kg $K_2SO_4$ $m^{-3}$ to a total volume of 10 $dm^3$. The medium was then vigorously aerated overnight and quietly aerated for at least 24 hours prior to use. Characteristics of both the STM and modified Flückiger medium are given in Table 17.

Chlorella pyrenoidosa was reared for food using a nutrient solution and procedure according to the Dutch Standardization Institute (1976). The algae were concentrated by centrifugation and the pellet resuspended in STM. The concentration was estimated by graphic interpolation of the extinction at 540 nm at a 1:25 dilution of this suspension in STM. The stock suspension usually had a concentration of about

Table 17. Composition of the Standard Test Medium (STM) and the modified Flückiger Medium.

| Salt | STM (g $m^{-3}$) | Mod. Flück. Med. (g $m^{-3}$) |
|---|---|---|
| $NaHCO_3$ | 100 | 200 |
| $CaCl_2.6H_2O$ | 187.5 | 444 |
| $K_2SO_4$ | - | 26 |
| $MgSO_4.7H_2O$ | 18.5 | - |
| KCL | 6 | - |
| $Na_2HPO_4.2H_2O$ | 23 | - |
| $KHPO_4$ | 2 | - |
| $CaCO_3$ | 2 | - |

Table 18. Feeding schedule for D. magna Straus with the monocellular alga Chlorella pyrenoidosa.

| Age of the daphnids in days | Number of algalcells given in $10^8$ cells/ $dm^3$ per 25 daphnids | Density of the daphnids in the culture vessels per $dm^3$ |
|---|---|---|
| 0 - 2 | 1 | 1000 |
| 3 - 4 | 2 | 1000 |
| 5 - 6 | 4 | 1000 |
| 7 - 8 | 6 | 25 |
| 8 - 9 | 8 | 25 |
| >10 | 10 | 25 |

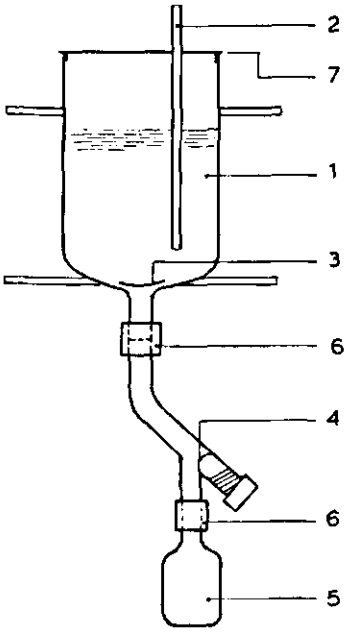



Figure 1. Sketch of a culture vessel used for the rearing of Daphnia magna. When (---) the vessel is darkened and the watch glass removed, the daphnids swim down into the bottle, which can be screwed off after the tap is closed. When the watch glass is replaced by a nylon sieve (1mm mesh) only the young daphnids can reach the bottle.
1 - all-glass culture vessel (10 $dm^3$)
2 - air inlet; one bubble every two seconds
3 - watch glass
4 - tap with Teflon screw-cork
5 - glass bottle
6 - screw couplings
7 - perspex top

$10^{12}$ cells/$m^3$. The algae were stored in the dark at 1 °C. Algae thus prepared and stored remained viable for at least 5 days. The daphnids were fed daily according to the scheme given in Table 18. They were reared in 10 $dm^3$ vessels (Fig.1) in 5 - 7 $dm^3$ of medium. The daphnids were kept at a density of 25 daphnids $dm^{-3}$.

The vessels were cleaned twice a week and the young removed. Thereafter the old medium was filtered over a sieve with a mesh size of 0.1 mm and 20 per cent of this old medium was used to make up the new medium (one part old supplied with 4 parts new STM). Daphnids were kept until the age of four weeks.

For the toxicity tests only daphnids younger than 24 hours of age and with mothers of 24 $\pm$ 4 days old were used.

*4.2.2 Other organisms*

*Asellus aquaticus L.* This species became available in large numbers in November 1976 during the routine cleaning of the MES[1]. Only specimens from the untreated MES were used for testing. Species identification based on the pattern of the head shield showed that all specimens belonged to the species A. aquaticus. They were kept at room temperature in large polythene containers of 80 x 40 x 15 cm filled up to a height of 10 cm with STM.

Elodea nuttallii, Potamogeton crispus and some small dead fish from the trout hatchery in the laboratory were supplied for food.

*Cloeon dipterum L.* The larvae of this mayfly species were collected from a local pond. They were kept in plastic aquaria (60 x 30 x 30 cm) with 5 cm STM and Elodea as substratum. For food Chlorella pyrenoidosa from the stock suspension of the Daphnia culture was provided as a film on the bottom. Temperature was kept at 18 $\pm$ 2 $^{o}$C. Specimens were identified at the end of the test, according to Macan (1970).

*Chaoborus crystallinus (de Geer)* The larvae were collected from a small local canal. They were kept in 20 $dm^3$ all glass aquaria filled with STM at room temperature. The larvae were fed on young daphnids from the culture and larvae of Artemia salina. All specimens were checked individually according to Johannsen (1973) at the end of the tests.

*Other test species* All other species were collected in either the untreated MES (Culex, Herpobdella, Tricladida) or the pond referred to above (Notonecta, Cloeon, Sigara). Unless stated otherwise, they were kept in STM at 18 $\pm$ 2 $^{o}$C, supplied with food and substratum where necessary.

4.3 ASSAY METHODS

*4.3.1 General test-conditions*

All toxicity tests were conducted in a climatic chamber at 18 $\pm$ 2 $^{o}$C. The temperature of the medium in the test beakers ranged from 17 - 19 $^{o}$C. The light regime was 14 hours light and 10 hours dark. In most tests STM was used, but some of the early tests with D. magna were conducted in modified Flückiger medium. The tests were run in 1 $dm^3$ glass beakers, filled with 0.8 $dm^3$ STM and covered with a watch glass. The test media were prepared as follows. Of each insecticide stock solutions were made of 800 g $m^{-3}$ ingredient in acetone. These solutions were diluted 1:1000 with STM. An

1 MES stands for Model ecosystem, see section 6.2.1

aliquot was pipetted out of these diluted solutions into the test beaker containing 0.4 $dm^3$ STM. Then the volume was made up to 0.75 $dm^3$ with STM. After the daphnids were transferred to this glass beaker the volume was adjusted to 0.8 $dm^3$ with STM. In each experiment two controls were run, one without acetone and the other with the highest acetone concentration used in the test range, but never more than 25 g $m^{-3}$. The acetone concentration in all insecticide concentrations of the test range was adjusted to that at the highest concentration of insecticide.
Four types of tests were performed:
- *acute* toxicity tests yielding the 48-hour $EC_{50}$
- *sub-acute* toxicity tests providing the 3-week $EC_{50}$ and for D. magna also the reproduction rate
- *comparative* toxicity tests in which all five insecticides were simultaneously tested in three concentrations for test periods up to three weeks
- *range-finding* toxicity tests yielding the 48 or 96-hours $EC_{50}$ value for the species which were difficult to maintain for longer periods or of which only limited numbers were available.

In all sub-acute tests the medium was renewed every Monday, Wednesday and Friday. In the sub-acute tests with daphnids the medium consisted of old medium from the Daphnia culture and new STM in a ratio of 1:3 (v/v), since tests performed with 100 per cent fresh medium resulted in a considerable mortality in the controls and breaking of the second antennae.

In the acute and range-finding tests the organisms were not fed. All species except the daphnids were acclimatized for at least 48 hours prior to testing in STM at 18 $\pm$ 2 °C, unless stated otherwise. During this period they were provided with substratum and food ad libitum.

### *4.3.2 Species-specific test-conditions*

*Daphnia magna* was fed daily according to the same scheme as used in the culture (Table 19). The amount of food was corrected for mortality only when the mortality rate was more than 10 per cent. Offspring was removed and counted when the medium was refreshed.

*Asellus aquaticus* was acclimatized in STM for at least one week in the climatic chamber. In the test a few sprigs of Elodea were used as substratum and food. Dead aselli were removed and their lengths measured. Sometimes, however, this was impossible due to scavenging by the living aselli.

*Cloeon dipterum* larvae were acclimatized for at least one week in STM at 18 $\pm$ 2 °C. Chlorella pyrenoidosa was applied as a film on the bottom of the beaker glass. A sprig of Elodea was provided as substrate. Dead specimens were removed, identified according to Macan (1970) and their body lengths were measured. Sometimes, however,

Table 19. Summary of the tests conducted with the different test species with a specification of the major test conditions.

| Species | Compounds tested | Type of test | | | | Number of organisms per concentration single/ duplicate | Criterion[1] | Medium |
|---|---|---|---|---|---|---|---|---|
| | | acute | sub-acute | comparative | range-finding | | | |
| D. magna | parathion | X | X | with all 5 | | 20 dupl. | mortality | STM/modified Flückiger |
| | azinphosmethyl | X | X | insecticides | | 20 dupl. | immobilization | |
| | parathionmethyl | X | X | | | 20 dupl. | reproduction | |
| | malathion | X | X | | | 20 dupl. | | |
| | diazinon | X | X | | | 20 dupl. | | |
| A. aquaticus | parathion | X | X | with all 5 | | 20 dupl. | mortality | STM |
| | azinphosmethyl | X | X | insecticides | | 20 dupl. | | |
| C. dipterum | parathion | X | X | with all 5 | | 20 dupl. | mortality | STM |
| | azinphosmethyl | X | X | insecticides | | 20 dupl. | | |
| C. crystallinus | parathion | X | X | with all 5 | | 20 dupl. | mortality | STM |
| | azinphosmethyl | X | X | insecticides | | 20 dupl. | | |
| H. octulata | parathion | | | | X | 10 s | mortality | STM |
| | azinphosmethyl | | | | X | 10 s | | |
| D. lugubris | parathion | | | | X | 10 s | mortality | STM |
| | azinphosmethyl | | | | X | 10 s | | |
| Notonecta sp. | parathion | | | | X | 10 s | mortality | STM |
| Sigara sp. | parathion | | | | X | 10 d | floating | natural water |
| Culex sp. | parathion | | | | X | | immobilization | natural water |

1 See definition of each criterium in the respective sections.

this was impossible since they were partially eaten by the specimens which were still alive.

*Chaoborus crystallinus* was acclimatized for at least 4 days in STM in the climatic chamber. Two hours before the tests started they were supplied with an abundance of newly hatched Artemia larvae. In the experiments only specimens with a full intestine were used as could be seen from the reddish colour due to the consumption of the Artemia larvae. In the sub-acute tests they were fed twice daily predominantly with daphnids from the culture.

A survey of the tests conducted with the different test species is given in Table 19, together with some test conditions.

### *4.3.3 $EC_{50}$ determination*

The percentage of effect per concentration was plotted on logarithmic-probability paper for determination of the $EC_{50}$ value for the different time intervals. The method of Litchfield & Wilcoxon (1949) for evaluating dose-effect experiments was used to determine the confidence limits of the $EC_{50}$. If the number of points between 0 and 100% were not sufficient to apply this method, a graphical interpolation was carried out between two adjacent points. In the tables the concentrations corresponding with these points are presented between square brackets.

The results of the comparative tests are presented in graphical form, by plotting median effective time against concentration. This median effective time ($ET_{50}$) is the time at which half the test population in a certain pesticide concentration exhibits the relevant effect. Usually this $ET_{50}$ value is found by interpolation on log-probality paper between two successive moments of observation and their corresponding percentages of effect.

## 4.4 RESULTS

### *4.4.1 Daphnia magna Straus*

*Symptoms of poisoning* The symptoms of poisoning by cholinesterase inhibitors were described by Wasserburger (1952) and Parker et al. (1970). From the many tests carried out in this study the following consecutive stages can be described.

1. Hyperactive swimming: the daphnids hang forward during rapid swimming, instead of having their normal vertical attitude.
2. Uncoordinated swimming: they make circular motions in all planes and forward and backward summersaults.
3. Paralysis: the animals lie on the bottom, but occasionally have short outbursts of uncoordinated swimming activity. Some of the daphnids show tremors.
4. Tremors: the daphnids stick to the bottom with heavy and constant tremors of the

second antennae, sometimes rotating slowly around their axis. In this stage they usually remain in one place.

5. Immobilization: the animals are generally immobile with some incidence of antennal movement.

In general all individuals exhibited all stages, but at high concentrations some of the stages may have been missed.

The measurement of effect was mainly based on parameter immobilization, corresponding with stages 4 and 5, although occasionally an individual exhibiting stage 3 may have been included. Stages 4 and 5 were invariable found to be moribund in both the acute and sub-acute tests. Whether or not the first two stages are to be considered as moribund probably depends on the time interval after which they are observed; the shorter the interval, the earlier a stage will be moribund.

The floating, described by Parker et al. (1970) was never observed and probably has to be attributed to surface-active compounds, unintentionally added in combination with the insecticides used in their studies.

*Toxicity data* The results of the acute and sub-acute tests are given in Table 20 and Fig.2. The data show that differences are minimal between duplicate series, but that different test series with the same compound may result in $EC_{50}$ values that differ a

Table 20. Estimated $EC_{50}$ immobilization values in $g\ m^{-3}$ for D. magna exposed to organophosphorus insecticides. Temperature 18 $\pm$ 2 °C. All tests were carried out in duplicate. Figures in parentheses represent P = 0.05 confidence limits. Figures between square brackets refer to concentration interval used for interpolation (see Section 4.3.3).

| Insecticide | 48 hour $EC_{50}$ | | 21 day $EC_{50}$ |
|---|---|---|---|
| | acute | sub-acute | |
| parathion | 1.3 (1.1-1.6) | 0.99 (0.72-1.4) | 0.33 (0.3-0.7) |
| | 1.3 (1.2-1.6) | 0.91 (0.60-1.2) | 0.35 (0.3-0.7) |
| | 1.4 (1.2-1.6) | | |
| | 1.3 (1.2-1.5) | | |
| | 0.7 (0.5-0.9) | | |
| | 0.7 (0.5-0.8) | | |
| azinphosmethyl | 1.6 (1.5-1.7) | 1.6 (1.2-2.1) | 0.28 (0.22-0.30) |
| | 1.6 (1.5-1.7) | 1.5 (1.2-2.0) | 0.26 (0.22-0.35) |
| diazinon | 1.5 (1.3-1.6) | 0.8 (0.7-1.0) | 0.22 (0.2-0.3) |
| | | 0.7 (0.6-0.9) | 0.24 (0.2-0.3) |
| malathion | 2.1 (0.14-14) | 1.6 (1.2-2.4) | 0.34 (0.3-0.6) |
| | 2.2 (1.6-3.1) | 1.7 (1.2-2.4) | 0.38 (0.3-0.6) |
| parathion-methyl | 9.1 (7-14) | 8.0 (4.8-6.9) | 2.0 (1.2-2.4) |
| | 8.5 (6-12) | 7.8 (4.8-9.6) | 2.0 (1.2-2.4) |

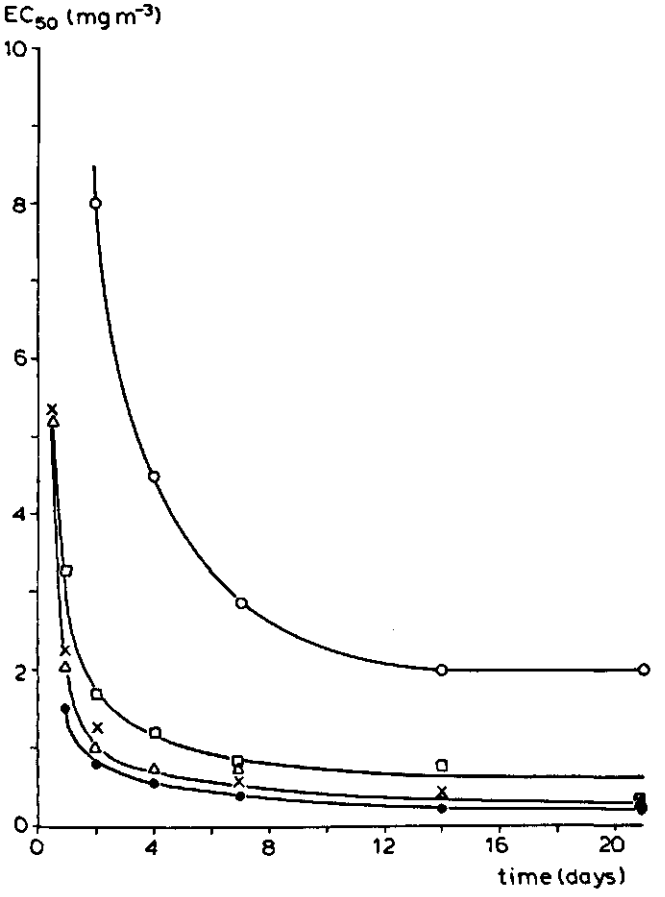



Figure 2. $EC_{50}$ values for five organophosphorus insecticides versus time of exposure. Values were obtained by calculation or interpolation (Section 4.3). (Δ) parathion, (X) azinphosmethyl, (●) diazinon, (□) malathion, (○) parathion-methyl.

factor two or more. Therefore all insecticides were tested in parallel tests, to obtain comparable results. (Fig.3).

In all tests parathion-methyl was the least toxic of all five compounds, followed by malathion. The most toxic compounds were parathion, azinphosmethyl and diazinon. Hardly any difference in toxicity was found among te latter compounds, although a preliminary conclusion from the comparative test might be that diazinon is slightly more toxic than the first two insecticides. It was also found that differences became smaller with longer exposure periods. All compounds except parathion-methyl can be considered as very toxic for Daphnia magna.

Data on reproduction and the approximate no-effect levels are presented in Table 21. Reproduction was only impaired with 0.4 mg $m^{-3}$ azinphosmethyl. Then the number of young produced was markedly less then in the controls, while at this level the reproducing adults did not show any symptoms of poisoning.

Table 21. Reproduction of D. magna during sub-acute exposure to organophosphorus insecticides. Figures represent the total number of offspring per daphnid over 21-days test period. When there was mortality among the adults, the numbers of offspring were calculated and presented in proportion to the number of adults which were still alive at the moment of removal of the young. Test were carried out in duplicate. Figures between parentheses indicate that 100 per cent mortality was reached before the end of the test.

| Parathion conc.mg $m^{-3}$ | | Azinphosmethyl conc.mg $m^{-3}$ | | Diazinon conc.mg $m^{-3}$ | | Malathion conc.mg $m^{-3}$ | | Parathionmethyl conc.mg $m^{-3}$ | |
|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 42.69 | 0.0 | 52.28 | 0.0 | 53.84 | 0.0 | 39.70 | 0.0 | 21.61 |
| | 50.70 | | 56.78 | | 50.95 | | 55.86 | | 41.06 |
| 0.1 | 50.46 | 0.1 | 60.22 | 0.1 | 49.62 | 0.15 | 57.34 | 0.15 | 42.70 |
| | 57.69 | | 57.12 | | 51.58 | | 61.20 | | 40.05 |
| 0.2 | 42.78 | 0.2 | 50.74 | 0.2 | 58.57 | 0.3 | 53.20 | 0.3 | 56.30 |
| | [1] | | 50.82 | | 59.66 | | 51.46 | | 60.33 |
| 0.3 | 60.63 | 0.4 | 27.44 | 0.3 | 38.58 [2] | 0.6 | 25.65 [2] | 0.6 | 44.05 |
| | 48.96 | | 28.36 | | 25.78 [2] | | 49.14 | | 48.83 |
| 0.7 | (2.40) | 0.6 | (2.08) | 0.4 | (2.00) | 1.2 | 0.00 | 1.2 | 44.69 |
| | (3.09) | | (6.12) | | 41.14 [2] | | 0.00 | | 50.10 |
| | | | | 0.6 | (1.00) | | | 2.4 | (17.85) |
| | | | | | (1.00) | | | | (17.64) |

no effect level for reproduction

| 0.3 | 0.2 | 0.2 | 0.3 | 1.2 |
|---|---|---|---|---|

no effect level for immobilization

| 0.2 | 0.1 | 0.2 | 0.15 | 1.2 |
|---|---|---|---|---|

1 Lost.
2 All adults either showed symptoms of stages 3, 4 or 5 (Section 4.4.1), or were dead.

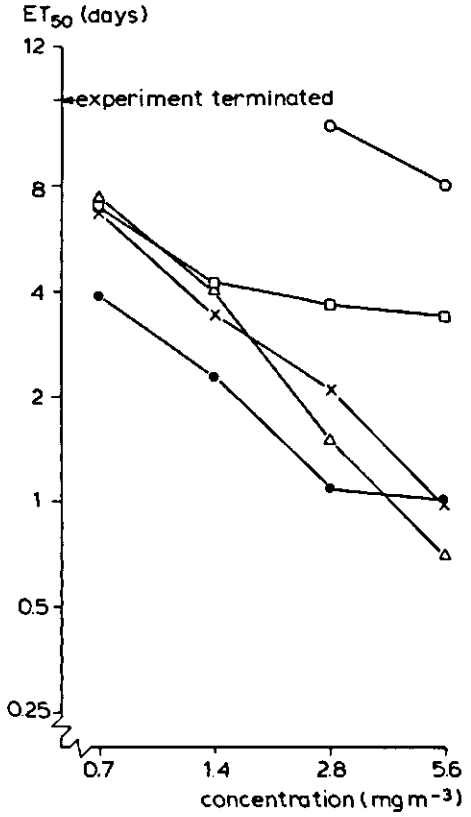



Figure 3. Comparative test on the toxicity of five organophosphorus insecticides to Daphnia magna. No mortality was observed in 0.7 mg $m^{-3}$ parathion-methyl at the end of the experiment. (Δ) parathion, (X) azinphosmethyl, (•) diazinon, (□) malathion, (o) parathion-methyl.

A lower birth rate was observed with diazinon and malathion. However, this coincided with toxic effects in the adults, which showed paralysis and tremors on Day 20 and 21, resulting in unborn and stillborn offspring. The very low reproduction figures at some of the highest concentrations were due to 100 per cent mortality of the adults before Day 14.

As can be seen in Table 22, the toxicity data obtained in the present study agree well with data found in the literature, in spite of differences in temperature and test species.

*4.4.2 Asellus aquaticus L.*

The symptoms of poisoning found were hyperactivity, followed by what could be described as uncoordinated 'dancing', with rapid gill movements. At a later stage the animals lay on their backs, wavering their legs. The gill and leg-movements gradually

Table 22. Comparative toxicities of organophosphorus insecticides for D. magna and D. pulex. Figures represent $EC_{50}$ immobilization values in mg $m^{-3}$.
(1) Values determined in this study
(2) Values reported by Sanders & Cope (1966).
(3) Values reported by references in U.S. Environmental Protection Agency (1973).

| | D. magna 48-hour $EC_{50}$ 18 ± 2 °C (1) | D. pulex 48-hour $EC_{50}$ 15.6 °C (2) | D. magna 21-day $EC_{50}$ 18 ± 2 °C (1) | D. magna 21-day $EC_{50}$ temp.? (3) |
|---|---|---|---|---|
| parathion | 1.3 | 0.60 | 0.34 | . |
| azinphosmethyl | 1.6 | . | 0.27 | . |
| diazinon | 1.5 | 0.90 | 0.23 | 0.26 |
| malathion | 2.2 | 1.8 | 0.36 | 0.6 |
| parathion-methyl | 8.8 | . | 2.0 | . |

Table 23. Estimated $LC_{50}$ values and no-effect levels in mg $m^{-3}$ for Asellus aquaticus exposed to parathion and azinphosmethyl at 18 ± 2 °C. Only part of the test at the high concentration rate of the sub-acute test was carried out in duplicate. All other tests were single. Figures in parentheses represent P = 0.05 confidence limits. Figures between square brackets refer to concentration interval used for interpolation (see Section 6.3).

| | Parathion | Average length (mm) | Azinphosmethyl | Average length (mm) |
|---|---|---|---|---|
| 48-hour $LC_{50}$ acute test | 20 (13-30) | 5.2 | 4.8 (4.0-5.6) | 5.0 |
| | 12 (18-18) | 3.1 | | |
| 48-hour $LC_{50}$ sub-acute test | 23 (17-32) | 4.9 | 6.7 (5.5-8.1) | 4.4 |
| | 22 [20-40] | 5.0 | 7.5 (6.2-9.2) | 4.4 |
| 21-day $LC_{50}$ | 4.8 (3.1-7.3) | 5.0 | 2.4 (1.7-3.5) | 4.6 |
| 21-day no effect level | 1.0 - 2.5 | 5.0 | 0.5 - 1.0 | 4.6 |

became slower until the intervals between the gill movements increased and became irregular, followed by death of the animals. Most stages of poisoning were found to be completely or partially reversible after the animals were transferred to STM without insecticide after an exposure period of 48 hours to parathion. No recovery was found with (nine) animals showing infrequent gill movements. The results of the acute and sub-acute tests are summarized in Table 23. These data indicate that azinphosmethyl is more toxic than parathion in all tests. The no-effect levels for mortality were 1.0 mg $m^{-3}$ and 0.5 mg $m^{-3}$ for parathion and azinphosmethyl, respectively. The level where no symptoms at all were observed was 1.0 mg $m^{-3}$ for parathion and 0.25 mg $m^{-3}$ for azinphosmethyl. From the results of the comparative test it can be concluded that azinphosmethyl is the most toxic of the five compounds tested and parathion-methyl the least toxic (Fig.4). Toxicity increases in the following order: parathion-methyl, diazinon, malathion, parathion, azinphosmethyl. A comparison with 48-hour $LC_{50}$ values for the American species A. brevicaudus (azinphosmethyl 21.0;

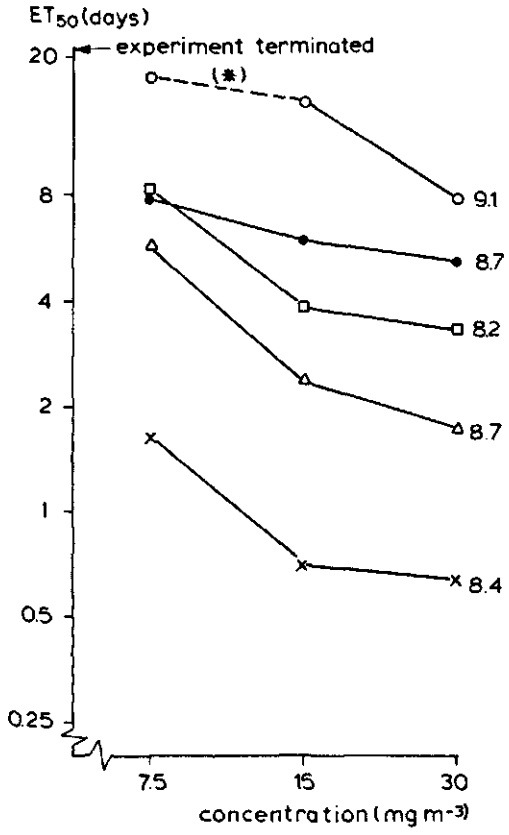



Figure 4. Comparative test on the toxicity of five organophosphorus insecticides to Asellus aquaticus. Figures indicate the average length (mm) of the organisms in each test series. (*) A sudden increase in mortality occurred in the concentrations 7.5 and 15 mg $m^{-3}$ of parathion-methyl, probably due to erroneous concentrations after the test medium was renewed at Day 15. (Δ) parathion, (X) azinphosmethyl, (•) diazinon, (□) malathion, (o) parathion-methyl.

malathion 600; malathion 3000 mg $m^{-3}$) indicates that A. aquaticus is probably far more susceptible to the toxic action of organophosphorus insecticides.

From the data of Lüdemann & Neumann (1962) it can be deduced that the 24-hour $LC_{50}$ values for A. aquaticus in these tests would be between 10 and 50 mg $m^{-3}$ for parathion, 50 and 100 mg $m^{-3}$ for diazinon and 1 and 50 mg $m^{-3}$ for malathion. These results seem in line with those obtained in the present study. For instance the 24-hour mortality in the acute tests with parathion amounted to 20 and 60 per cent at 40 mg $m^{-3}$ with an average length of 2.9 and 5.4 mm, respectively.

Table 24. Estimated $LC_{50}$ values and no-effect levels in mg $m^{-3}$ for Cloeon dipterum, exposed to parathion and azinphosmethyl at 18 ± 2 °C. Acute tests carried out in duplicate. Figures in parentheses represent P = 0.05 confidence limits. Figures between square brackets refer to concentration-interval used for interpolation (see Section 6.3).

| | Parathion | Average length (mm) | Azinphosmethyl | Average length (mm) |
|---|---|---|---|---|
| 48-hour $LC_{50}$ (acute test) | 2.5 (1.7-3.5) | 6.1 | 13.3 (10.8-16.4) | 6.2 |
| | 2.6 (2.0-3.4) | 6.4 | 12.0 (9.9-14.1) | 6.0 |
| 48-hour $LC_{50}$ (sub-acute test) | 1.7 [1.25-2.5] | 6.0 | 11.6 [8.0-16.0] | 5.8 |
| 21-day $LC_{50}$ | 0.38 (0.28-0.52) | 6.0 | 3.4 [2.0-4.0] | 5.8 |
| 21-day no-effect level | 0.15 - 0.1 | 6.0 | 2.0 - 4.0 | 5.8 |

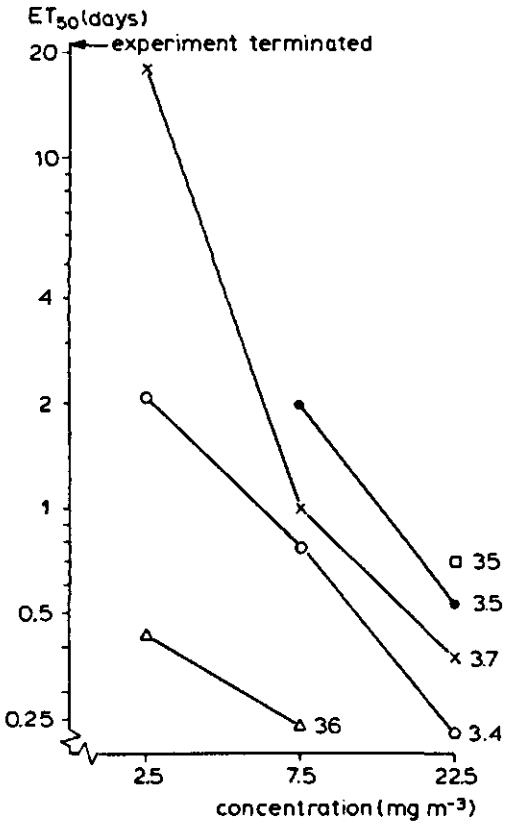



Figure 5. Comparative test on the toxicity of five organophosphorus insecticides to Cloeon dipterum. The following percentages mortality were found on Day 21: diazinon 2.5 mg $m^{-3}$: 11.1%; malathion 7.5 and 2.5 mg $m^{-3}$: 33.3% and 0% respectively. Numbers indicate the average length (mm) of the organisms for each test series. (Δ) parathion, (X) azinphosmethyl, (•) diazinon, (□) malathion, (o) parathion-methyl.

*4.4.3 Cloeon dipterum L.*

The symptoms of poisoning were roughly the same as those described for Asellus except for the gill movements which were not observed in sufficient detail. No attempt was made to estimate the moribund stage of poisoning.

The results of the acute and sub-acute tests are summarized in Table 24. Parathion is far more toxic than azinphosmethyl, not only after acute exposure, but also after sub-acute exposure and then the difference is even more pronounced. The no-effect level for parathion is competitive with the corresponding value for D. magna. The comparative tests show an increasing toxicity in the order malathion, diazinon, azinphosmethyl, parathionmethyl and parathion (Fig.5).

*4.4.4 Chaoborus crystallinus (de Geer)*

The first symptom of poisoning was hyperactivity, with the larvae and pupae rushing through the glass beaker with rapid spasmodic and jerky movements. The following stage was that they hung against the watersurface, still capable of diving from time to time, but apparently unable to stay deeper in the water. Finally both larvae and pupae lost their balance and started floating on their side. Some individuals everted the alimentary canal through the mouth, a phenomenon which has also been reported fore stonefly naiads (Jensen & Gaufin, 1966). This floating is permanent and irreversible because all the animals die in this position. Results of the acute and sub-acute tests are summarized in Table 25. All larvae were about 10 mm long and showed little variation in length.

Since approximately 10 - 15 per cent of the larvae emerged during the sub-acute tests, mortalities were calculated in relation to the number of larvae and pupae remaining at a given point of time. The data show that parathion is far more toxic than azinphosmethyl in both the acute and sub-acute tests. For parathion a no-effect level could not be established since 0.25 mg $m^{-3}$ was the lowest concentration in the series. The next higher concentration (0.5 mg $m^{-3}$) showed 100 per cent mortality at day 14 and 16. For azinphosmethyl a no-effect level of 2.0 mg $m^{-3}$ was found. Mortality

Table 25. Estimated $EC_{50}$ floating values and no-effect levels in mg $m^{-3}$ for Chaoborus crystallinus exposed to parathion and azinphosmethyl at 18 ± 2 °C. Figures in parentheses P = 0.05 confidence limits. Figures between square brackets refer to concentration interval used for interpolation (see Section 6.3).

| | Parathion | Azinphosmethyl |
|---|---|---|
| 48-hour $EC_{50}$ (acute test) | 1.0 (0.75-1.4) | 67 (50-89) |
| 48-hour $EC_{50}$ (sub-acute test) | 1.4 (0.99-2.1) | 33 (22-52) |
| 21-day $EC_{50}$ | 0.25 | 10.6 [8.0-16.0] |
| | 0.20 [0.0-0.25] | 10.2 [8.0-16.0] |
| 21-day no-effect level | <0.25 | 2.0 - 4.0 |

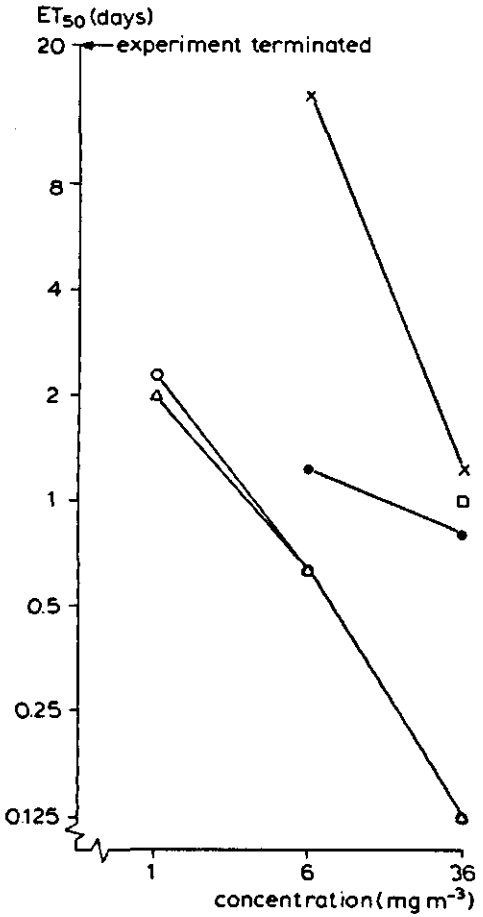



Figure 6. Comparative test on the toxicity of five organophosphorus insecticides to Chaoborus crystallinus. The following mortality percentages were found on Day 21: azinphosmethyl 1 mg $m^{-3}$: 31.6%; diazinon 1 mg $m^{-3}$: 15.8%; malathion 6 and 1 mg $m^{-3}$: 26.4% and 20%, respectively. (Δ) parathion, (X) azinphosmethyl, (•) diazinon, (□) malathion, (o) parathion-methyl.

at 4.0 mg $m^{-3}$ was 4.7 and 14.7 per cent on day 14 and 21, respectively. The comparative test shows that parathion and its methyl-analogue are equally toxic. The order of increasing toxicity is malathion, azinphosmethyl, diazinon, parathion and parathion-methyl (Fig.6).

*4.4.5 Other species*

The results of the acute tests with various other aquatic invertebrates are presented in Table 26.

*Culex sp.* The difference in susceptibility between larvae and pupae amounts to about three orders of magnitude. In order to test their reaction the pupae were gently

Table 26. Acute toxicities of parathion and azinphosmethyl to various species of aquatic invertebrates. Figures in parentheses represent P = 0.05 confidence limits.

| Organism | Criterium of effect | Exposure time (hours) | $EC_{50}$ | |
|---|---|---|---|---|
| | | | parathion | azinphosmethyl |
| Culex sp. larvae | immobilization | 48 | 1.1 (0.7-1.7) | . |
| Culex sp. pupae | immobilization | 48 | >1000 | . |
| Notonecta sp. adults + larvae | mortality | 48 | 5.1 (3.9-6.6) | . |
| Sigara sp. adults + larvae | floating | 48 | 2.6 (2.0-3.4)<br>2.3 (1.8-2.8) | . |
| Herpobdella octoculata | mortality | 96 | >160 | >160 |
| Dugesia lugubris | mortality | 96 | >160 | >160 |

touched with a Pasteur pipette. At 1 mg $m^{-3}$ they showed a slower reaction than in the control, but none of the individuals died within the test period.

*Notonecta sp.* Although it is virtually impossible to identify species of Heteroptera in the larval stages, it is most likely that all specimen used in the test belong to the species N. glauca since all adults found at the sampling site belonged to this species. The individuals suffered from extra stress due to a panic escape reaction upon every movement in the test room.

*Sigara sp.* For the same reason as given for Notonecta sp. all larvae probably belonged to the species S. lateralis. The individuals that started floating could not dive any more and were considered moribund.

The tests with the three aforementioned species were carried out in filtered water from MES IV.

*Herpobdella octoculata* With 160 mg $m^{-3}$ parathion twenty percent mortality was found in this leech species. The other individuals at this concentration and some at 80 mg $m^{-3}$ exhibited uncoordinated swimming when touched gently with a Pasteur pipette in order to test their reaction. After exposure to azinphosmethyl mortality amounted to twenty per cent at 160 mg $m^{-3}$. Uncoordinated swimming was exhibited by individuals exposed to the concentrations 40, 80 and 160 mg $m^{-3}$, respectively.

*Dugesia lugubris* Both in the control and in the test concentrations the individuals usually were motionless. They adhered to the wall of the glass beaker and moved only incidently. Their reaction to gentle stimulation with a pipette was invariable slow

Table 27. Order of decreasing toxicity of five organophosphorus insecticides to four species of aquatic invertebrates, based on the comparative toxicity tests.

| | Parathion | Azinphos-methyl | Diazinon | Malathion | Parathion-methyl |
|---|---|---|---|---|---|
| Daphnia magna | 1-3[1] | 1-3[1] | 1-3[1] | 4 | 5 |
| Asellus aquaticus | 2 | 1 | 4 | 3 | 5 |
| Cloeon dipterum | 1 | 3 | 4 | 5 | 2 |
| Chaoborus crystallinus | 1-2[1] | 4 | 3 | 5 | 1-2[1] |

[1] ex equo

in all concentrations as well as in the controls. Up to the maximum concentration of 160 mg $m^3$ used in the test no adverse effects could be observed.

## 4.5 GENERAL DISCUSSION

Of the four species that were extensively tested with respect to their sensitivity to a number of organophosphorus compounds D. magna was the most and A. aquaticus the least sensitive. There does not seem to be a marked difference in susceptibility between Cloeon dipterum larvae and the larvae of Chaoborus crystallinus in the acute test. The susceptibility of the last two species with regard to sub-acute toxicity and no-effect level for parathion closely resembles that of Daphnia magna.

It can be concluded from the comparative tests that parathion is the most toxic of the five compounds tested (Table 27). Parathion-methyl appears to be by far the least toxic for the crustaceans, while malathion is least toxic for the insect larvae. The toxicity of the other compounds does not vary markedly from species to species. Of the species only tested in the range-finding tests, the parathion $EC_{50}$ values for the insects, viz. Culex sp., Notonecta sp. and Sigara sp. are of the same order as for Cloeon dipterum and Chaoborus crystallinus. It cannot be excluded that adults might escape poisoning by flying away from a contaminated water body. However, there is no information available which supports this hypothesis. For the adult leeches and flatworms tested there seems to be little danger of their becoming poisoned by organophosphorus insecticides in the lower range of concentrations applied in the present study.

The conclusion can be drawn that chronic exposure to concentrations in the order of tenths of mg $m^{-3}$ of organophosphorus insecticides may endanger the survival and the reproductive potential of at least some species of Cladocera and aquatic insects.

# 5 Half-lives of five organophosphorus insecticides in the waterphase of freshwater aquaria

## 5.1 AIM OF THE EXPERIMENT

The aim of the experiment was threefold. First to get an impression of the dissipation rates that could occur in the outdoor MES, and so arrange a scheme for maintaining a certain concentration. Second to compare the rate of dissipation for the five insecticides used, and third to estimate the influence of water plants and bottom sediment on that rate.

## 5.2 MATERIALS AND METHODS

Of three 85 $dm^3$ all-glass aquaria, two (numbers 1 and 2) were provided with a 7 - 8 cm bottom layer of sand and very fine mud. This material was obtained from the same pond as referred to in Section 4.2.2. The water of this pond was analysed for cholinesterase inhibitors. However, the outcome of these measurements was negative. All three aquaria were then filled with water from the untreated outdoor MES and placed in a climatic room at a temperature of 20 $\pm$ 1 $^{o}C$. In Aquaria 1 and 2 220 gram (wet weight) of Elodea densa were planted. The third aquarium (No.3) was not provided with either bottom material or water plants so that the influence of sediment and plants on the dissipation rate could be evaluated. The aquaria were covered with a Plexiglass top to reduce evaporation but still permitting gas exchange and photochemical reaction. Two fluorescent daylight lamps of 40 watt each were placed 30 cm above the plastic cover of the aquaria to give 14 hour diurnal illumination. After a fortnight of acclimatization, the water was analysed for cholinesterase inhibitors by TLC. The results of these analyses were negative. The aquaria were subsequently treated with parathion, azinphosmethyl, diazinon, malathion and parathion-methyl, all at a concentration of 1 mg $m^{-3}$, calculated for the watervolume above the bottom layer. The compounds were injected from a stock solution of 100 mg $dm^{-3}$ in acetone and subsequently stirred with a small battery mixer. One $dm^3$ aliquots were withdrawn from the aquaria after 1, 20, 50, 90, 152, 216, 355, 525 and 1005 hours after dosing. Zooplankton in these samples was sieved off with a 0,1 mm sieve, killed in 5 per cent formaldehyde and counted.

The water samples were extracted and analysed by gas-liquid chromatography according to the procedures given in Chapter 3. During the experiment pH and the concentration of dissolved oxygen were measured at intervals.

*5.2.1 Calculation of the half-life*

The half-life time of the insecticide can be considered as a measure for the rate of dissipation in the water phase. Although several factors contribute to dissipation, according to Grahl (1973), the overall process can be characterized by one rate constant, resulting in a first order equation:

$$dc/dt = kc \qquad (1)$$

in which

c = mass concentration of the insecticide

t = time

k = rate coefficient of dissipation

Integration of Eqn 1 gives

$$k = -\ln (c/c_0)/t \qquad (2)$$

in which

$c_0$ = concentration at time zero.

Figure 7-9. Rate of decline of the concentration of five organophosphorus insecticides in the water phase of aquarium systems with (Fig.8,9) and without (Fig.7) bottom material and water plants. Curved were fitted by linear least squares analysis. (Δ) parathion, (+) azinphosmethyl, (X) diazinon, (□) malathion, (o) parathion-methyl, (•) initial concentration (calculated).

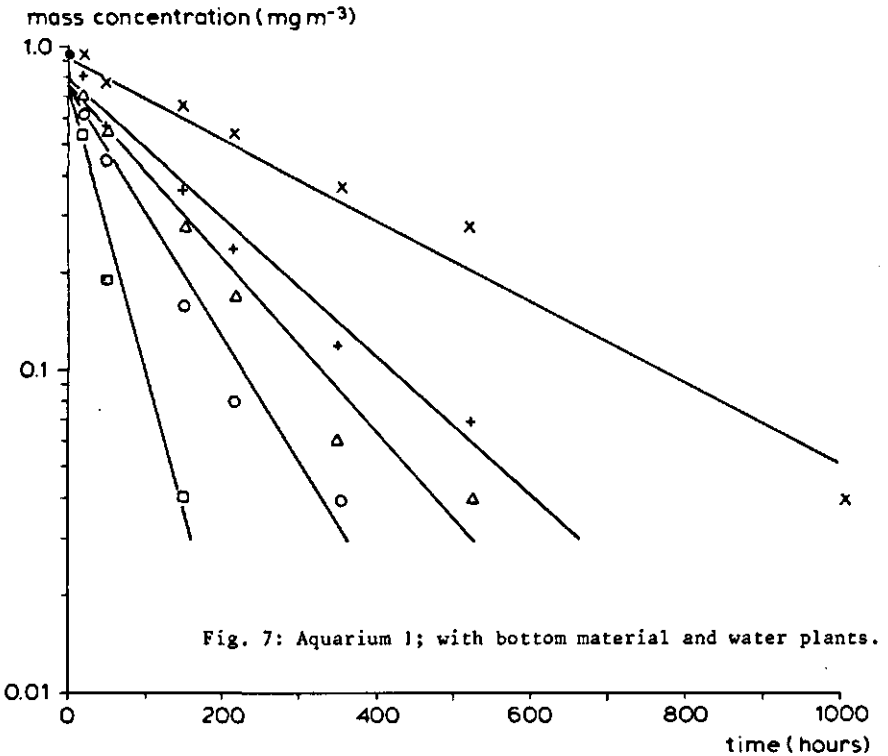



Fig. 7: Aquarium 1; with bottom material and water plants.

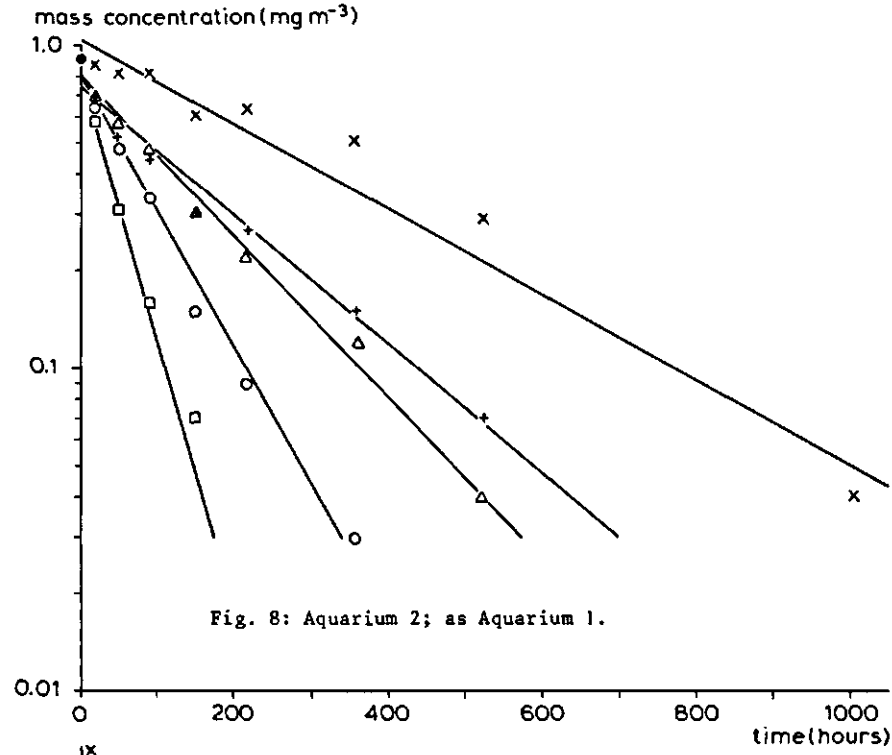



Fig. 8: Aquarium 2; as Aquarium 1.

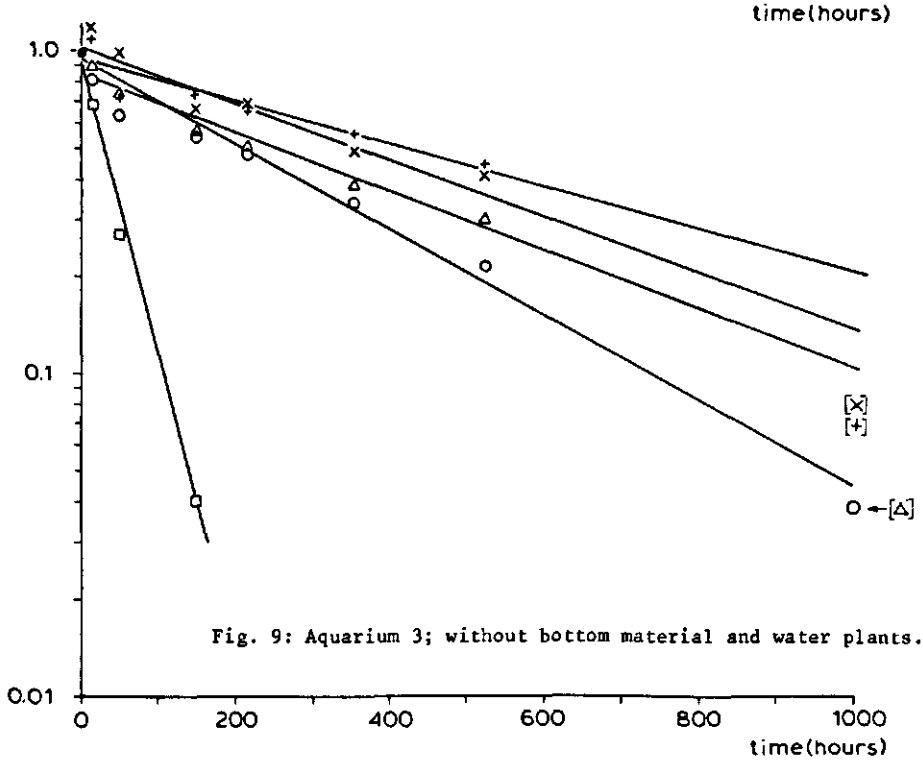



Fig. 9: Aquarium 3; without bottom material and water plants.

Thus the slope of a ln c versus time plot provides k. The half-life of the insecticides can then be found with

$$t_{\frac{1}{2}} = 0.693/k$$

## 5.3 RESULTS

The concentration of the five insecticides is plotted on a log scale against time in the Figures 7-9. In these graphs the concentrations measured at t = 1 are not plotted, because the results indicate that at that moment the insecticides were not yet fully mixed with the water phase. The calculated concentrations were used for the concentration at t = o. The derived half-lives are presented in Table 28. The results show a fair agreement between the Aquaria 1 and 2. However, half-lives in these two aquaria differ considerably from those in Aquarium 3, with the exception of the half-life for malathion which is almost the same in all three aquaria. These differences can only be attributed to the presence of bottom material and water plants in Aquaria 1 and 2, both being absent in Aquarium 3. Moreover the higher pH in Aquarium 3 might have favoured hydrolysis in this aquarium, (see Table 29). The pathways that may have

Table 28. Time in hours required for halving the initial concentration of five insecticides in the water phase of aquarium systems with and without bottom material and water plants.
Aquarium 1: water, bottom material, water plants
Aquarium 2: idem
Aquarium 3: water.
Figures in parentheses give the half-life times as a percentage of the corresponding half-lives in Aquarium 3.

| Insecticide | Initial concentration ($mg\ m^{-3}$) | | | Half-life (hours) | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| parathion | 0.98 | 0.95 | 0.92 | 112 (34) | 122 (37) | 333 |
| azinphosmethyl | 0.98 | 0.95 | 0.92 | 138 (28) | 151 (31) | 488 |
| diazinon | 0.98 | 0.95 | 0.92 | 227 (63) | 231 (64) | 361 |
| malathion | 0.98 | 0.95 | 0.92 | 34 (103) | 41 (124) | 33 |
| parathion-methyl | 0.98 | 0.95 | 0.92 | 78 (34) | 99 (43) | 231 |

Table 29. Average pH and concentration of dissolved oxygen in the aquaria during the experimental period.

| Aquarium | pH | | | Dissolved oxygen ($g\ m^{-3}$) | | |
|---|---|---|---|---|---|---|
| | average | standard deviation | number of measurements | average | standard deviation | number of measurements |
| 1 | 7.4 | 0.23 | 21 | 8.0 | 1.63 | 25 |
| 2 | 7.7 | 0.52 | 22 | 7.6 | 2.29 | 26 |
| 3 | 8.2 | 0.21 | 21 | 9.2 | 0.52 | 22 |

contributed to this rapid dissipation in the presence of mud and biota, are microbial degradation and adsorption to particulate matter and biota. During the experiment the biomass of Elodea increased considerably, resulting in a greater adsorption surface.

In Table 28 the relative half-lives for the various insecticides are given in parentheses as percentages of the half-life in Aquarium 3. These figures give an indication to what extent these pathways (apart from hydrolysis) may have contributed to the dissipation from the water phase, viz. the smaller the percentage, the higher the contribution. There is a meaningful contribution for azinphosmethyl, parathion and parathion-methyl and, to a lesser extent, for diazinon. In both the aquaria with plants and sediments the order of the half-lives for the five insecticides is the same: diazinon > azinphosmethyl > parathion > parathion-methyl > malathion.

On the basis of the results of this experiment a calculation could be made of the frequency by which the two pesticides parathion and azinphosmethyl had to be applied to the MES (see Section 6.2.4).

The results of the zooplankton countings do not permit more than some preliminary conclusions (see Appendix B).

# 6 Model ecosystems (MES) studies

## 6.1 INTRODUCTION

Over the past decade model ecosystems (MES) have come to play an increasingly important role in biological and toxicological investigations of both aquatic and terrestrial ecosystems. In general a MES can be regarded as an attempt to create a natural ecosystem on a model scale. These models can vary from very simple systems to systems which closely resemble natural ecosystems with regard to structural and functional relationships. Aquatic MES usually consist of aquaria in which some of the various components of natural ecosystems like bottom material, water, plants and animals are brought together to simulate a natural situation. Both the behaviour and fate of a chemical or its toxic effects on biota can be studied in such a system. The MES used as instruments in biological and toxicological studies are mainly laboratory MES, also referred to in the literature as microcosms or micro-ecosystems. These are in contrast to so-called outdoor MES, which are generally larger and may even consist of parts of natural ecosystems isolated from the main environment by artificial enclosures. Reviews on these microcosms were given by Taub & Pearson (1974), Metcalf (1977), Ringelberg & Kersting (1978). The first two reviews deal with the environmental behaviour of radionuclides and trace metals and the degradation of insecticides, respectively. The last study reviews the microcosms in relation to aquatic biology in general, without special reference to toxicological studies of chemicals.

Among the microcosms used as tools in toxicological studies the systems developed by Metcalf and coworkers are those most frequently used (Metcalf, 1977). With these systems information can be obtained about the following parameters: degradation pathways, transport, distribution over the compartments of the system and the potential for bioconcentration and biomagnification. In general, emphasis is on the behaviour of the compound rather than on the toxic effects, mainly because most laboratory MES are too simple for any detailed investigation of effects on populations or community structure. If emphasis is on the effects on biota, another type of MES should be used, especially where it is the aim to study long-term ecological changes, such as shifts in community size and structure as a result of the introduction of a chemical. Then, several conditions have to be fulfilled:

- Several trophic and other relationships representative of the natural ecosystem should be incorporated because the more meaningful ecological effects of toxic compounds are likely to result from changes in competition for nutrients, food-selection and other processes which determine community structure (Menzel & Case, 1977).

- The system should be able to sustain itself over a long period of time. This requires sufficient mineralization and recycling of nutrients and a continuous survival of the species, (Ringelberg & Kersting, 1978). The system should be operatable long enough to study any alterations in, for instance, life-cycle biology and the seasonal changes in population since these will be slow and often initially invisible, especially in response to exposure to chronic low levels of specific toxic chemicals.
- The system should be sufficiently large. This condition is not only to enable the species to escape overgrazing and overpredation, but also to create a certain representative diversity of species. Finally the system should also be large enough to permit a regular chemical and biological sampling, without disturbing the system.
- The system should be operatable in a way to distinguish between natural changes, such as seasonal changes due to climate, and those induced by the chemical introduced.

This last condition requires that an adequate control situation is available which preferably should be identical to the system treated. Such a control system could either be an 'internal' or 'external' blank. An internal blank implies that the changes observed in the period of treatment are compared with the pre-treatment situation. Here sufficient base-line information on the system should be available to serve as a reference for the development of the system that would have taken place without experimental intervention. For an external blank the development of the treated system is compared with that of a system which closely resembles, and preferably is identical to, the treated system. The more complex the system, the more difficult it is to obtain a blank. Therefore controls and experimentally manipulated systems are replicated so that the experiment can be statistically evaluated.

It does not seem possible to meet the four requirements on a laboratory scale. More promising seems an approach in which outdoor MES are involved. Such an outdoor MES for marine environments was developed in the framework of a research programme called Controlled Ecosystem Pollution Experiment (CEPEX) and was described by Reeve et al. (1976) and Menzel & Case (1977). In these experiments large bags of flexible transparent plastic are used to enclose a large water column (up to 2000 $m^3$) in order to capture a representative part of the aquatic ecosystem. The impounded ecosystems can either be treated with a chemical or left untreated, thus serving as replicate manipulated systems or controls, respectively. The basic idea of CEPEX was to study the effects of chemicals on a system of an almost natural degree of complexity with adequate controls. This idea also applies to the MES used in the present study, which can be considered as an example of an outdoor system which closely resembles a freshwater ditch community in the Netherlands. These MES consisted of polythene or Perspex containers of 3x1x1 meter, which were dug into the soil in a trial field of the Agricultural University. After the introduction of bottom material, water and biota, the systems were allowed to develop undisturbedly for 18 months before the trials started. During two consecutive summers the containers were divided crosswise into halves with a waterproof partition. The aim was to obtain two almost identical subsystems of which one was used for treatment with an organophosphorus insecticide

while the other served as control. It was anticipated that only minor differences would develop in these subsystems during summer if there was no experimental intervention. In this way the systems were considered to meet the four basic prerequisites discussed above.

## 6.2 MATERIALS AND METHODS

### *6.2.1 Design of the MES*

Four plastic containers were dug into the soil on a plot near the laboratory (Fig.10). Three containers were made of white polythene and were 3x1x1 meter. The fourth container was made of transparent Perspex and was 2.70x0.87x0.90 meter. The containers were surrounded by a wooden construction to protect the sides against water pressure from the inside and the pressure of the soil from the outside. The rims protruded approximately 30 cm above the soil surface to avoid contamination from the surrounding soil. Stainless-steel wire-nettings (mesh size 11 mm) were placed on top of the containers to prevent contamination of the water by leaves, litter and animals. The containers were provided with a bottom layer of approximately 0.1 m thick. The bottom material was collected from a comparatively clear watercourse without significant urban influences, called the 'Tutenburgse Wetering', situated near Giesbeek (Province of Gelderland). The watercourse had been enlarged three years before sampling during land consolidation work. The upper 10 cm of the bottom material from the watercourse was collected with a basket-type cutter having a fine-meshed wire-netting inside the basket. This material was partially dried in air and then sterilized for one day by steaming under a sheet. Subsequently the containers were filled with tap water, originating from deep groundwater. The polythene containers were filled in October 1975, the Perspex one in August 1976. The volume of water added was measured with a water-meter, the rise in water level with a stainless-steel rule, fixed to the east-side of each container. From the calibration curve, the volume in each of the containers could be inferred at any time from the water level.

About three weeks after the containers were filled with water, small plants of water thyme (Elodea nuttallii), hornwort (Ceratophyllum demersum), water starwort (Callitriche sp.) and pondweed (Potamogeton crispus, P. berchtoldii) were introduced. For each container the same wet-weight of plants was used, to promote a similar development. The plants were collected from a small watercourse along the river Linge near Heteren. They were kept in buckets and rinsed with tap water for three successive days to remove possible contaminants. The fourth container was supplied with plants obtained from the other three MES. Snails, leeches, flatworms and oligochaete worms were introduced through the plant material. Furthermore they were inoculated with about 2 $dm^3$ well-mixed fresh bottom sludge, containing micro-organisms, small crustaceans etc. All four containers were divided into two subsystems with a crosswise waterproof partition (Fig.10) at the start of the experimental periods which usually

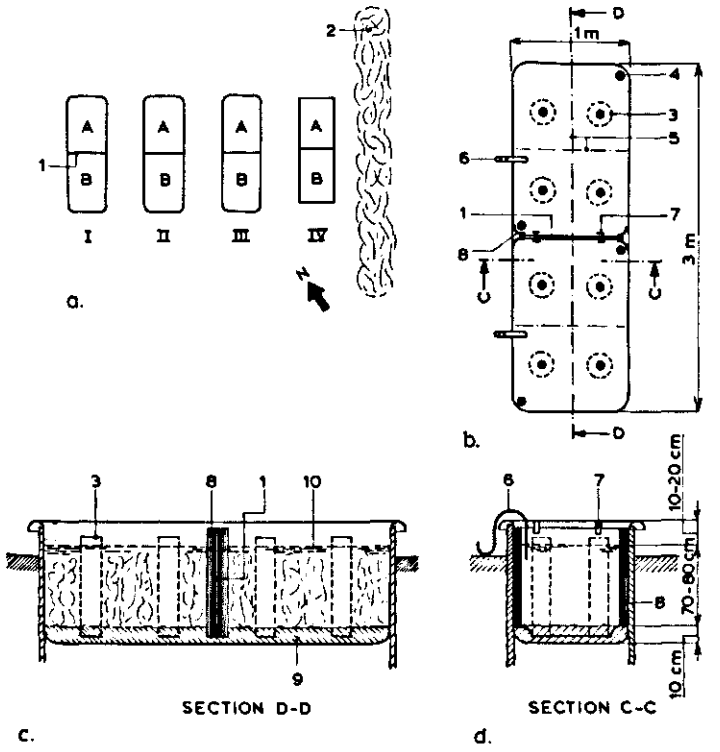



Figure 10. Sketch of the outdoor model ecosystems (MES) used in the present study. (a) arrangement of the MES, (b) top view, (c) side view, (d) front view.
1 = waterproof partitioning-sheet
2 = beech-hedge
3 = cylinders of stainless-steel wire-netting (mesh 1 cm)
4 = sampling points
5 = fish-line
6 = over-flow
7 = stainless-steel sheet-holder
8 = rubber flaps
9 = bottom material
10 = water surface

lasted from May to October. Thereafter the sheets were removed in order to restore pre-experimental conditions. In each subsystem four cylinders of stainless-steel wire-netting were placed upright in the water and partially sunk into the bottom layer. These cylinders which were 90-cm long and had a diameter of 15 cm were necessary for a proper sampling of biota (see Section 6.2.5).

### *6.2.2 Physico-chemical measurements*

Physico-chemical water parameters were recorded for the following purposes:
- to compare the physico-chemical characteristics of the MES-water with those of

Table 30. Physico-chemical water parameters measured in the outdoor MES in 1977 and 1978.

| Parameter | Unit | Frequency |
|---|---|---|
| pH | . | daily |
| dissolved oxygen | $g\ m^{-3}$ | daily |
| min. and max. temperature | $^{o}C$ | daily |
| Total phosphate (as $PO_4$) | $g\ m^{-3}$ | every two weeks |
| Orthophosphate (as $PO_4$) | $g\ m^{-3}$ | every two weeks |
| $NO_3^-$ | $g\ m^{-3}$ | every two weeks |
| $NO_2^-$ | $g\ m^{-3}$ | once a month |
| $NH_4^+$ | $g\ m^{-3}$ | once a month |
| Hardness (as CaO) | $g\ m^{-3}$ | every two weeks |
| $HCO_3^-$ | $mol\ m^{-3}$ | every two weeks |
| $Ca^{2+}$ | $g\ m^{-3}$ | once a month |
| $Mg^{2+}$ | $g\ m^{-3}$ | once a month |
| $K^+$ | $g\ m^{-3}$ | once a month |
| $Na^+$ | $g\ m^{-3}$ | once a month |
| $Cl^-$ | $g\ m^{-3}$ | every two months |
| Electric conductivity | $mS\ m^{-1}$ | twice a year |

surface waters in the Netherlands,
- to see whether or not the separate development of the subsystems would result in different physico-chemical characteristics of their waters,
- to signalize conditions which might be unfavourable for any of the organisms monitored and thus interfere with the experiments.

A survey of the physico-chemical measurements is given in Table 30. Concentration of dissolved oxygen, pH and temperature were measured daily between 8h00 and 8h30. Occasionally measurements were made at 4-hour intervals from 6h00 until 18h00. Dissolved oxygen was measured by a WTW Oxy 56 portable oxygen-meter with membrane-electrode (purchased from Wissenschaftlich-Technische Werkstätte GmbH, Weilheim, West Germany). For the pH measurements a portable pH-meter WTW pH 56 was used. Both pieces of apparatus had an accuracy of 0.1 unit.

### *6.2.3 Vegetation assessment*

A survey on the vegetation in the MES was made three times a year; at the end of May, half-way August and at the end of October for each subsystem separately. Of each plant species the covering percentage in both the floating and submerse layer was

estimated. To facilitate this estimation, nylon fish-line was stretched crosswise and lengthwise over the MES to divide the surface into eight equal parts, which were separately examined (Fig.10). The percentage was related to the total watersurface of a subsystem minus that within the cylinders.

*6.2.4 Insecticide applications*

A scheme for the insecticide treatment of the MES in the two summer seasons during which trials were carried out is given in Table 31. For the application of the insecticides to a subsystem of the MES the required dose was calculated for the total water volume of the compartment concerned at that moment. With a stainless-steel bucket a volume of about 15 $dm^3$ was withdrawn from the subsystem. This water sample was spiked with insecticide from a 800 g $m^{-3}$ stock solution in acetone, stirred and poured back into the subsystem in five dashes, about equally distributed over the water surface. The bucket was subsequently rinsed twice with 2 $dm^3$ water from the subsystem and the rinsing was poured back. It was anticipated that this procedure would thoroughly mix the insecticide over the system. Some temporary short disturbance of the vegetation could not be avoided. Only where duckweed covered the water surface did the disturbance last some time (order of days) because part of this weed became entangled in the submerse vegetation. The same procedure but without insecticide was performed with the corresponding control system.

Table 31. Scheme for the insecticide treatment of the model ecosystems.

| Year | MES | Insecticide | Intended concentration (mg $m^{-3}$) |
|---|---|---|---|
| 1977 | I A | parathion | 1 |
| | I B | - | - |
| | II A | - | - |
| | II B | - | - |
| | III A | azinphosmethyl | 1 |
| | III B | - | - |
| | IV A | - | - |
| | IV B | - | - |
| 1978 | I A | azinphosmethyl | 1 |
| | I B | - | 1 |
| | II A | parathion | 0.2 |
| | II B | - | - |
| | III A | parathion | 1 |
| | III B | - | - |
| | IV A | parathion | 0.5 |
| | IV B | - | - |

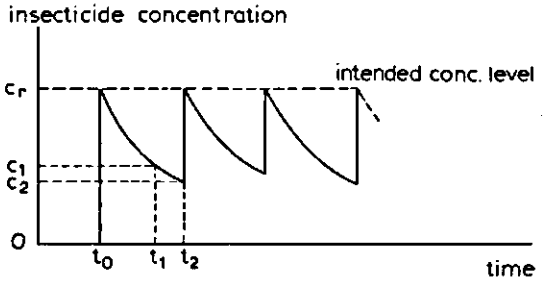



Figure 11. Concentration pattern of parathion and azinphosmethyl, respectively, in the water phase of the model ecosystems (MES). $c_r$ = intended concentration. $c_1$ = concentration measured by GLC. $c_2$ = calculated concentration.

To maintain a constant insecticide concentration the following procedure was used (Fig.11). At $t_o$ the insecticide was added to the system to make up the required concentration $c_r$. At $t_1$, usually 48 hours later, an aliquot of 1 $dm^3$ was sampled, extracted and analysed by GLC, yielding $c_1$. The rate coefficient of dissipation was calculated from Equation 2 (Section 5.2.1) with $c=c_1$ and $t=t_1-t_o$. Subsequently $c_2$, the calculated concentration at the time of redosing, was derived from the same equation by using $t=t_2-t_1$, usually a period of 24 hours. Thus 24 hours after sampling the initial concentration could be restored by adding $(c_r-c_2)v$, in which $v$ is the water volume of the system at $t_2$. This procedure resulted in a scheme consisting of two analyses per week (Monday, Thursday) and two re-applications of insecticide (Tuesday, Friday) per treated subsystem. In case of abnormally high $c_1$-values the normal re-application procedure was used. However, if $c_1$ was unusually low, less then the calculated dosage was added in order to avoid overdosing in case the actual concentration present at the time of analysis would have been underestimated. In each analysis a water sample of an untreated subsystem too was analysed for insecticide. The untreated subsystems were analysed in rotation, which implies a frequency of at least once a fortnight. The sampling technique will be described in Section 6.2.5.

*6.2.5 Population assessment of zooplankton and macrofauna*

For sampling of both zooplankton and water for insecticide analysis a 1-m long, 44 cm i.d. transparent Perspex tube was used (Fig.12). Through this tube a nylon cord was fixed to a rubber ball with a diameter of 5 cm, loosely set in a ring outside the tube. By pulling the nylon cord the rubber ball could be drawn out of the ring to lock the tube. An iron-wire construction was fitted to the lower side of the tube to prevent bottom mud from being sampled too. Without this construction it was virtually impossible to count a sample quantitatively due to the fine detritus. For the purpose of water sampling for insecticide analysis, the open tube was sunk slowly and vertically into one of the cylinders. When the wire construction reached the bottom, the tube was locked and the sampled water column was poured into a 2 $dm^3$ glass beaker.

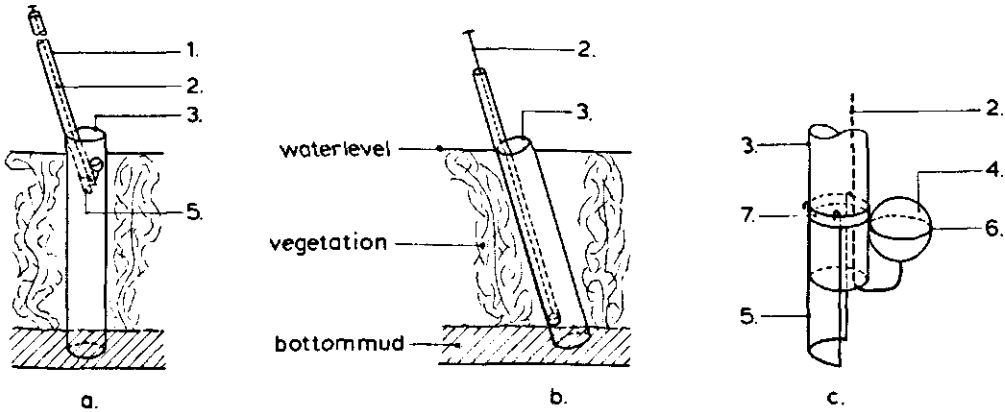



Figure 12. Sampling procedure with Perspex tube. The sampling tube is placed inside the cylinders of stainless-steel wire-netting (a), which is then pulled to the side while at the same time the tube is pushed downwards to the bottom. When the bottom is reached the nylon rope is pulled to lock the tube (b). Figure c represents a more detailed sketch of the bottom end of the sampling tube.
1 = sampling tube
2 = nylon rope
3 = cylinder of stainless-steel wire-netting
4 = rubber ball
5 = iron wire construction to prevent the bottom end of the tube from coming in contact with the mudlayer
6 = holder for the rubber ball
7 = clamp for wire-construction and ball-holder

From this sample a 1 $dm^3$ subsample was used for the GLC analysis.

For zooplankton sampling, the cylinder was slanted into the vegetation, the Perspex tube was rapidly sunk into the cylinder and closed when the wire construction reached the bottom (Fig.12). With the water flow the zooplankton drifted from the vegetation into the cylinder, where it was subsequently sampled. The cylinders were found to be necessary because of the dense vegetation which developed during the summer. Without them the tube pushed most of the vegetation downwards and restoration of the vegetation structure then took approximately one week. Repeated sampling thus would have resulted in a permanently compressed layer of water plants in the lower half of the container.

An extra advantage of these cylinders was the diversification of the MES by the creation of open spots, since vegetation did not grow inside the cylinders. Populations of Daphnia pulex and D. longispina were found almost exclusively inside these cylinders. A zooplankton sample of a subsystem was made up of six subsamples. The arrangement of these points is given in Fig.10. The total volume of water sampled amounted to approximately 7 $dm^3$. This volume of water was collected in a stainless-steel bucket, sieved through a Hydro-Bios plankton-net with an unscrewable tip cup with gauze bottom (mesh 55 µm). During sieving the lower part of the net was suspended in the water to minimize withdrawal of water from the MES and disturbance of the

system. The plankton was killed with 5 per cent formaldehyde and separated into two fractions with a double sieve of 1 mm and 0.01 mm mesh, respectively. The plankton was counted in the total fractions. The crude fraction usually contained some macrofauna and a few larger specimens of Daphnia, Simocephalus and Cyclopoida and was preserved in 90 per cent ethanol. This sample was counted in a Petri dish. The finer sample was preserved in 7% formaldehyde and counted on a slide, covered with a cover slip. This slide (7.5 x 2.5 cm) was divided into longitudinal strips of 2 mm which were successively examined under the microscope.

In addition to the collection of macrofauna in the weekly zooplankton samples, a momentary survey of the macrofauna population was made at the time the MES were cleaned during November 1976 and 1977. At that time half of the vegetation of the MES was removed to prevent the vegetation becoming choked the year following, as well as to simulate the cleaning regime normally carried out in ditches in the Netherlands. The aquatic plants collected in this way were then searched for macrofauna which were subsequently identified and counted.

## 6.3 RESULTS

### *6.3.1 Development of the MES in the pre-experimental period*

In the course of 1976 the first three MES developed into basins with a varied abundance of flora and fauna. They showed similarity to a ditch system of a relatively undisturbed nature, as occasionally observed under field conditions.
The water was invariably transparant, permitting a clear view of the bottom and submerged vegetation. Despite the attempt to create similar systems, the MES differed in both abundance and composition of the flora. In all three basins Elodea nuttallii was the dominant species: in MES I, II and III it comprised 70, 50 and 95 per cent respectively, of the higher water plants. Ceratophyllum demersum, being one of the more dominant species of plants in later years, was virtually absent in the pre-experimental period. Most diverse in plant species was MES II. The vegetation was dense in all MES except for MES III.

In the summer of 1976 different sampling and counting techniques for zooplankton and macrofauna were tried out. The results showed that there were no meaningful differences in composition of zooplankton and macrofauna. Neither did the species composition of the zooplankton of the system alter very much in the following years, with the exception of Ceriodaphnia quadrangula that was abundant in MES I and II in 1976 but was not found anymore in 1977 and 1978.

### *6.3.2 Physico-chemical measurements*

*Water temperature* The maximum and minimum water temperatures for the seasons 1977 and 1978 are presented in Fig.13 and Table 32. The figure gives the temperature pat-

Table 32. Average pH and the minimum and maximum temperature of the water in the MES durirg the experimental season in 1977 and 1978.

| MES | Average pH | | Average min. temp. (°C) | | Average max. temp. (°C) | |
|---|---|---|---|---|---|---|
| | 1977 | 1978 | 1977 | 1978 | 1977 | 1978 |
| I A | 8.9 | 9.3 | 13.2 | 13.0 | 16.6 | 15.6 |
| I B | 9.1 | 9.3 | | | | |
| II A | 7.6 | 8.0 | 12.9 | 13.2 | 15.9 | 15.3 |
| II B | 8.3 | 8.3 | | | | |
| III A | 8.1 | 8.9 | 13.3 | 12.9 | 16.7 | 15.4 |
| III B | 8.9 | 9.0 | | | | |
| IV A | 9.4 | 8.4 | 13.0 | 12.9 | 15.0 | 14.7 |
| IV B | 9.5 | 8.4 | | | | |

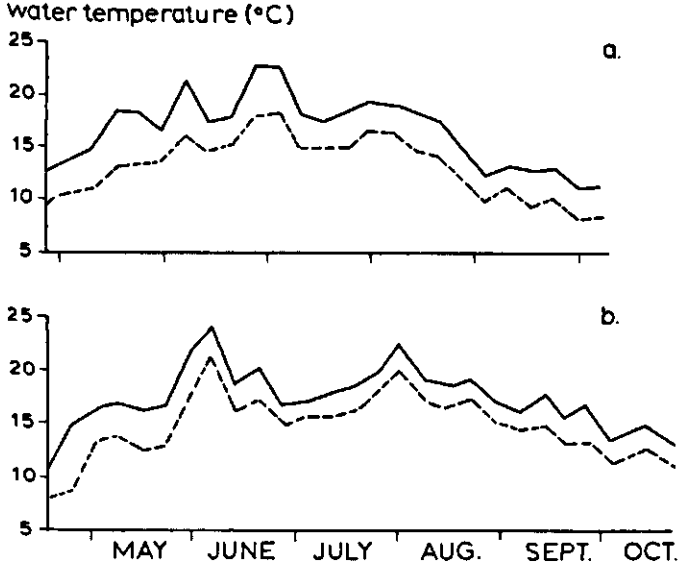



Figure 13. Minimum and maximum temperature of water in MES I in 1977(a) and 1978(b), respectively.

tern for MES I as being representative for the other MES as well, which is not surprising since the atmospheric temperature is the major determinant of this pattern. Among the different MES there are, however, differences in the daily amplitude, as can be derived from Table 32; the amplitude of MES I, II and III being higher than that of MES IV. This difference is mainly caused by the shading of this last MES by a beech-hedge (see Fig.10). A shading effect by duckweed cover is reflected in the lower maximum temperatures of MES II in both 1977 and 1978.

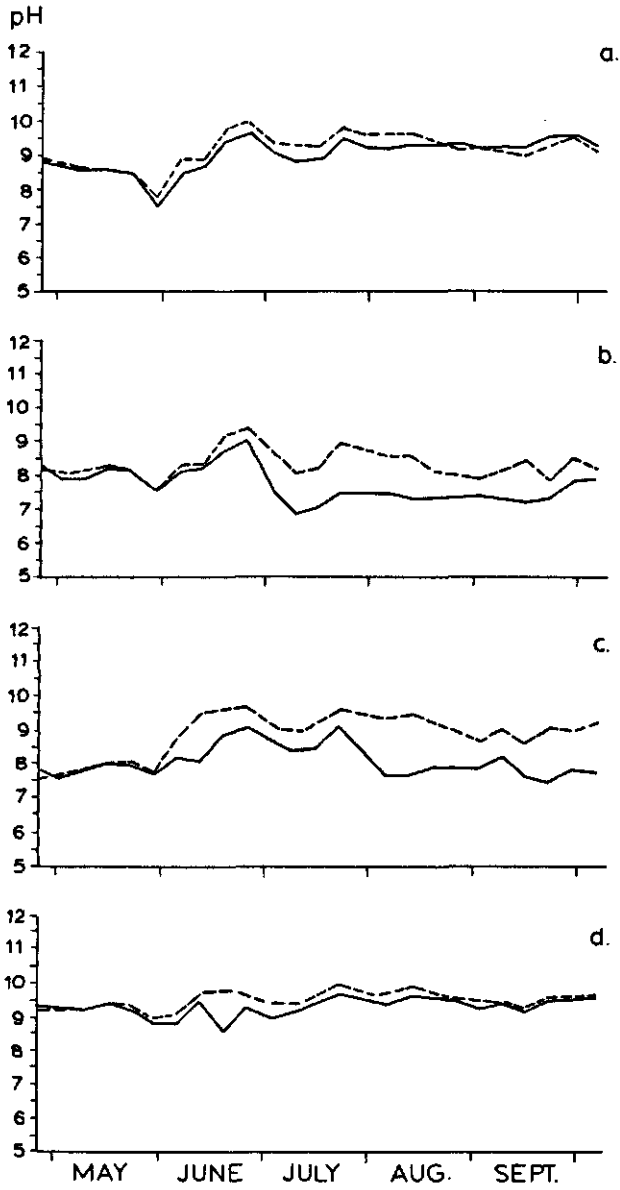



Figure 14. Values for pH in the water of MES I/IV (Figs. a/d, respectively) in 1977. —— treated subsystem (except MES II, IV) --- control.

*Measured pH values* The seasonal developments of this parameter for all MES are presented in Fig.14 a-d and Fig.15 a-d for 1977 and 1978, respectively. The average pH values for both seasons are given in Table 32. The patterns differ per season and per system. Among the corresponding subsystems the differences in pH are generally small.

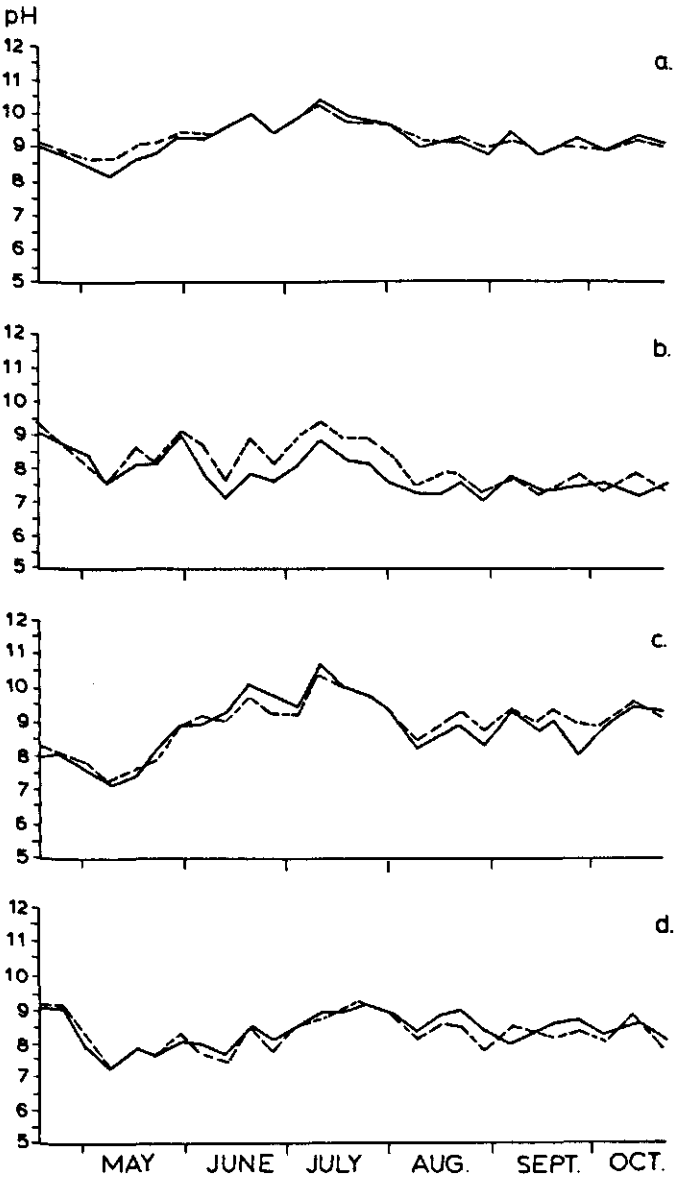



Figure 15. Values for pH in the water of MES I/IV (Figs. a/d, respectively) in 1978. — treated subsystem --- control.

On several occasions pH was measured at 4-hour intervals during the day. Some of these data are tabulated in Table 33 to illustrate that even on sunny days the diurnal fluctuation generally did not exceed 2 pH-units, and also that occasionally pH values exceeded a value of 10.

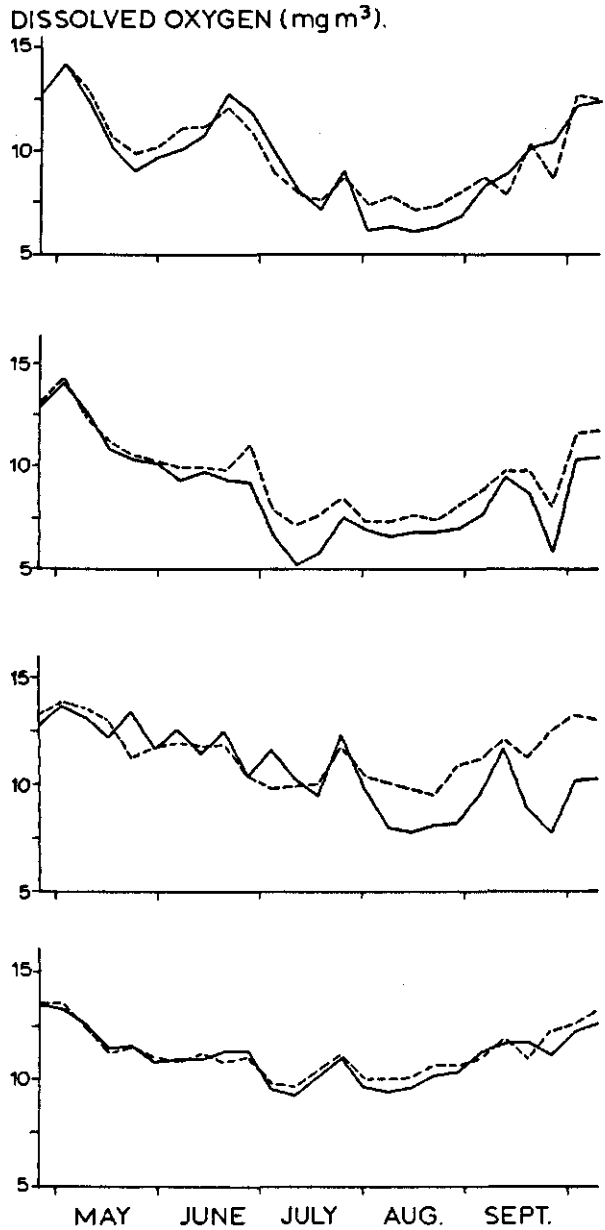



Figure 16. Dissolved oxygen in MES I/IV (Figs. a/d, respectively) in 1977. —— treated subsystem (except MES II, IV) --- control.

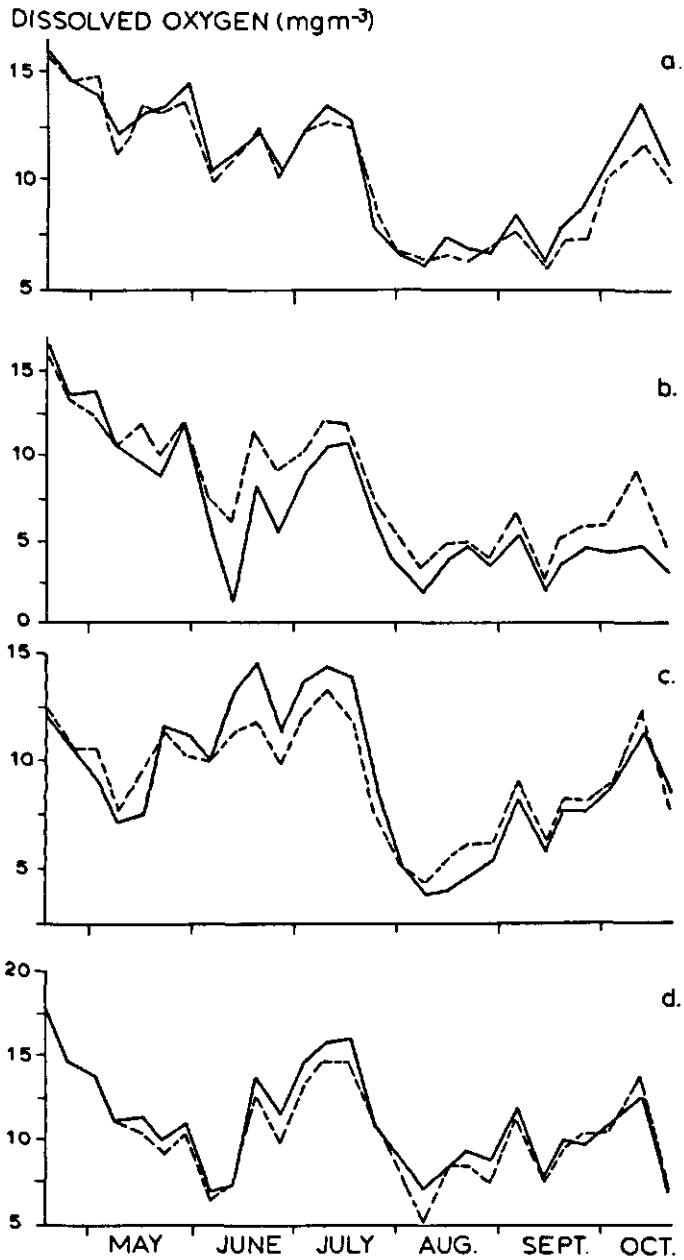



Figure 17. Dissolved oxygen in MES I/IV (Figs. a/d, respectively) in 1978. —— treated subsystem --- control.

Table 33. Development of the pH in the different subsystems of the MES on one cloudy and two sunny days.

| Day | Time | MES I | | MES II | | MES III | | MES IV | | Weather conditions |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | A | B | A | B | A | B | |
| 20 July | 6.00 | 8.4 | 9.0 | 6.6 | 7.5 | 6.7 | 8.8 | 9.0 | 9.5 | cloudy |
| 1977 | 10.00 | 8.8 | 9.4 | 7.0 | 7.8 | 6.9 | 8.9 | 9.0 | 9.4 | max. temp. 24 °C |
| | 14.00 | 9.0 | 9.4 | 7.4 | 8.6 | 7.4 | 9.0 | 9.0 | 9.5 | hours sunshine: 4 |
| | 18.00 | 9.0 | 9.3 | 7.7 | 9.2 | 7.9 | 9.2 | 8.9 | 9.4 | |
| 4 July | 6.00 | 9.6 | 9.9 | 8.9 | 9.4 | 9.4 | 9.4 | 9.7 | 10.0 | sunny |
| 1977 | 10.00 | 10.0 | 10.4 | 9.4 | 9.7 | 9.6 | 9.8 | 9.8 | 10.1 | max. temp. 27 °C |
| | 14.00 | 10.2 | 10.5 | 9.6 | 9.9 | 9.7 | 10.3 | 9.9 | 10.2 | hours sunshine: 14 |
| | 18.00 | 10.1 | 10.5 | 9.7 | 9.9 | 10.2 | 10.4 | 10.0 | 10.1 | |
| 31 May | 6.00 | 8.0 | 8.1 | 8.9 | 9.1 | 8.9 | 8.6 | 8.0 | 8.2 | sunny |
| 1978 | 10.00 | 9.3 | 9.5 | 9.2 | 9.4 | 9.1 | 9.0 | 8.3 | 8.4 | max. temp. 27.5 °C |
| | 14.00 | 9.6 | 9.8 | 9.5 | 9.6 | 9.2 | 9.4 | 8.5 | 8.8 | hours sunshine: 14 |
| | 18.00 | 9.6 | 9.7 | 9.6 | 9.7 | 9.5 | 9.6 | 8.5 | 9.0 | |

*Dissolved oxygen* The seasonal fluctuations in the concentrations of dissolved oxygen are presented in Fig.16 a-d and Fig.17 a-d for 1977 and 1978, respectively. Concentrations were generally high and often the water in the MES was supersaturated with oxygen.

*Other parameters* The other physico-chemical variables measured are summarized in Table 34. In general both the differences between corresponding subsystems and among separate MES were small. MES III differed from the others in its low concentrations of orthophosphate in 1977. Five times out of 14 no orthophosphate could be measured in Subsystem A and 6 times out of 14 in Subsystem B. The potassium content of the water in all the MES was usually less than 1 g $m^{-3}$ and often under the detection limit of 0.01 g $m^{-3}$.

### *6.3.3 Vegetation assessment*

The vegetational composition of the MES is presented in Table 35. It shows that the systems varied in composition and abundance. The vegetation also varied from year to year. In 1977 hardly any Elodea was observed, but it was rather abundant in 1978. In 1977 and 1978 Ceratophyllum demersum dominated in all MES. Since the start of the MES, species like Ranunculus aquatilis and Nasturtium microphyllum gradually have disappeared, while Callitriche sp. in 1978 was only found in MES II.

Generally the vegetation was dense. However, large open spaces were present in MES III in 1977 and in MES II and IV in 1978. In the first two MES large quantities of filamentous algae were present. In Subsystem A of MES III Oedogonium sp. formed large algal mats in contrast to only one small mat in B. Similar mats were removed

Table 34. Chemical characteristics of water samples from the four outdoor MES in 1977 and 1978.

| Characteristics | Unit | | MES I | | MES II | | MES III | | MES IV | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | '77 | '78 | '77 | '78 | '77 | '78 | '77 | '78 |
| Total phosphorus-P | $g\ m^{-3}$ | A | 1.8 | 0.94 | 1.6 | 1.1 | 1.4 | 0.94 | 1.8 | 1.2 |
| | | B | 1.6 | 1.2 | 1.5 | 0.75 | 1.4 | 1.1 | 1.9 | 1.6 |
| Orthophosphate-P | $g\ m^{-3}$ | A | 0.40 | 0.52 | 0.14 | 0.30 | 0.08 | 0.28 | 0.39 | 0.54 |
| | | B | 0.44 | 0.60 | 0.12 | 0.19 | 0.04 | 0.52 | 0.32 | 0.86 |
| $NO_3^-$-N | $g\ m^{-3}$ | A | 0.13 | 0.05 | 0.10 | 0.07 | 0.10 | 0.06 | 0.08 | 0.08 |
| | | B | 0.16 | 0.06 | 0.09 | 0.07 | 0.09 | 0.06 | 0.07 | 0.07 |
| $NO_2^-$-N | $g\ m^{-3}$ | A | 0.03 | <0.01 | 0.02 | <0.01 | 0.02 | <0.01 | 0.02 | <0.01 |
| | | B | 0.02 | <0.01 | 0.01 | <0.01 | 0.01 | <0.01 | 0.02 | <0.01 |
| $NH_4^+$-N | $g\ m^{-3}$ | A | 0.27 | 0.18 | 0.29 | 0.33 | 0.16 | 0.22 | 0.20 | 0.21 |
| | | B | 0.27 | 0.17 | 0.29 | 0.21 | 0.20 | 0.21 | 0.14 | 0.27 |
| Hardness (as CaO) | $g\ m^{-3}$ | A | 74 | 56 | 72 | 54 | 80 | 46 | 67 | 57 |
| | | B | 78 | 56 | 68 | 50 | 67 | 45 | 64 | 65 |
| $HCO_3^-$ | $mol\ m^{-3}$ | A | 4.2 | 4.5 | 3.5 | 5.6 | 3.9 | 3.8 | 3.3 | 5.3 |
| | | B | 4.3 | 4.5 | 3.2 | 4.0 | 2.4 | 3.9 | 3.0 | 5.9 |
| $Ca^{2+}$ | $g\ m^{-3}$ | A | 29 | 21 | 28 | 20 | 30 | 18 | 30 | 22 |
| | | B | 32 | 17 | 27 | 19 | 22 | 17 | 30 | 27 |
| $Mg^{2+}$ | $g\ m^{-3}$ | A | 13 | 11 | 12 | 11 | 14 | 9.4 | 11 | 11 |
| | | B | 13 | 11 | 12 | 9 | 14 | 8.6 | 7.2 | 11 |
| $K^+$ | $g\ m^{-3}$ | A | 0.66 | <0.01 | 0.35 | <0.01 | 1.3 | <0.01 | 0.04 | <0.01 |
| | | B | 0.67 | <0.01 | 0.36 | <0.01 | 1.0 | <0.01 | 0.07 | <0.01 |
| $Na^+$ | $g\ m^{-3}$ | A | 9.6 | 5.2 | 9.5 | 7.6 | 12.0 | 7.6 | 7.2 | 5.4 |
| | | B | 9.5 | 5.4 | 9.0 | 6.2 | 10.6 | 6.6 | 6.6 | 7.4 |
| $Cl^-$ | $g\ m^{-3}$ | A | 9.2 | 5.5 | 9.4 | 6.6 | 10.0 | 7.3 | 8.0 | 3.5 |
| | | B | 9.5 | 6.7 | 9.9 | 6.2 | 10.9 | 6.3 | 6.7 | 5.2 |
| Electric conductivity | $mS\ m^{-1}$ | A | 23 | 18 | 20 | 22 | 24 | 26 | 20 | 15 |
| | | B | 23 | 18 | 19 | 20 | 21 | 25 | 20 | 15 |

Table 35. Survey on the floating and submerged vegetation in the MES at the end of the experimental seasons in 1977 and 1978. Figures represent percentages of the total surface area minus the area enclosed by the sampling cylinders.
- not present
+ observed during the experimental season, but not found at the time of survey

| | | MES I | | MES II | | MES III | | MES IV | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1977 | 1978 | 1977 | 1978 | 1977 | 1978 | 1977 | 1978 |
| *Floating vegetation (incl. emerging vegetation)* | | | | | | | | | |
| Duckweed (Lemna minor + Spirodela polyrhizza) | A | - | + | 50 | 50 | <1 | <1 | 20 | 70 |
| | B | <1 | + | 30 | 5 | <1 | <1 | 20 | 60 |
| Water starwort (Callitriche sp.) | A | - | - | <1 | 5 | - | - | - | - |
| | B | - | - | <1 | - | - | - | - | - |
| Total | A | <1 | - | 50 | 55 | <1 | <1 | 20 | 70 |
| | B | - | - | 30 | 5 | <1 | <1 | 20 | 60 |
| *Submerged vegetation* | | | | | | | | | |
| Hornwort (Ceratophyllum demersum) | A | 100 | 100 | 50 | 15 | 5 | <1 | 70 | 10 |
| | B | 80 | 95 | 60 | 50 | 35 | 40 | 50 | 10 |
| Pondweed (Potamogeton crispus) | A | <1 | <1 | <1 | - | <1 | 30 | 25 | 5 |
| | B | 10 | 5 | 15 | - | 40 | 15 | 40 | 5 |
| Pondweed (Potamogeton berchtoldii) | A | - | + | 40 | 15 | 15 | 50 | + | - |
| | B | 10 | + | 15 | <1 | 10 | - | - | - |
| Waterthyme (Elodea nuttallii) | A | + | + | + | 15 | + | 20 | 5 | <1 |
| | B | + | + | <1 | 15 | + | 40 | 10 | <1 |
| Water buttercup (Ranunculus aquatilus) | A | - | - | 5 | - | - | - | - | - |
| | B | - | - | <1 | - | - | - | - | - |
| Water starwort (Callitriche sp.) | A | - | - | <1 | 5 | - | - | - | - |
| | B | - | + | <1 | - | - | - | - | - |
| Ivy-leaved duckweed (Lemna trisulca) | A | - | - | 5 | <1 | - | - | <1 | + |
| | B | + | - | 10 | <1 | - | <1 | + | + |
| Chara sp. | A | - | - | - | <1 | <1 | - | - | - |
| | B | - | - | - | <1 | <1 | - | - | - |
| Oedogonium sp. | A | - | + | - | + | 50 | - | <1 | + |
| | B | - | - | - | 30 | 5 | - | + | - |
| Total | A | 100 | 100 | 100 | 50 | 70 | 100 | 100 | 15 |
| | B | 100 | 100 | 100 | 85 | 90 | 95 | 100 | 15 |

Table 36. Average insecticide concentrations in the model ecosystems and incidental extremes. No irregular concentrations were found in MES II A in 1978.

| Subsystem | Year | Period of treatment | Insecticide | Intended conc. mg $m^{-3}$ | Average conc. mg $m^{-3}$ | Incidental extremes | Date of these concentrations |
|---|---|---|---|---|---|---|---|
| I A | 1977 | 25-4 → 15-8 | parathion | 1 | 0.70 | 1.5 | 24-5 |
| | | | | | | 1.1 | 8-7 |
| | | | | | | 0.12 | 20-7 |
| | | | | | | 0.15 | 8-8 |
| | 1978 | 30-5 → 18-8 | azinphosmethyl | 1 | 0.61 | 0.10 | 16-6 |
| | | | | | | n.d [1] | 20-6 |
| | | | | | | 1.1 | 8-8 |
| II A | 1977 | no treatment | | | | | |
| | 1978 | 13-6 → 18-8 | parathion | 0.2 | 0.10 | - | - |
| III A | 1977 | 25-4 → 15-8 | azinphosmethyl | 1 | 0.81 | 1.2 | 24-5 |
| | | | | | | 1.2 | 26-5 |
| | | | | | | 1.1 | 6-6 |
| | 1978 | 30-5 → 18-8 | parathion | 1 | 0.73 | 1.0 | 13-6 |
| IV A | 1977 | no treatment | | | | | |
| | 1978 | 30-5 → 18-8 | parathion | 0.5 | 0.37 | 0.05 | 16-6 |

1. After 16 June no insecticide was applied for fear that the 0.1 mg $m^{-3}$ measured on that date would be an underestimation of the actual concentration. The concentration was near zero for a week.

from MES III in the routine cleaning in 1976, when they were evenly distributed over the whole system. At the end of the experimental season in 1977 they again were removed and did not re-appear in 1978. In MES II in 1978 a filamentous green alga covered walls and submerged water plants in both subsystems but was more abundant in II B at the end of the season.

### *6.3.4 Insecticide concentrations*

The average concentrations of insecticide in the MES are presented in Table 36. Also those concentrations which were exceptionally high or low are tabulated. A check was made on the horizontal and vertical distribution of the insecticides over the subsystems. The results are presented in Table 37. They do not indicate that either horizontal or vertical concentration gradients did exist.

The calculated half-lives of the applied insecticides in the different subsystems are presented in Table 38. For parathion the half-lives were generally of the order of 3 to 4 days with a lower value for Subsystem IIA. The half-lives for azinphosmethyl amounted to 7 days in 1977 and nearly 3 days in 1978.

In general an equally distributed and fairly constant insecticide concentration could be maintained.

Table 37. Assessment of the horizontal and vertical distribution of the insecticides parathion and azinphosmethyl in the model ecosystems. Measurement of the horizontal distribution was made six hours after application. Vertical distribution was measured along with the normal analysis.

| Insecticide | Calculated conc. mg $m^{-3}$ | Measured conc. mg $m^{-3}$ | Water sampled | Date |
|---|---|---|---|---|
| *Horizontal distribution* | | | | |
| parathion | 0.87 | 0.93 | inside cylinder | 10 June '77 |
| | | 0.89 | between vegetation | |
| | 0.96 | 0.88 | inside cylinder | 15 July '77 |
| | | 0.90 | between vegetation | |
| azinphosmethyl | 0.76 | 0.98 | inside cylinder | 15 July '77 |
| | | 0.80 | between vegetation | |
| | 0.94 | 0.94 | inside cylinder | 9 Aug. '77 |
| | | 0.92 | between vegetation | |
| *Vertical distribution* | | | | |
| parathion | not calculated | 0.40 | lower part water column | 5 Oct. '77 |
| | | 0.37 | upper part water column | |
| | | 0.37 | total column | |
| azinphosmethyl | not calculated | 0.26 | lower part water column | 25 Oct. '77 |
| | | 0.25 | upper part water column | |
| | | 0.26 | total water column | |

Table 38. Average half-lives of parathion and azinphosmethyl in the water phase of the outdoor MES. Half-lives of more than twice the average have been excluded.

| Insecticide | Intended conc. (mg $m^{-3}$) | MES | Year | Average half-life ± S.D. (hours) | Average max. water temp. | Average pH |
|---|---|---|---|---|---|---|
| parathion | 1 | I A | 1977 | 75 ± 26 | 16.6 | 8.9 |
| | 1 | III A | 1978 | 85 ± 34 | 15.4 | 8.9 |
| | 0.5 | IV A | 1978 | 95 ± 40 | 14.7 | 8.4 |
| | 0.2 | II A | 1978 | 36 ± 10 | 15.3 | 8.0 |
| azinphosmethyl | 1 | III A | 1977 | 168 ± 50 | 16.7 | 8.1 |
| | 1 | I A | 1978 | 68 ± 34 | 15.6 | 9.3 |

*6.3.5 Population assessment of zooplankton and macrofauna*

A list of illustrations of the main species studied is presented in Appendix C. Other species found in the MES but not studied are listed in Appendix D. The changes in the abundance of species observed in relationship with the insecticides applied are summarized in Table 39. Marked reductions in the abundancies of Daphnia spp. were observed during the applications of parathion (0.5 and 1 mg $m^{-3}$) and azinphosmethyl at 1 mg $m^{-3}$. Strong reductions in abundancy were also recorded for Simocephalus vetulus in response to 1 mg $m^{-3}$ parathion and to a lesser extent for 1 mg $m^{-3}$ azinphosmethyl. Numbers of Chydorus sphaericus decreased markedly in the presence of 1 mg $m^{-3}$ parathion and somewhat less at a concentration of 0.5 mg $m^{-3}$. Populations of Chaoborus crystallinus rapidly decreased at a concentration of 1 mg $m^{-3}$ parathion but not at 0.5 mg $m^{-3}$. Only a slight decrease in numbers was observed for Graptoleberis with 1 mg $m^{-3}$ parathion. After the introduction of the insecticides the abundance of Cyclopoida (nauplii and copepodits included) and of Ostracoda did not decrease. More detailed information can be derived from Figs.18-33, which are put together in Appen-

Table 39. Summary of the changes in abundance of zooplankton and macrofauna species in relation to the insecticides applied. (-) no reduction in numbers compared to be control; (±) hardly any reduction in numbers; (+) strong reduction in numbers as compared to the control, but still present in fair numbers; (++) none or only occasional specimens were sampled, while large numbers were present in the control.

| Organism | Parathion 1 mg $m^{-3}$ | Parathion 0.5 mg $m^{-3}$ | Parathion 0.2 mg $m^{-3}$ | Azinphosmethyl 1 mg $m^{-3}$ |
|---|---|---|---|---|
| Simocephalus vetulus | ++ | - | - | +(+)[1] |
| Daphnia spp. | ++ | ++ | - | ++ |
| Chydorus sphaericus | ++ | + | - | - |
| Graptoleberis testudinaria | ± | - | - | - |
| Cyclopoida | - | - | - | - |
| Ostracoda | - | - | - | - |
| Chaoborus crystallinus | ++ | - | - | - |

1. ++ in 1977, + in 1978.

Table 40. Survey of the different figures which show the development of the zooplankton species in the MES.

| Species | MES I | | MES II | | MES III | | MES IV | |
|---|---|---|---|---|---|---|---|---|
| | 1977 | 1978 | 1977 | 1978 | 1977 | 1978 | 1977 | 1978 |
| Simocephalus vetulus | 18a | 19a | 18b | 19b | 18c | 19c | 18d | 19d |
| Daphnia spp. | 21a | 22a | - | 22b | - | - | 21b | 22c |
| Chydorus sphaericus | 23a | 24a | 23b | 24b | 23c | 24c | 23d | 24d |
| Graptoleberis testudinaria | 25a | 26a | 25b | 26b | 25c | 26c | 25d | 26d |
| Peracantha truncata | - | - | 27a | 27c | - | - | 27b | 27d |
| Cyclopoida | 28a | 29a | 28b | 29b | 28c | 29c | 28d | 29d |
| Ostracoda | 30a | 31a | 30b | 31b | 30c | 31c | 30d | 31d |
| Chaoborus crystallinus | 32a | 33a | 32b | 33b | 32c | 33c | 32d | 33d |

dix E (see Table 40). In these figures the numbers of specimens collected per sample are plotted versus time for each species and for each subsystem. Curves of corresponding subsystems are drawn in the same graph, with a solid line for the A subsystems and a broken line for the B subsystems. These curves will be discussed in detail in Section 6.4.4.

With regard to the, mainly benthic, macrofauna, the numbers in the samples were so small that no attempt was made to present these data graphically. Therefore these numbers are summed per subsystem per year and presented in Table 41.

## 6.4 DISCUSSION

### *6.4.1 Physico-chemical measurements*

The MES proved to be sufficiently large to keep diurnal temperature fluctuations of the water within a narrow range. The highest water temperature ever recorded in the MES was 27.5 $^{o}$C on June 30, 1976 at the end of a very warm day.

The pH values recorded were rather high compared with those of most surface waters in the Netherlands, but this is not abnormal in ditches with an abundance of higher water plants (see for instance Beltman, 1976). The even higher values recorded later in the day (Table 33) may have favoured hydrolysis of the applied insecticides.

Although the measured concentrations of dissolved oxygen were generally high, with the exception of some periods in MES II in 1978 (Fig.17b), it cannot be excluded that near the bottom oxygen concentrations occasionally were so low as to be unfavourable for bottom dwelling organisms.

For the other physico-chemical parameters, as summarized in Table 34, the values measured in the MES fall within the ranges generally encountered in surface waters in the Netherlands (e.g. Werkgroep Biologische Waterbeoordeling, 1977). There are however a few exceptions, notably potassium, orthophosphate and chloride content and hardness of water. The potassium content, which in the MES was usually under 1 g $m^{-3}$, is low in relation to the range of 1-15 g $m^{-3}$ normally recorded in surface waters in

Table 41. Numbers of macrofauna sampled together with the zooplankton during the experimental seasons in 1977 and 1978.

| Organism | | MES I | | MES II | | MES III | | MES IV | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1977 | 1978 | 1977 | 1978 | 1977 | 1978 | 1977 | 1978 |
| Tricladida | A | 0 | 6 | 0 | 7 | 1 | 7 | 2 | 14 |
| | B | 0 | 4 | 2 | 7 | 3 | 21 | 2 | 14 |
| Hirudinea | A | 0 | 3 | 5 | 4 | 3 | 0 | 4 | 3 |
| | B | 0 | 4 | 3 | 1 | 7 | 5 | 4 | 7 |
| Asellus sp. | A | 8 | 12 | 99 | 34 | 20 | 64 | 11 | 26 |
| | B | 6 | 14 | 61 | 28 | 41 | 122 | 6 | 26 |
| Ephemeroptera | A | 2 | 6 | 0 | 0 | 0 | 0 | 35 | 0 |
| | B | 6 | 15 | 1 | 7 | 12 | 1 | 29 | 16 |
| Odonata | A | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| | B | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 |
| Oligochaeta | A | 0 | 0 | 0 | 1 | 4 | 0 | 0 | 0 |
| | B | 0 | 1 | 0 | 0 | 13 | 0 | 0 | 0 |
| Chaoborus sp. | A | 40 | 522 | 286 | 593 | 259 | 142 | 112 | 262 |
| | B | 230 | 379 | 283 | 811 | 260 | 464 | 83 | 389 |
| Heteroptera | A | 1 | 0 | 13 | 13 | 2 | 0 | 0 | 0 |
| | B | 2 | 3 | 2 | 17 | 0 | 1 | 0 | 2 |
| Coleoptera | A | 0 | 3 | 1 | 4 | 2 | 3 | 0 | 4 |
| | B | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 |
| Gastropoda | A | 37 | 23 | 49 | 37 | 16 | 92 | 20 | 61 |
| | B | 32 | 24 | 54 | 20 | 11 | 55 | 14 | 63 |
| Hydracarina | A | 16 | 19 | 40 | 85 | 42 | 33 | 83 | 49 |
| | B | 30 | 21 | 59 | 113 | 41 | 25 | 36 | 65 |

the Netherlands. Since potassium is a macronutrient for water plants it may have been limiting in some of the MES for some periods. The same can be said of the orthophosphate concentration especially in MES III. As far as the ion content of the water in the MES is concerned this water can be regarded as oligo-ionic (<20 mS $m^{-1}$) or beta-meso ionic (20-50 mS $m^{-1}$). Such low values are comparatively rare in Dutch surface waters. In the MES they were a result of the low chloride content. In general the chloride content of Dutch surface waters ranges from 20-300 g $m^{-3}$. Consistent with the low chloride content is the low electric conductivity of 15-25 mS $m^{-3}$, the normal range of Dutch freshwaters being 25-100 mS $m^{-1}$. The hardness of the water in the MES with values generally far below 80 g $m^{-3}$ (as CaO) was a little low compared with values over 100 g $m^{-3}$ for most Dutch surface waters.

Finally it can be said that the data obtained for $NH_4^+$ and $NO_2^-$ concentrations were low and very unlikely to represent toxic concentrations.

treatment was stopped the population increased up to the level of the control population. This result supports the hypothesis that the absence of a recovery in 1977 was due to the ciliate bloom rather than to a direct effect of azinphosmethyl. A bloom of Chroomonas sp. similar to that in 1977 occurred in this same pond with 1 mg $m^{-3}$ parathion, but was of shorter duration. No meaningful differences were observed between corresponding subsystems in either the untreated MES (Figs.19b,d) or in the MES treated with 0.2 or 0.5 mg $m^{-3}$ parathion.

*Daphnia spp.* Daphnia was not present in each MES every year. In MES I in 1977 a population of D. longispina appeared after the treatment with 1 mg $m^{-3}$ parathion was stopped and probably contributed to the termination of the algal bloom. (Fig.21a). Not a single specimen was found in the control. A population of D. longispina in the untreated MES IV in 1977 showed a parallel development in both subsystems.

After the introduction of 1 mg $m^{-3}$ of azinphosmethyl an almost complete disappearance of D. pulex took place (Fig.22a). In the control the population was stable throughout the experimental season. The population in the treated part of the MES did not recover after the treatment was stopped. Hence under the conditions in this MES the Daphnia species concerned are more susceptible than Simocephalus vetulus. A similar effect was found during the treatment with 0.5 mg $m^{-3}$ parathion (Fig.22c) Again no recovery took place after the treatment was stopped.

Evaluation of the 0.2 mg $m^{-3}$ parathion treatment in relation to the Daphnia population was difficult due to the late emergence of the population in both subsystems of MES II (Fig.22b). However the fact that the population emerged in spite of the treatment indicates that this concentration probably did not affect the species involved (D. longispina and D. pulex curvirostris). This is in agreement with the no-effect levels of 0.3 mg $m^{-3}$ found in the 3-week laboratory toxicity test with D. magna (Table 21).

*Chydorus sphaericus* Introduction of parathion at a concentration of 1 mg $m^{-3}$ in MES IA in 1977 was followed by a strong reduction in numbers of this species in the samples (Fig.23a). After the treatment was stopped, the population showed a tendency to recover. However recovery might have been tempered by the onset of a seasonal decline as can be read from the other curves in Fig.23. In 1978 at this same concentration the effects of 1977 could not be reproduced (Fig.24c) for the same reasons as mentioned for the Simocephalus population in this MES. However a similar sharp reduction in numbers was found after introduction of 0.5 mg $m^{-3}$ parathion in 1978, although less pronounced.

For parathion at a concentration of 0.2 mg $m^{-3}$ the dip in numbers in July at first sight might be regarded as an effect with a relatively long latency period due to the low concentration applied. However a similar dip was observed for Simocephalus (Fig.19b). Since in the latter case a complete recovery took place within the period of treatment and no effect was observed at the next higher level of 0.5

mg $m^{-3}$ (Fig.19d), the dip probably cannot be attributed to parathion. It is, therefore, conceivable that the decline of the Chydorus curve also cannot be attributed to parathion, even though Chydorus is probably more susceptible to this insecticide than Simocephalus, as can be seen by comparing Fig.24d with Fig.19d. In Fig.19d no effect of parathion on Simocephalus is seen.

Without treatment similar populations developed in corresponding subsystems (Figs.23b,d). No effect was observed for azinphosmethyl at a concentration of 1 mg $m^{-3}$ in 1978 (Fig.24a). The gradual decrease in numbers in 1977 in relation to the control (Fig.23c), therefore most likely has to be attributed to the ciliate bloom (Section 6.3.3) instead of being a direct effect of the insecticide.

*Graptoleberis testudinaria* Neither parathion nor azinphosmethyl did affect the populations of this species (Figs.25a,c and Figs.26a-d). Population developments in corresponding subsystems also were quite similar (Figs.25c,d).

*Peracantha truncata* The results of the insecticide-treated MES are inconclusive as far as this species is concerned, because the populations started to develop late (Figs.27c,d) or were almost completely absent during the experimental season as was the case in MES II and IV in 1977 and in MES III in 1978. Similar population developments were observed in corresponding subsystems of the untreated MES (Fig.27a).

*Cyclopoida* Cyclopoida did not seem to experience any deleterious effect from the insecticide concentrations applied in the MES-experiments. This is in agreement with literature data (Section 2.3.3). Lüdemann and Neumann (1962) found no effect on Cyclops strenuus at 1 g $m^{-3}$ parathion in a test period of 24 hours. No-effect levels of 6 other organophosphorus insecticides for this species ranged from 5 to 8 g $m^{-3}$. Baytex was found not to cause mortality in Microcyclops bicolor at concentrations up to 0.5 g $m^{-3}$.

All corresponding subsystems showed a similar population development over the season and levels were equal in most cases. In the MES treated with parathion at concentration levels of 1 and 0.5 mg $m^{-3}$ the numbers in the treated subsystems were even higher than in the controls. A tentative explanation of this phenomenon might be that the supply of food for the herbivorous part of the Cyclopoida population relatively increased because of the reduction in the populations of the Cladocera. Also a reduction in predation pressure by the larvae of Chaborus crystallinus as a result of its population decline may have contributed (Figs.32a,33c). The decrease in numbers of Cyclopoida at the end of 1977 in MES IA (Fig.28a) could then be attributed to a reduced availability of food as a result of the recovery of Simocephalus (Fig.19a) and the emergence of Daphnia longispina (Fig.21a). Such events did not occur in either MES IIIA or IVA in 1978 and this might explain why in these MES the numbers of Cyclopoida remained above control levels even after the treatment was stopped.

*Ostracoda* No effect was found for either insecticide used, which result is in line with the literature data (Section 2.3.3). Hardly any mortality was observed in Chlamydotheca arcuata after a 48-hour exposure to 2 g $m^{-3}$ fenthion (Khudaira & Ruber,1974). Baytex was found to cause 30 and 100 per cent mortality in Cypridopsis vidua at concentrations of 1 and 2 g $m^{-3}$, respectively, in a 24-hour test.

Population developments among different MES were also similar but the patterns differed between the two years (Figs.30,31).

*Chaoborus crystallinus* Both in 1977 and 1978 application of parathion at 1 mg $m^{-3}$ resulted in a reduction in the numbers of Chaoborus larvae (Figs.32a,33c). Later in the period of treatment the larvae were almost invariably found in the fine fraction of the zooplankton samples, which indicated that they were newly hatched ones. In all other MES the populations of corresponding subsystems developed similarly, which shows that parathion at 0.5 and 0.2 mg $m^{-3}$ and azinphosmethyl at 1 mg $m^{-3}$ did not affect the larvae in the MES. These results are in accordance with the laboratory toxicity data obtained in this study (Section 4.4.4). In all other MES the population developments between corresponding subsystems were similar.

*Other species* Asellus spp., Gastropoda (snails) and Hydracarina (watermites) did not seem to be affected by any of the insecticide concentrations. Although Cloeon dipterum was not collected in any sample from the subsystems treated with parathion in 1978, or with azinphosmethyl in 1977, no conclusions can be drawn from this result, because by far the most larvae sampled in the corresponding control subsystems were collected after the treatment was stopped. The lower total numbers for Chaoborus crystallinus in MES IA (1977) and IIA (1978) correspond with the lower numbers in the treatment period (Figs.32a and 33c, respectively). For the other species even the total numbers were too small to draw any definite conclusions. Ciliata and Rotatoria species were often present in the samples in large numbers, but were not counted for lack of manpower. However, also for these organisms the population development seemed roughly similar in corresponding subsystems.

From the numbers of organisms collected during the autumn cleaning in November 1977 and 1978, no conclusions can be drawn about any effect of insecticide treatment. They only provide a momentary survey of the numbers of macrofauna present in the MES. A table listing the organisms collected at the time of the cleaning is presented in Appendix F.

### *6.4.5 Similarities and differences between corresponding subsystems*

Corresponding subsystems of the untreated MES showed a similar development for nearly all physico-chemical and biological parameters. Only the pH in MES II in 1977 largely differed between Subsystems A and B (Fig.14b). Such similarities were also observed between corresponding subsystems of MES that were treated with insecticide,

for those parameters which were not affected by the insecticide treatment. On the other hand differences were observed in parameters which were not likely to be affected by the applied insecticides. One example is the completely different vegetation in MES IIIA compared with that in IIIB in 1978. This difference might have been caused by the ciliate bloom; at the time the MES was subdivided no submerged plants were visible at all and it might have been that the system had not yet taken its final course of development. Subdivision then resulted in the divergent development of the vegetation in the subsystems. Such dissimilar development might be averted in future studies by postponing the subdivision until the vegetation has grown to a substantially larger biomass. Since in MES as used in this study the vegetation probably is a major conditioning parameter for the zooplankton, a later subdivision may also promote similar development of the population of the different zooplankton species.

The limited number of MES did not permit a statistical evaluation of the results. Nevertheless the changes in zooplankton development in the treated subsystems compared with those of the controls, could in most cases be attributed to the insecticide treatment on the basis of circumstantial evidence. Such evidence is based on the observed general similarity in development between corresponding subsystems and on the rapid and clear changes in population development, in response to such treatment.

## 6.5 CONCLUSIONS

The results show that MES of the type used in the present study largely meet the requirements set in Section 6.1.

- Several trophic and other relationships are present in the MES. The different species of plants, zooplankton and macrofauna that take part in these relationships are common and widespread in the Netherlands (Werkgroep Biologische Waterbeoordeling,1977; De Lange,1976; Notenboom-Ram et al.,1976).
- The MES can be kept intact for a period of at least 3-4 years without any apparent sign of long-term deterioration. It therefore seems that one of the main problems in model ecosystem research, namely the generally short lifetime of the systems, does not play a role in this type of MES.
- The MES proved to be large enough to maintain a diverse and equilibrated zooplankton and macrofauna community and to permit an adequate biological and chemical sampling programme. However, with the exception of Chaoborus larvae, no adequate monitoring of the macrofauna community could be accomplished with the sampling method in this study.
- Also the MES provided the possibility to distinguish between natural phenomena and those induced by the insecticide treatments even though the limited numbers of MES did not permit a statistical evaluation. The experiments showed that under near-natural conditions 0.5 mg $m^{-3}$ parathion and 1 mg $m^{-3}$ parathion and azinphosmethyl can strongly reduce populations of Cladocera for at least as long as these concentra-

tions are maintained. Populations of Chaoborus crystallinus are strongly reduced by 1 mg $m^{-3}$ parathion. In the MES experiments the populations sometimes recovered after the treatment was stopped and sometimes did not. But it is not clear what this means for field situations. Sometimes phytoplankton blooms occurred which seemed to be caused by the absence of an adequate herbivorous zooplankton population due to a reduction in the numbers of Cladocera. Cyclopoida and Ostracoda were not sensitive to either parathion or azinphosmethyl in the concentrations applied. Although no detailed conclusions can be made about other macrofauna than Chaoborus, it can be concluded that there were no severe effects. Parathion at 0.2 mg $m^{-3}$ most likely represents a no toxic effect level for all species studied. To what extent blooms of ciliates and filamentous algae are to be attributed to the insecticides applied cannot be assessed from the present data. Nor can any definite conclusion be made as to whether or not the small dimensions of the MES have promoted these blooms.

# 7 Comparison of the MES-experiments with the laboratory toxicity trials

In general there was a good agreement between the results of the MES-experiments and those obtained in the laboratory toxicity tests. In order to illustrate this point, the effects on populations of several species in the MES, which were ascribed to the insecticide treatment, were compared with the sub-acute laboratory toxicity trials. For this purpose predictions based on the maximum concentrations causing no effect in the laboratory tests ('no-effect concentrations') were compared with the outcome of the MES-experiments. For these predictions the 3-week no-effect concentrations obtained with Daphnia magna were considered to be representative not only for all Daphniidae (e.g. Daphnia spp., Simocephalus) but also for the Chydoridae (e.g. Chydorus, Graptoleberis) (Table 42). For Daphnia spp. the observed effects fully complied with the predictions. Discrepancies between predicted and observed effects for 0.5 mg $m^{-3}$ parathion with regard to S. vetulus, G. testudinaria and C. crystallinus only reflect minor quantitative differences; the predicted effects occur at 1 mg $m^{-3}$. The predictions with regard to an effect of 1 mg $m^{-3}$ azinphosmethyl on C. sphaericus

Table 42. Predicted and observed effects of parathion and azinphosmethyl in the MES used in the present study. First sign represents predicted effects (Chapter 7), second sign gives the observed effects.
(+) effect, (-) no effect, (±) dubious effect, (o) data inconclusive.

| Species | Parathion | | | Azinphosmethyl |
|---|---|---|---|---|
| | 0.2 mg $m^{-3}$ | 0.5 mg $m^{-3}$ | 1 mg $m^{-3}$ | 1 mg $m^{-3}$ |
| Daphnia spp. | -/- | +/+ | +/+ | +/+ |
| Simocephalus vetulus | -/- | +/- | +/+ | +/+ |
| Chydorus sphaericus | -/- | +/+ | +/+ | +/- |
| Graptoleberis testudinaria | -/- | +/- | +/± | +/- |
| Asellus aquaticus | -/o | -/o | -/o | +/o |
| Chaoborus crystallinus | -/- | +/- | +/+ | -/- |
| Cloeon dipterum | -/o | +/o | +/o | -/o |

and G. testudinaria did not come true. However, the question remains whether a slightly higher concentration of this insecticide would have produced the predicted effect. For A. aquaticus and C. dipterum the results of the MES-experiments were inconclusive.

From the overall good agreement between the results from the laboratory tests and the MES-experiments one should not draw the conclusion that in general simple laboratory tests can provide sufficient information for the ecological hazard assessment for other groups of compounds as well. For other compounds the comparability of results observed for the organophosphorus insecticides tested in this study may not exist. But more important, and this also applies to parathion and azinphosmethyl: the results of the MES-experiments have the advantage of being obtained under near-natural environmental conditions and thus provide a more solid basis for extrapolation to field situations.

On the basis of the present study it is, therefore, recommended that the present tentative limit ('voorlopige grenswaarde') for the concentration of cholinesterase inhibitors in surface waters in the Netherlands should be lowered from 1 mg $m^{-3}$ (Ministerie van Verkeer en Waterstaat, 1975) to 0.1 mg $m^{-3}$ paraoxon equivalents. For comparison: the criteria for freshwater and marine aquatic life as set by the US Environmental Protection Agency (1976) for parathion, azinphosmethyl (Guthion) and malathion are 0.04, 0.01, and 0.1 mg $m^{-3}$, respectively. These criteria are based on the 96 hour $LC_{50}$ values of several species of microcrustaceans multiplied by an arbitrary application factor of 0.1. The results of the present study suggest that for organophosphorus insecticides no-effect levels for field situations might be calculated from 48 hour $EC_{50}$ values by multiplication by a factor 0.1.

# 8 Conclusions and recommendations

- The results obtained in the MES-experiments show that with a limited number of systems, in spite of the implicit statistical limitations, invaluable information can be obtained about the vulnerability of a range of species within a complex ecological setting.
- The results of the present study suggest that for organophosphorus insecticides no-effect levels for field situations might be calculated from 48-hour $EC_{50}$ values for Daphnia magna Straus by multiplication by a factor 0.1.
- It is recommended that the present tentative limit ('voorlopige grenswaarde') for the concentration of cholinesterase inhibitors in surface waters in the Netherlands should be lowered from 1 mg $m^{-3}$ (Ministerie van Verkeer en Waterstaat, 1975) to 0.1 mg $m^{-3}$ paraoxon equivalents.

Finally, it is my opinion that, because of our small and fragmentary knowledge of the total complexity of relationships in freshwater ecosystems, freshwater quality criteria with regard to toxic substances should be tested in model ecosystems of a relatively high natural complexity and under near-natural test conditions before they come into force (see also Golterman, 1976). The type of MES used in the present study could be developed into a valuable model for such a purpose.

# Summary

A number of organophosphorus insecticides are continuously present as contaminants in surface waters in the Netherlands. Similar findings have been reported from other European countries. The levels reported can be considered as relatively high, because they are of the same order as the levels found to be toxic to several aquatic organisms under laboratory conditions.

It was the aim of the present study to assess the hazards of long-term exposure of freshwater ecosystems to organophosphorus insecticides, with special emphasis on the most susceptible groups. Since such an assessment cannot be based on laboratory toxicity data alone, an attempt was made to develop a test model incorporating some of the major environmental variables to narrow down the risk margin in the extrapolation of laboratory data to field situations and to enable a more realistic hazard assessment.

After the introduction in Chapter 1, the literature on the toxicology of organophosphorus insecticides in relation to freshwater ecosystems is surveyed in Chapter 2. In this chapter the following subjects are covered: the environmental fate, including volatilization, distribution over the ecosystems, (photo) chemical and microbial breakdown, and the toxicity to aquatic organisms. From the literature data the conclusion is drawn that the concentrations in the surface waters in the Netherlands may represent a toxicological risk for Cladocera (waterfleas) and certain aquatic insects.

Chapter 3 gives the analytical-chemical methods as applied in this study, for the measurement of organophosphorus insecticides.

In Chapter 4 detailed information on the laboratory toxicity tests is given. The insecticides tested were parathion, azinphosmethyl, diazinon, malathion and parathion-methyl. These compounds were tested in static 48-hour and 21-day toxicity trials on Daphnia magna, (water flea), Asellus aquaticus (water hoglouse), Cloeon dipterum (mayfly larva), and Chaoborus crystallinus (phantom midge larva). Of these species only Daphnia was reared in the laboratory. All other test species were collected in the field or from outdoor model ecosystems (MES). In addition comparative tests were carried out in which all five insecticides were tested simultaneously on each species. Toxicity ranges were assessed for Herpobdella octoculata (leech), Dugesia lugubris (flatworm), Notonecta sp. and Sigara sp. (water bugs), and Culex sp. (mosquito larva). A summary of the tests conducted and the major test conditions is presented in Table 19. It can be concluded from the comparative tests that parathion is the most toxic of the five compounds tested (Table 27). Parathion-methyl appears to be by far the least toxic for the insect larvae. The toxicity of the other compounds does not

vary markedly from species to species. Of the species tested only in the range-finding tests, the parathion $EC_{50}$ values for the insects, viz. Culex sp., Notonecta sp. and Sigara sp. are of the same order as for Cloeon dipterum and Chaoborus crystallinus. The conclusion can be drawn that chronic exposure to concentrations of the order of tenths of mg $m^{-3}$ of organophosphorus insecticides may endanger the survival and the reproductive potential of Cladocera and aquatic insects.

Chapter 5 describes the study of the rate of dissipation of the five organophosphorus insecticides from the water phase of three freshwater aquaria. Aquarium 1 and 2 were provided with a bottom layer of sand and very fine mud and planted with Elodea densa. Aquarium 3 was not provided with either bottom material or water plants so that the influence of sediment and plants on the dissipation rate could be evaluated. The five insecticides were applied to the aquaria at a concentration of 1 mg $m^{-3}$ each. One $dm^3$ aliquots were withdrawn from the aquaria after 1, 20, 50, 90, 152, 216, 355, 525 and 1005 hours after dosing and after extraction analysed by gas-liquid chromatography. The half-lives of the insecticides were calculated, assuming a first order reaction (Table 28). The presence of plants and bottom material resulted in a meaningful increase in dissipation rate for azinphosmethyl, parathion,parathion-methyl and diazinon but not for malathion. In both the aquaria with plants and sediment the order of the half-lives for the five insecticides is the same: diazinon > azinphosmethyl > parathion > parathion-methyl > malathion. On the basis of the results of this experiment a calculation could be made of the frequency by which the two insecticides parathion and azinphosmethyl had to be applied to the MES.

In Chapter 6 a report is given of the MES-studies. In Section 6.1 the use of MES in toxicological research is briefly discussed. It is stated that long-term ecological effects of chemicals on freshwater ecosystems can only be studied if several requirements, which cannot possibly be met on a laboratory scale, are fullfilled. For that reason outdoor MES were designed to study the effects of parathion and azinphosmethyl under near-natural conditions. Four plastic containers of 3 x 1 x 1 meter were dug into the soil on a plot near the laboratory (Fig.10). Subsequently the containers were provided with bottom material, water, water plants and aquatic invertebrates. All four containers were divided into two subsystems with a crosswise waterproof partition at the start of the experimental season, which usually lasted from May to October. Thereafter the sheets were removed in order to reunite the subsystems and restore pre-experimental conditions.

During the experimental period in 1977 two MES were treated with 1 mg $m^{-3}$ parathion and azinphosmethyl, respectively,while no insecticide was applied to the other MES. In 1978 all four MES were treated with parathion (1, 0.5 and 0.2 mg $m^{-3}$) or azinphosmethyl (1 mg $m^{-3}$). In all the treated MES, one subsystem received the insecticide treatment while the corresponding subsystem served as control. During the experimental periods an extensive monitoring programme was carried out which consisted of physico-chemical measurements, vegetation assessment, insecticide analyses and population assessment of zooplankton and macrofauna. The purpose of this programme

was to see whether or not differences could be observed between the development of the parameters in corresponding subsystems and if so, which of these differences could be attributed to insecticide treatments.

The results are described and discussed in Section 6.3 and 6.4, respectively, while conclusions are presented in Section 6.5. It was found that corresponding subsystems of the untreated MES showed a similar development for nearly all physico-chemical and biological parameters. Such similitaries were also observed between corresponding subsystems of MES that were treated with insecticide, for those parameters which were not affected by the insecticide treatment. On the other hand differences were observed in parameters which were not likely to be affected by the applied insecticides. However, these last dissimilarities might be averted in future studies by postponing the subdivision until the vegetation as the major conditioning parameter has grown to a substantially larger biomass.

Although the limited number of MES did not permit a statistical evaluation of the results, the changes in zooplankton development in the treated subsystems compared with those of the controls could nevertheless in most cases be attributed to the insecticide treatment on the basis of circumstantial evidence. A survey of these changes in zooplankton development is given in Table 39. Marked reductions in the abundance of Daphnia spp. were observed during the applications of parathion (0.5 and 1 mg $m^{-3}$) and azinphosmethyl (1 mg $m^{-3}$). Strong reductions in abundance were recorded for Simocephalus vetulus in response to 1 mg $m^{-3}$ parathion and to a lessier extent for 1 mg $m^{-3}$ azinphosmethyl. Numbers of Chydorus sphaericus decreased markedly in the presence of 1 mg $m^{-3}$ parathion and somewhat less at a concentration of 0.5 mg $m^{-3}$. Populations of Chaoborus crystallinus rapidly decreased at a concentration of 1 mg $m^{-3}$ parathion but not at 0.5 mg $m^{-3}$. Only a slight decrease in numbers was observed for Craptoleberis with 1 mg $m^{-3}$ parathion. After the introduction of the insecticides the abundance of Cyclopoida (nauplii and copepodits included) and of Ostracoda did not decrease. Sometimes phytoplankton blooms occurred which seemed to be caused by the absence of an adequate herbivorous zooplankton population due to a reduction of the numbers of Cladocera. Although no detailed conclusions can be made about macrofauna other than Chaoborus, it can be concluded that there were no severe effects. The MES proved to be suitable for producing invaluable information about the vulnerability for organophosphorus insecticides for a range of species in a complex ecological setting.

In Chapter 7 a comparison is made between the outcome of the MES-experiments and the results of the laboratory toxicity trials. In general there was a good agreement as is illustrated in Table 42.

In Chapter 8 the conclusions and recommendations are given.

# Samenvatting

Een aantal organofosforinsekticiden is voortdurend als ontreiniging aanwezig in Nederlandse oppervlaktewateren. Soortgelijke bevindingen zijn gemeld uit andere Europese landen. De gerapporteerde niveaus kunnen als tamelijk hoog worden beschouwd, wanneer men in aanmerking neemt dat ze van dezelfde grootte-orde zijn als de niveaus die onder laboratoriumomstandigheden schadelijk zijn gebleken voor diverse waterorganismen.

Doel van de onderhavige studie was een schatting te maken van de risico's van langdurige blootstelling van zoetwater-ecosystemen aan organofosforinsekticiden, waarbij de nadruk lag op de voor deze stoffen gevoeligste groepen. Aangezien het niet mogelijk is een dergelijke risicoschatting uitsluitend te baseren op toxiciteitsgegevens verkregen in het laboratorium, werd een poging ondernomen om een testmodel te ontwikkelen waarin een aantal van de belangrijkste milieuvariabelen konden worden verenigd. Dit teineinde de risicomarge, die bij de extrapolatie van laboratoriumgegevens naar veldsituaties optreedt, te verkleinen en de mogelijkheid te scheppen voor een realistischer risicoschatting.

Na de inleiding in hoofdstuk 1, wordt in hoofdstuk 2 een overzicht gegeven van de literatuur betreffende de toxicologie van organofosforinsekticiden in relatie tot zoetwater-ecosystemen. In dit hoofdstuk worden de volgende onderwerpen behandeld: het lot van de stof in het milieu, inclusief vervluchtiging, verdeling over het ecosysteem, (foto) chemische en microbiële afbraak en de toxiciteit voor waterorganismen. Uit de literatuurgegevens wordt de conclusie getrokken dat de concentraties, zoals aangetroffen in het Nederlandse oppervlaktewater, een toxicologisch risico kunnen vormen voor Cladocera (watervlooien) en bepaalde in het water levende insekten.

Hoofdstuk 3 geeft de analytisch chemische methoden die zijn toegepast voor het meten van organofosforinsekticiden.

In hoofdstuk 4 wordt uitvoerig ingegaan op de toxiciteitstoetsen in het laboratorium. De toetsen werden uitgevoerd met de insekticiden parathion, azinfosmethyl, diazinon, malathion en parathion-methyl. Deze stoffen werden getoetst in statische 48-uur- en 21-dagen-toetsen en uitgevoerd met de proefdieren Daphnia magna (watervlo), Asellus aquaticus (waterpissebed), Cloeon dipterum (eendagsvlieglarve) en Chaoborus crystallinus (pluimmuglarve). Van deze soorten werd alleen Daphnia in het laboratorium gekweekt. Alle andere toetsorganismen werden in het veld verzameld of uit de buitengelegen modelecosystemen (MES) gehaald. Daarnaast werden er vergelijkende toxiciteitstoetsen uitgevoerd, waarin alle vijf insekticiden simultaan op elke soort werden uitgetest. Voor Herpobdella octoculata (bloedzuiger), Dugesia lugubris (platworm),

Notonecta sp. en Sigara sp. (waterwantsen) en Culex sp. (steekmuglarve) werden 'range-finding' toetsen uitgevoerd. Een overzicht van deze toetsen en van de belangrijkste toetsomstandigheden is gegeven in tabel 19. Uit de resultaten van de vergelijkende toetsen kan worden geconcludeerd dat parathion het meest toxisch is van de vijf getoetste verbindingen (tabel 27). Parathion-methyl blijkt veruit de minst giftige te zijn voor de insektelarven. De overige stoffen verschillen per toetsorganisme onderling niet duidelijk in toxiciteit. De $EC_{50}$-waarden van parathion voor de insektelarven van Culex sp., Notonecta sp. en Sigara sp. zijn van dezelfde grootteorde als voor de larven van Cloeon dipterum en Chaoborus crystallinus. Geconcludeerd kan worden, dat chronische blootstelling aan concentraties van tienden van mg $m^{-3}$ van organofosforinsekticiden de voortplanting en overleving voor cladoceren en in het water levende insekten in gevaar kan brengen.

In hoofdstuk 5 wordt het onderzoek beschreven naar de snelheid waarmee de vijf organofosforinsekticiden verdwijnen uit de waterfase van drie zoetwateraquaria. Aquarium 1 en 2 werden voorzien van een bodemlaag van zand en fijn slib en beplant met Elodea densa. In aquarium 3 ontbraken deze elementen teneinde de invloed van sediment en planten op de verdwijnsnelheid te kunnen bepalen. De vijf insekticiden werden elk in een concentratie van 1 mg $m^{-3}$ in de aquaria gedoseerd. Na 1, 20, 50, 90, 152, 216, 355, 525 en 1005 uur werd telkens 1 $dm^3$ water uit de aquaria geëxtraheerd en gaschromatografisch geanalyseerd. De halfwaardetijden van de insekticiden werden berekend op basis van een eerste-orde-reactie (tabel 28). De aanwezigheid van planten en bodemmateriaal resulteerde in een belangrijke toename in de verdwijnsnelheid van azinfosmethyl, parathion, parathion-methyl en diazinon, maar niet in die van malathion. In beide aquaria met planten en bodemmateriaal was de volgorde van halfwaardetijden dezelfde: diazinon > azinfosmethyl > parathion > parathion-methyl > malathion. Op basis van de resultaten van dit experiment kan worden berekend met welke frequentie parathion en azinfosmethyl in de MES zouden moeten worden gedoseerd.

In hoofdstuk 6 wordt verslag gedaan van het onderzoek met de MES. In paragraaf 6.1 wordt het gebruik van MES in het toxologisch onderzoek kort bediscussieerd. Gesteld wordt dat lange-termijneffecten van chemicaliën op zoetwater-ecosystemen slechts dan kunnen worden bestudeerd, wanneer wordt voldaan aan een aantal eisen welke waarschijnlijk niet op laboratoriumschaal kunnen worden gerealiseerd. Om die reden werden MES ontworpen welke buiten waren gesitueerd en die dienden ter bestudering van de effecten van parathion en azinfosmethyl onder zo natuurlijk mogelijke omstandigheden. Hiertoe werden vier plastic bakken van 3m x 1m x 1m ingegraven op een terrein dicht bij het laboratorium (fig. 10). Vervolgens werden de bakken voorzien van bodemmateriaal, water, waterplanten en zoetwater-evertebraten. Alle vier de bakken werden bij aanvang van de periode van proefnemingen, welke meestal duurde van mei tot oktober, door middel van een waterdicht schot verdeeld in twee subsystemen. Na afloop van die periode werden de schotten weer verwijderd teneinde de subsystemen te herenigen en terugkeer te bewerkstelligen naar omstandigheden vergelijkbaar met die van vóór de proefnemingen.

Gedurende de experimenteerperiode in 1977 werden twee MES behandeld met 1 mg $m^{-3}$ van respectievelijk parathion en azinfosmethyl, terwijl in de andere MES geen behandeling met insekticide werd toegepast. In 1978 werden alle vier de MES behandeld met parathion (1, 0,5 en 0,2 mg $m^{-3}$) of azinfosmethyl (1 mg $m^{-3}$). Bij alle behandelde MES werd het insekticide toegediend aan één subsysteem, terwijl de corresponderende helft als controle fungeerde. Gedurende de proefnemingen werd een uitgebreid bemonsteringsprogramma uitgevoerd, omvattende fysisch-chemische bepalingen, vegetatieopnamen, insekticide-analyses en tellingen van zoöplankton en macrofauna. Doel van dit programma was te zien of er verschillen zouden zijn waar te nemen in de ontwikkeling van de parameters van corresponderende subsystemen en zo ja, welke van deze verschillen zouden kunnen worden toegeschreven aan de behandeling met insekticide.

De resultaten worden beschreven en bediscussieerd in respectievelijk paragraaf 6.3 en 6.4, terwijl de conclusies worden geformuleerd in paragraaf 6.5. Er werd gevonden dat vrijwel alle fysisch-chemische en biologische parameters in de corresponderende subsystemen van de onbehandelde MES zich op dezelfde wijze ontwikkelden. Soortgelijke overeenkomsten werden ook waargenomen tussen corresponderende subsystemen van MES welke met insekticide werden behandeld, voor die parameters waarvan een beïnvloeding door het insekticide niet waarschijnlijk was. Anderzijds vertoonden sommige van dergelijke parameters juist wel verschillen. Deze verschillen echter zouden in toekomstige proefnemingen kunnen worden voorkomen door de tweedeling van de MES uit te stellen tot de vegetatie, als de belangrijkste conditionerende parameter, zich zou hebben ontwikkeld tot een aanzienlijk grotere biomassa.

Ofschoon het beperkte aantal MES geen statistische evaluatie van de resultaten toestond, waren er toch voldoende aanwijzingen om de veranderingen in de ontwikkeling van het zoöplankton in de behandelde subsystemen, in vergelijking tot die in de controles, in de meeste gevallen toe te kunnen schrijven aan de behandeling met insekticide. Een overzicht van deze veranderingen in de ontwikkeling van het zoöplankton is te vinden in tabel 39. Er werd een sterke afname van de omvang van de populaties van Daphnia spp. waargenomen gedurende de behandeling met parathion (0,5 en 1 mg $m^{-3}$) en azinfosmethyl (1 mg $m^{-3}$). Sterk verminderde aantallen werden ook gesignaleerd voor Simocephalus vetulus bij een concentratie van 1 mg $m^{-3}$ parathion en in mindere mate bij de dosering met 1 mg $m^{-3}$ azinfosmethyl. De aantallen van Chydorus sphaericus daalden sterk bij een concentratie van 1 mg $m^{-3}$ parathion en in iets mindere mate bij 0,5 mg $m^{-3}$. Populaties van Chaoborus crystallinus namen snel in omvang af bij 1 mg $m^{-3}$ parathion, maar niet bij 0,5 mg $m^{-3}$. Slechts een lichte daling in aantallen werd waargenomen voor Graptoleberis testudinaria bij 1 mg $m^{-3}$ parathion. Voor de Cyclopoida (inclusief nauplii en copepodieten) en Ostracoda werd geen afname van de populatieomvang waargenomen als reactie op de insekticiden. In enkele gevallen trad algenbloei op, welke, naar het scheen, werd veroorzaakt door de afwezigheid van voldoende herbivoor zoöplankton tengevolge van een afname van de cladoceren-populatie. Ofschoon geen gedetailleerde gevolgtrekkingen kunnen worden gemaakt ten aanzien van andere macrofaunasoorten dan Chaoborus, kan toch worden gesteld dat zich geen ingrijpende effecten

hebben voorgedaan.

In hoofdstuk 7 worden de resultaten van de MES-experimenten vergeleken met die van de toxiciteitstoetsen in het laboratorium. Over het algemeen stemden de resultaten goed overeen, zoals valt af te lezen uit tabel 42.

In hoofdstuk 8 worden de conclusies en aanbevelingen gegeven. Deze luiden:
- De resultaten verkregen met de MES-experimenten tonen aan dat met een beperkt aantal systemen, in weerwil van de daaraan inherente statistische beperkingen, zeer waardevolle informatie kan worden verkregen omtrent de kwetsbaarheid van een reeks organismen in een complexe ecologische context.
- De resultaten van de onderhavige studie suggereren dat voor organofosforinsekticiden no-effect-concentraties voor veldsituaties zouden kunnen worden afgeleid van 48-uur $EC_{50}$ waarden voor Daphnia magna Straus, door deze waarden te delen door 10.
- Aanbevolen wordt, de voorlopige grenswaarde voor de concentratie van cholinesteraseremmers in Nederlandse oppervlaktewateren te verlagen van 1 mg $m^{-3}$ (Indicatief Meerjarenprogramma 1975-1979 van het Ministerie van Verkeer en Waterstaat) naar 0.1 mg $m^{-3}$ paraoxon-equivalenten.

Tenslotte spreekt de auteur als zijn mening uit dat, in verband met onze beperkte en fragmentarische kennis van het complexe geheel van relaties in zoetwater-ecosystemen normen met betrekking tot toxische stoffen in het zoetwater, alvorens van kracht te worden, eerst zouden moeten worden getoetst in modelecosystemen met een relatief grote mate van natuurlijke complexiteit en onder zo natuurlijk mogelijke omstandigheden. Naar zijn mening zouden de MES gebruikt in de onderhavige studie tot een waardevol model voor een dergelijke toetsing kunnen worden ontwikkeld.

Appendix A. List of the important crustacean plankton in the freshwaters of the Netherlands. (After Gulati, 1978).

Phylum: Arthropoda

class : Crustacea

I. Subclass: Malacostraca
division: Pericarida
order : Mysidaceae (*Neomysis integer*)
order : Isopoda (*Asellus spp*)
order : Amphipoda (*Gammarus spp*)

II. Subclass: Branchiopoda
1. order: Anostraca (*Chirocephalus sp*)
2. order: Phyllopoda
1. suborder: Notostraca (*Lepidurus spp, Triops sp*)
2. suborder: Conchostraca (brackish water forms)
3. suborder: Cladocera
1. Haplopoda (*Leptodora kindtii*)
2. Eucladocera
1. superfamily Sidoidea (*Sida sp, Diaphanosoma sp*)
2. superfamily Daphnoidea
1 family Chydoridae (*Alona spp, Craptoleberis sp, Alonella spp, Peracantha sp, Pleuroxus spp, Chydorus spp*)
2 family Macrothricidae (*Iliocryptus sp*)
3 family Bosminidae (*Bosmina spp*)
4 family Daphniidae (*Daphnia spp, Simocephalus spp, Ceriodaphnia spp*)

III. Subclass: Maxillopoda.
1. order: Copepoda
1. suborder: Cyclopoida
2. suborder: Calanoida
3. suborder: Harpactoida

IV. Subclass: Ostracoda

Appendix B. Number of microcrustaceans in the water samples taken from the test aquaria for insecticide analysis at different times after the insecticides were introduced.

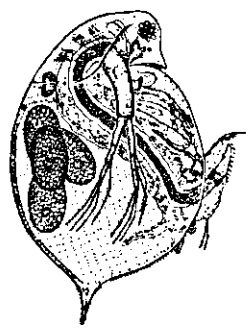
| | Time (hours) | Simocephalus vetulus | Chydorus sphaericus | Graptoleberis testudinaria | Cyclopoida |
|---|---|---|---|---|---|
| Aquarium 1 | 0 | 3 | 155 | - | 75 |
| | 1 | - | 88 | - | 55 |
| | 20 | 2 | 11 | 1 | 40 |
| | 50 | 2 | 3 | - | 93 |
| | 90 | 1 | 5 | 1 | 139 |
| | 152 | 5 | 2 | 28 | 203 |
| | 216 | 5 | 4 | 23 | 88 |
| | 355 | 24 | 3 | 26 | 224 |
| | 525 | 46 | 5 | 25 | 140 |
| | 1002 | 14 | - | 11 | 59 |
| Aquarium 2 | 0 | 16 | 206 | 10 | 298 |
| | 1 | 6 | 205 | 10 | 178 |
| | 20 | 5 | 32 | 7 | 246 |
| | 50 | 1 | - | 13 | 285 |
| | 90 | - | 2 | 4 | 155 |
| | 152 | 2[1] | 2[1] | 30[1] | 455[1] |
| | 216 | | | | |
| | 355 | - | 1 | 12 | 242 |
| | 525 | 13 | 2 | 5 | 90 |
| | 1002 | 35 | - | 22 | 118 |
| Aquarium 3 | 0 | - | 998 | 86 | 31 |
| | 1 | 1 | 1058 | 82 | 42 |
| | 20 | - | 17 | 12 | 30 |
| | 50 | - | - | 4 | 25 |
| | 90 | - | - | 7 | 31 |
| | 152 | - | - | 6 | 31 |
| | 216 | - | - | 2 | 29 |
| | 355 | - | - | 4 | 12 |
| | 525 | 1 | - | 4 | 7 |
| | 1002 | - | - | 3 | 13 |

[1] The 2 samples were accidently put together.

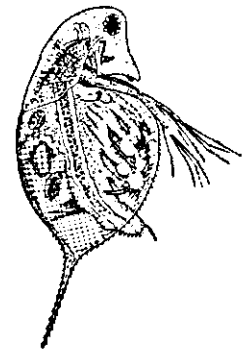
The results of the zooplankton countings do not permit more than some preliminary conclusions: - Cyclopoida do not seem te be affected; - populations of Chydorus sphaericus are heavily reduced in numbers; - populations of Graptoleberis testudinaria do not seem to be affected in the aquaria with sediment and plants. However, numbers in the aquarium without these matters were clearly reduced.

Appendix C. Main aquatic invertebrates studied in the MES-experiments. Permission to reproduce figures □ granted by the Franckh'schen Verlagshandlung, KOSMOS-Verlag, Stuttgart, from Streble/Krauter, Das Leben im Wassertropfen, 1976. ○ from H.C. Redeke, Hydrobiologie van Nederland - De zoete wateren. Backhuys & Meesters, Amsterdam, 1975.

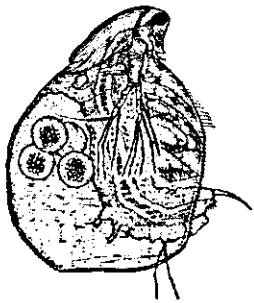
□Daphnia magna
≤6 mm



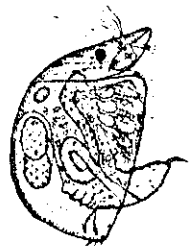
□Daphnia pulex
≤4 mm



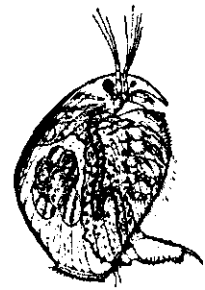
□Daphnia longispina
≤2.5 mm



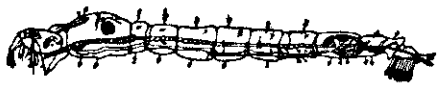
□Simocephalus vetulus
≤3 mm



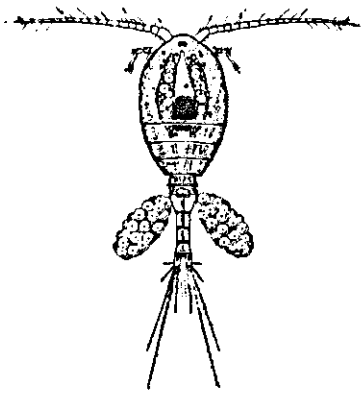
□Graptoleberis testudinaria
≤0.7 mm



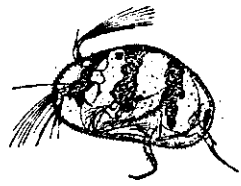
□Peracantha truncata
≤0.65 mm



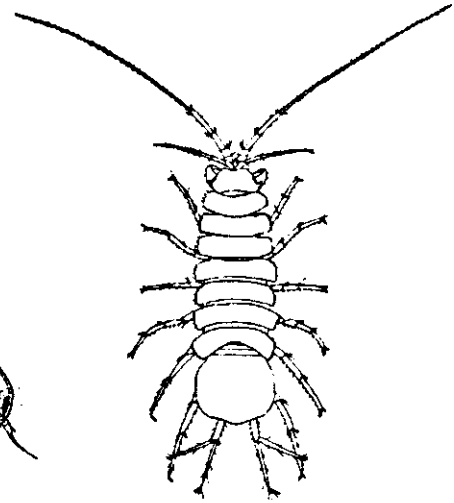
○Chaoborus crystallinus ≤14 mm



□Copepod 1-4 mm
(Macrocyclops albidus)



□Ostracod 0.5-1.2 mm
(Cypridopsis vidua)



○Asellus aquaticus
≤20 mm

Appendix D. List of aquatic zooplankton and macrofauna species collected from the MES during 1976-1978.

*Tricladida*

Dendrocoelum lacteum
Dugesia lugubris
Planaria torva
Polycelis nigra
P. tenuis

*Ciliata*

Spirostomum ambiguum
Stentor polymorphus

*Rotatoria*

Brachionus sp.
Keratella quadrata
Lecane sp.
Polyarthra sp.

*Mollusca*

Bythinia tentaculata
Lymnea peregra
L. stagnalis
Physa fontinalis
Planorbis corneus
P. planorbis
P. vortex
Valvata cristata

*Hirudinea*

Glossiphonia complanata
G. heteroclita
Helobdella stagnalis
Herpobdella octoculata
H. testacea

*Oligochaeta*

Chaetogaster sp.
Lumbriculus variegatus
Stylaria lacustris
Tubifex sp.

*Cladocera - Daphniidae*

Ceriodaphnia quadrangula
Daphnia pulex curvirostris
Daphnia pulex pulex
Simocephalus vetulus

*Cladocera - Chydoridae*

Alona guttata
Alonella (exiqua?)
Chydorus sphaericus
Graptoleberis testudinaria
Peracantha truncata
Pleuroxus aduncus

*Ostracoda*

Candona candida
Cypridopsis vidua
Notodromas monacha

*Copepoda - Cyclopoida*

Acanthocyclops vernalis
Cyclops furcifer
C. strenuus
Eucyclops speratus
Macrocyclops albidus

*Copepoda - Harpactoida*

*Isopoda*

Asellus aquaticus
Proasellus meridianus

*Ephemeroptera*

Cloeon dipterum

*Odonata - Zygoptera*

*Diptera*

Ceratopogonidae
Culex sp.
Chaoborus crystallinus
Chironomus sp.

*Coleoptera*

Ilibius sp.
Haliplus spp.
Helephorus guttatus
Hydroporus erythrocephalus
H. planus
Laccophilus minutus

*Heteroptera*

Corixa punctata
Gerris lacustris
Notonecta glauca
Sigara lateralis
Sigara striata

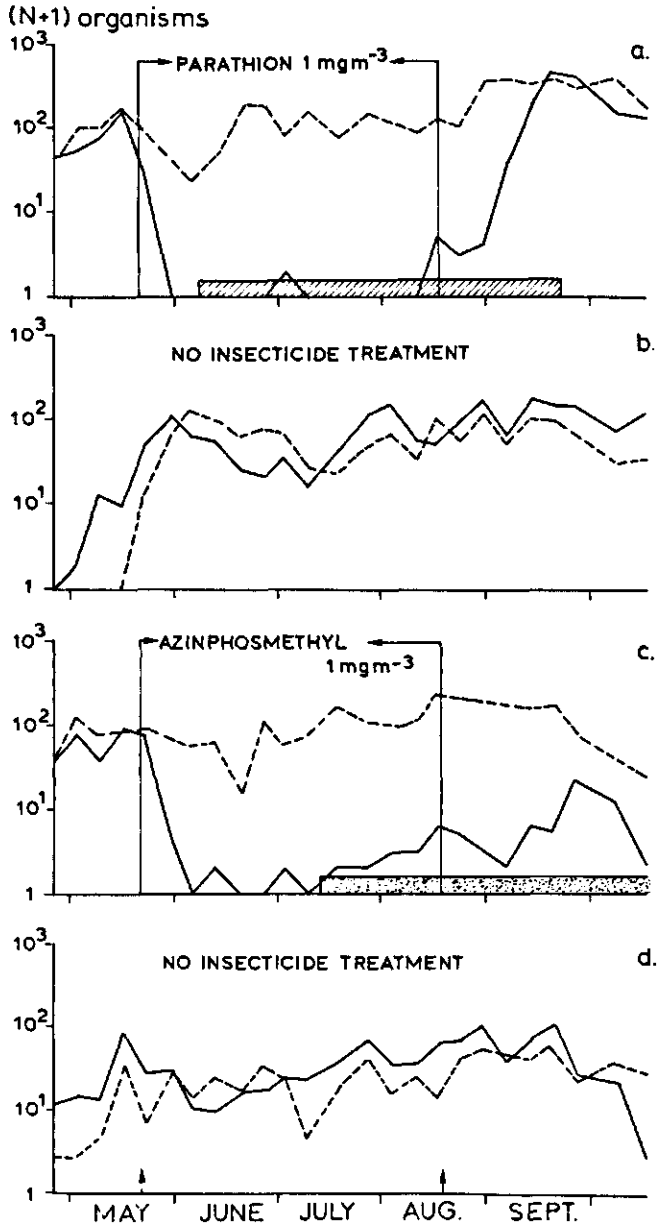



Figure 18. Numbers of Simocephalus vetulus present in the zooplankton samples from MES I/IV (Figs. a/d, respectively) in 1977. Period of insecticide treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. —— treated subsystem (except MES II and IV) --- control.

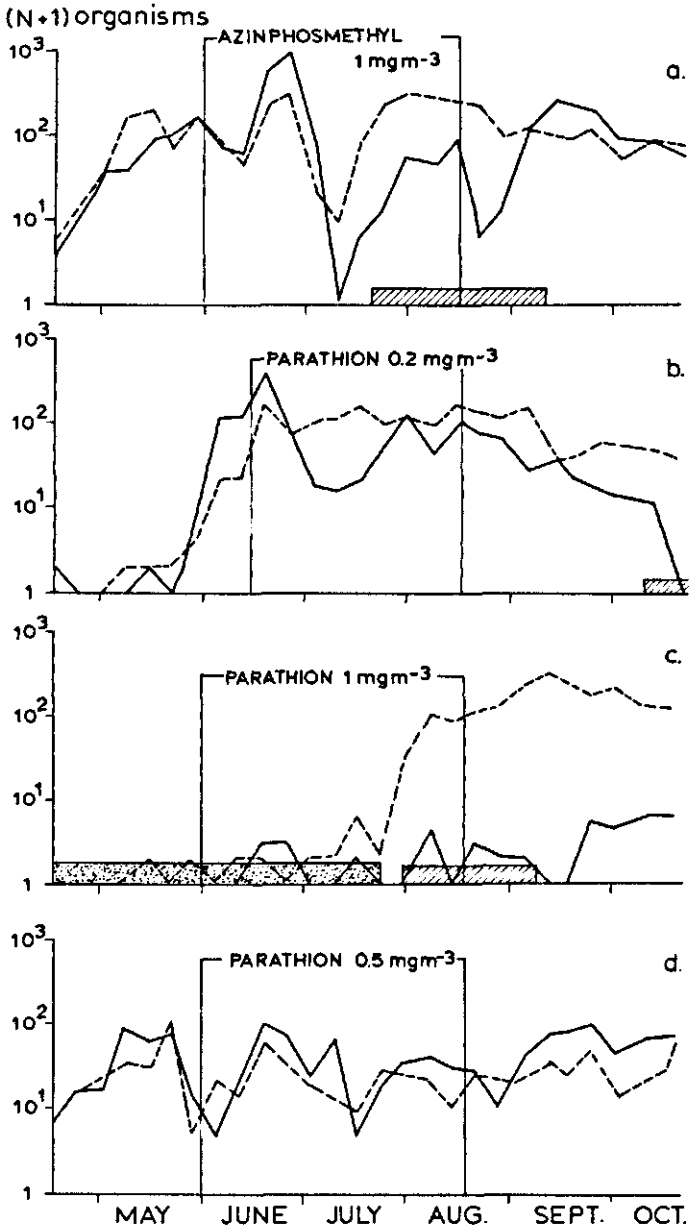



Figure 19. Numbers of Simocephalus vetulus present in the zooplankton samples from MES I/IV (Figs. a/d, respectively) in 1978. Period of insecticide treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. — treated subsystem --- control.

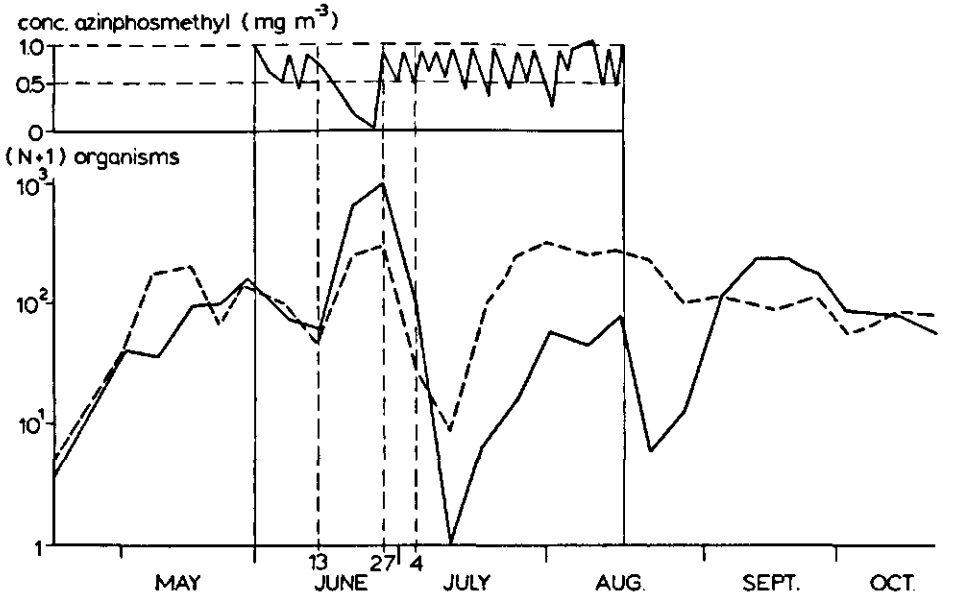



Figure 20. Numbers of Simocephalus present in the zooplankton samples from MES I in 1978. Period of insecticide treatment is indicated by vertical lines. Insert shows the pattern of the concentration of azinphosmethyl in the water phase. —— treated subsystem --- control.

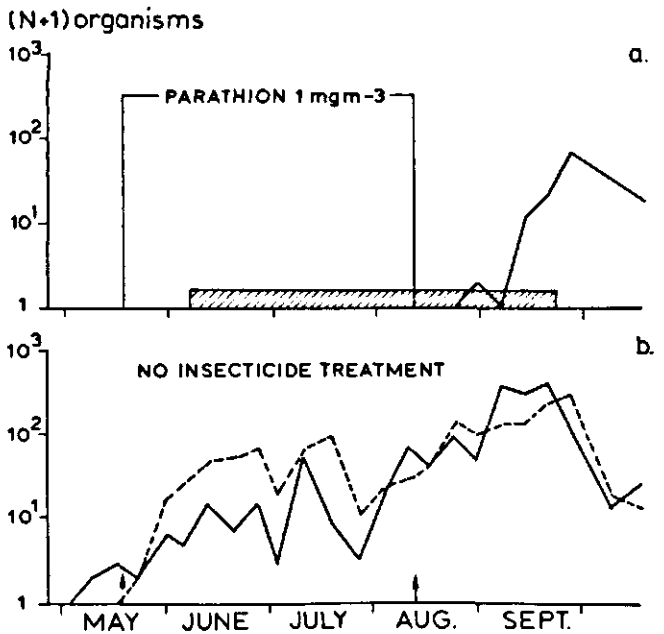



Figure 21. Numbers of Daphnia longispina present in the zooplankton samples from MES I (Fig.a) and MES IV (Fig.b) in 1977. Period of treatment is indicated by vertical lines. Horizontal bar indicates a period of algal bloom. —— treated subsystem (except MES IV) --- control.

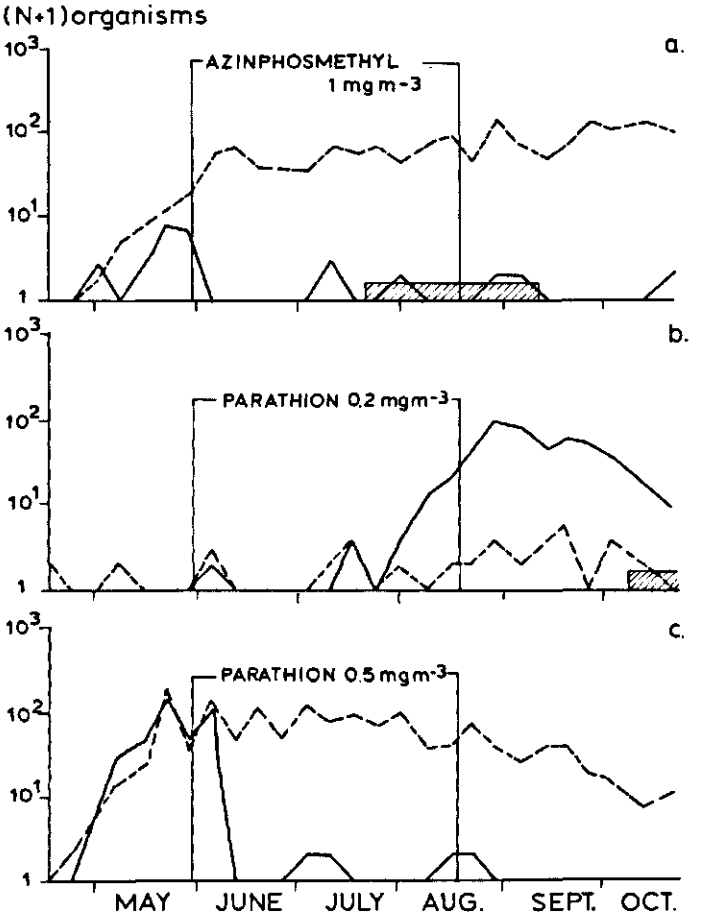



Figure 22. Numbers of Daphnia longispina and D. pulex present in the zooplankton samples from MES I, II and IV (Figs. a/c, respectively) in 1978. For MES II numbers of D. pulex curvirostris are included in D. longispina numbers. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. —— treated subsystem --- control.

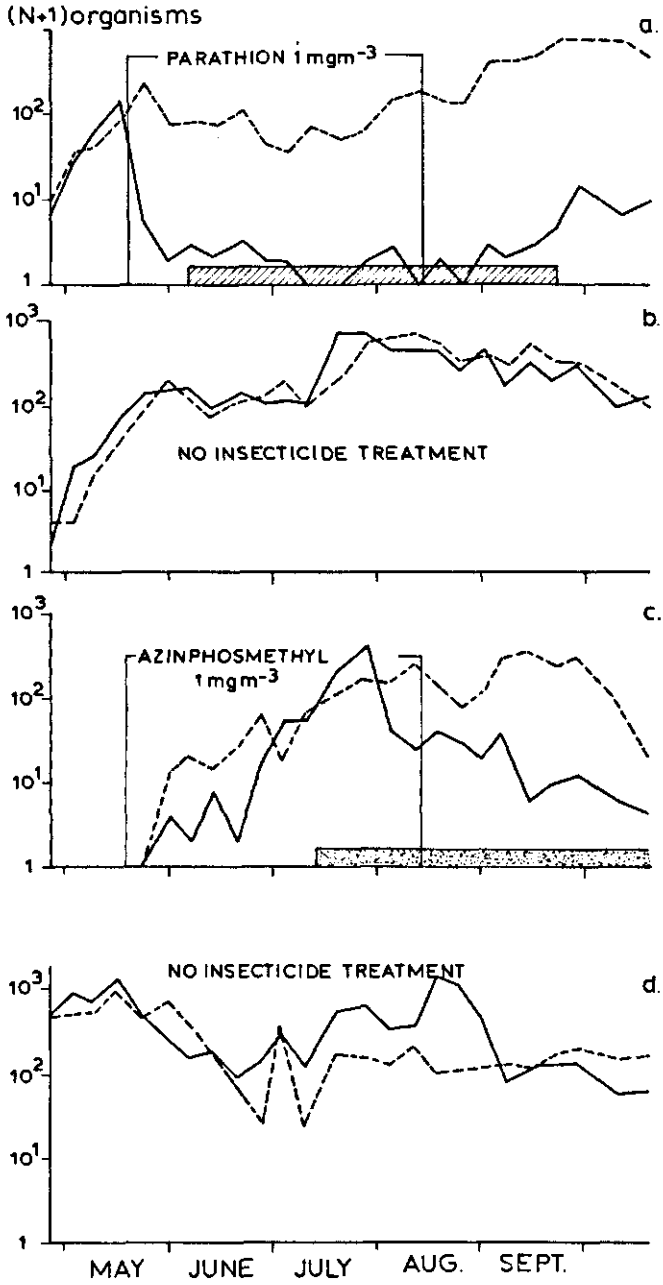



Figure 23. Numbers of Chydorus sphaericus present in the zooplankton samples from MES I/IV (Fig. a/d, respectively) in 1977. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. —— treated subsystem (except MES II and IV) --- control.

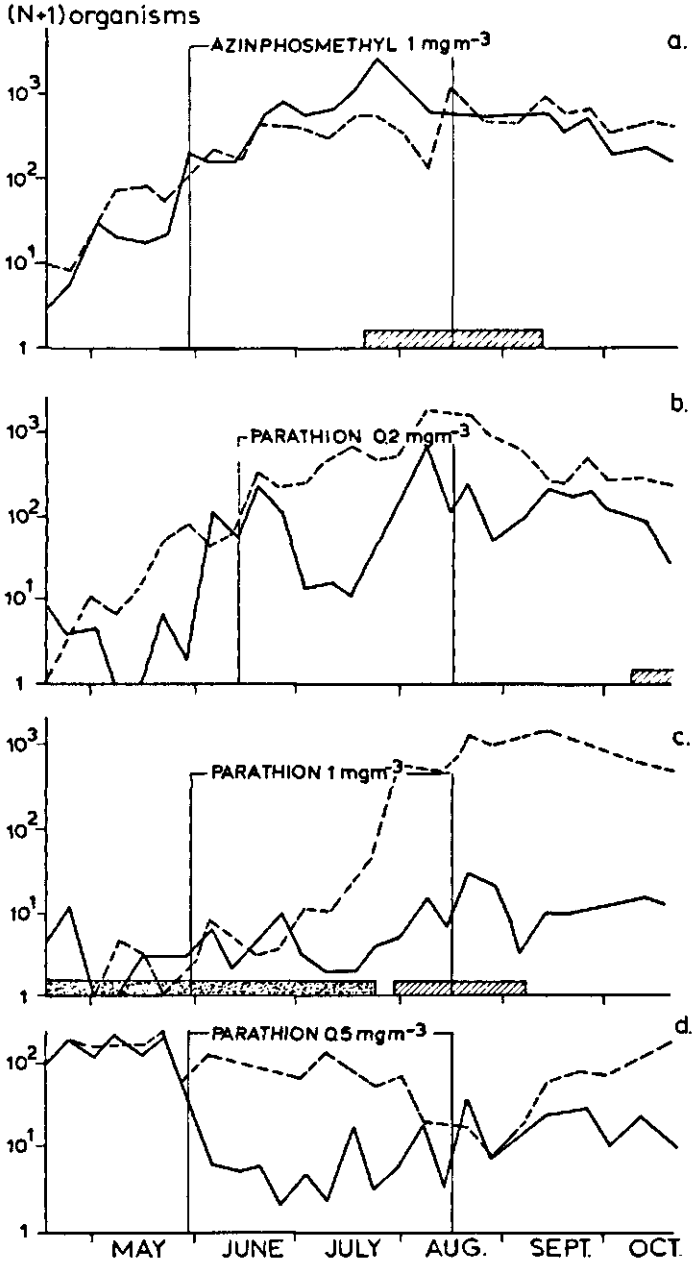



Figure 24. Numbers of Chydorus sphaericus present in the zooplankton samples from MES I/IV (Figs. a/d, respectively) in 1978. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. — treated subsystem --- control.

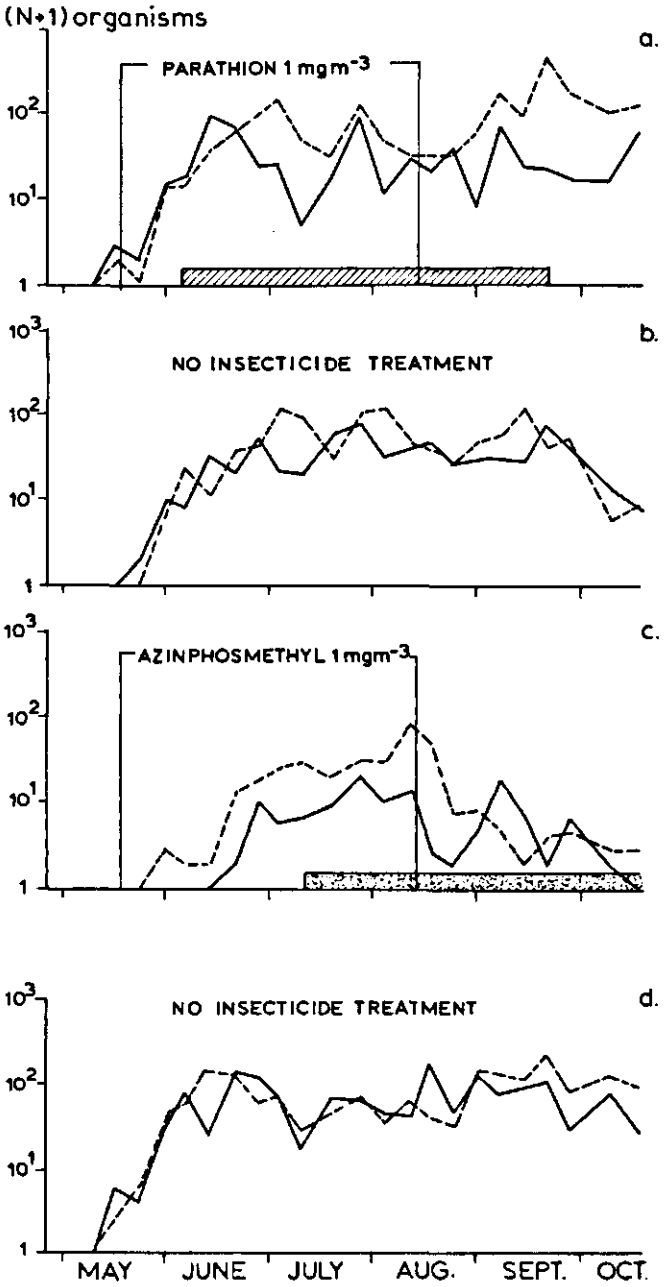



Figure 25. Numbers of Graptoleberis testudinaria present in the zooplankton samples from MES I/IV (Figs. a/d, respectively) in 1977. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. —— treated subsystem (except MES II, IV) --- control.

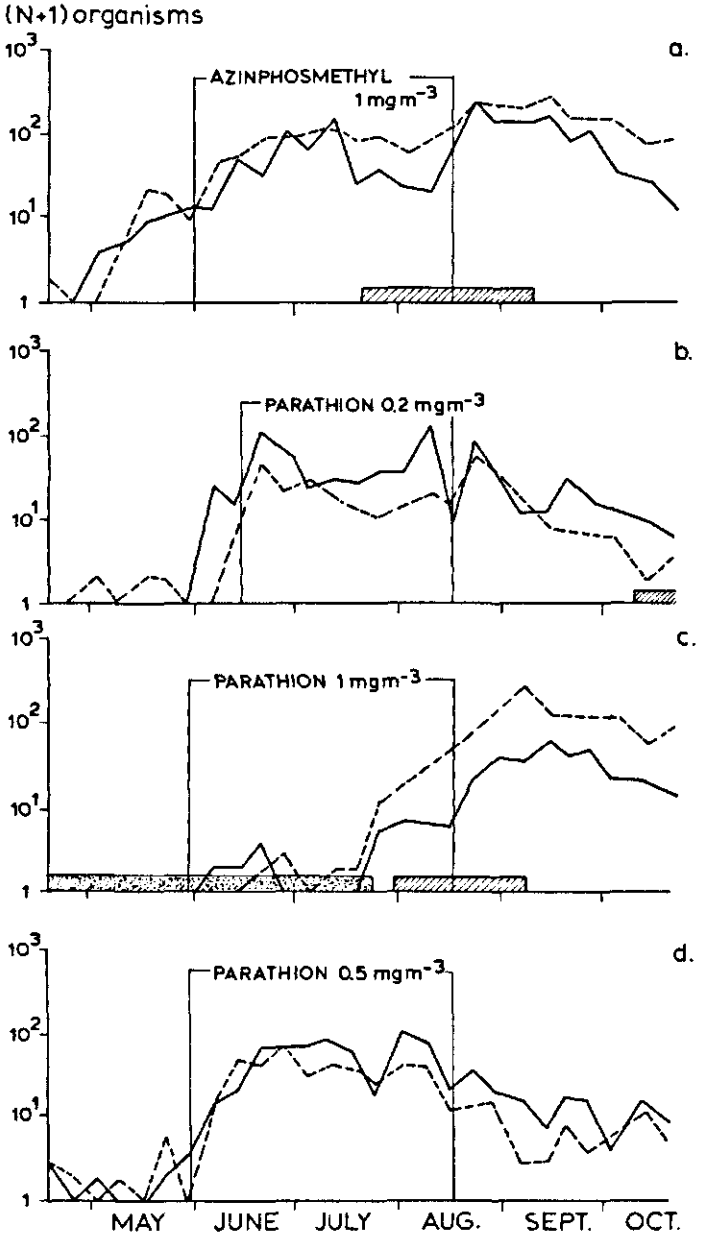



Figure 26. Numbers of Graptoleberis testudinaria present in the zooplankton samples from MES I/IV (Figs. a/d, respectively) in 1978. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. —— treated subsystem --- control.

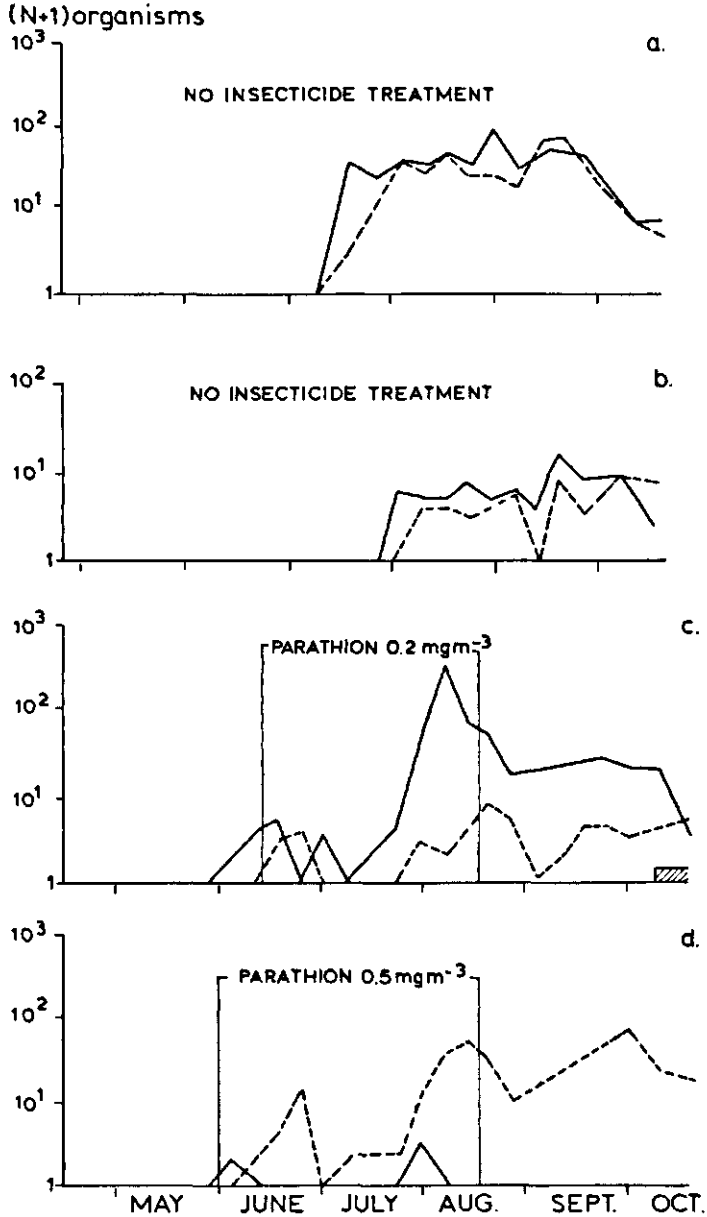



Figure 27. Numbers of Peracantha truncata present in the zooplankton samples from MES II, IV (Figs. a,b, respectively) in 1977 and MES II, IV (Figs. c,d, respectively) in 1978. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom. --- treated subsystem (except MES II, IV in 1977) --- control.

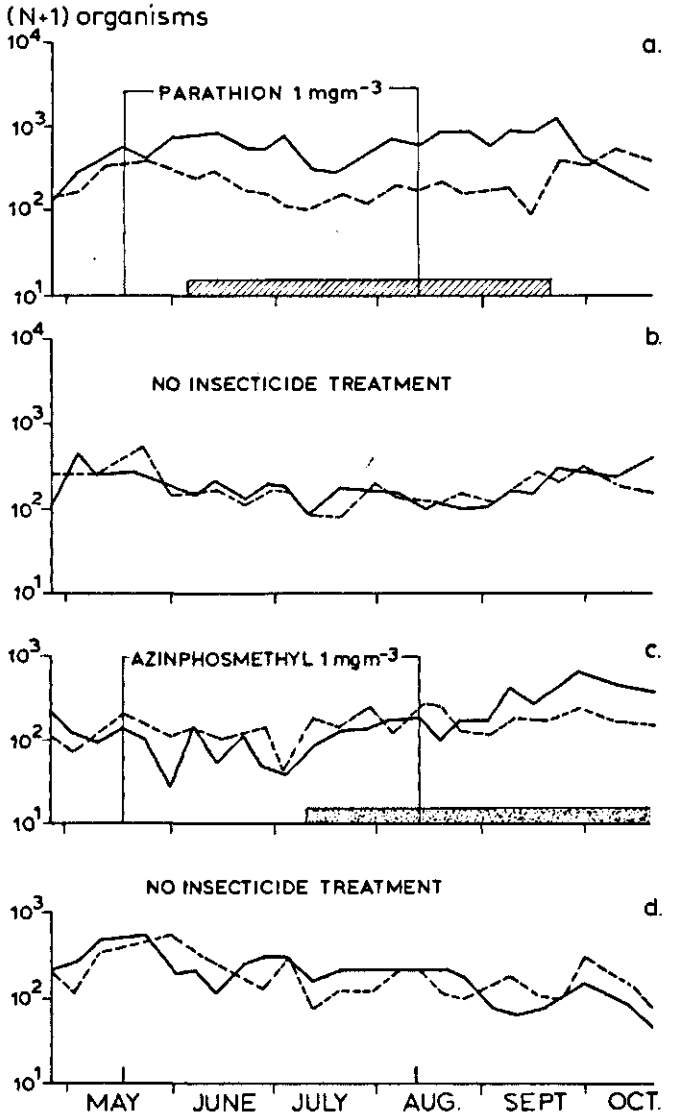



Figure 28. Numbers of Cyclopoida (all stages) present in the zooplankton samples from MES I/IV (Figs. a/d, respectively) in 1977. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. —— treated subsystem (except MES II, IV) --- control.

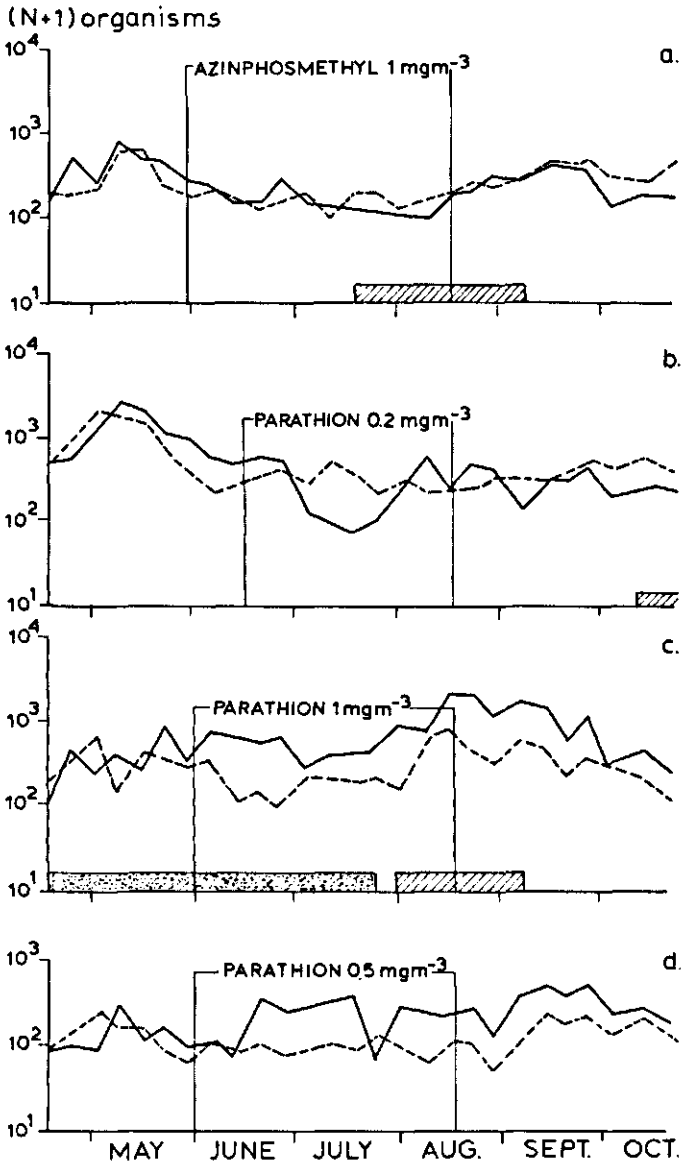



Figure 29. Numbers of Cyclopoida (all stages) present in the zooplankton samples from MES I/IV (Figs. a/d, respectively) in 1978. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. — treated subsystem --- control.

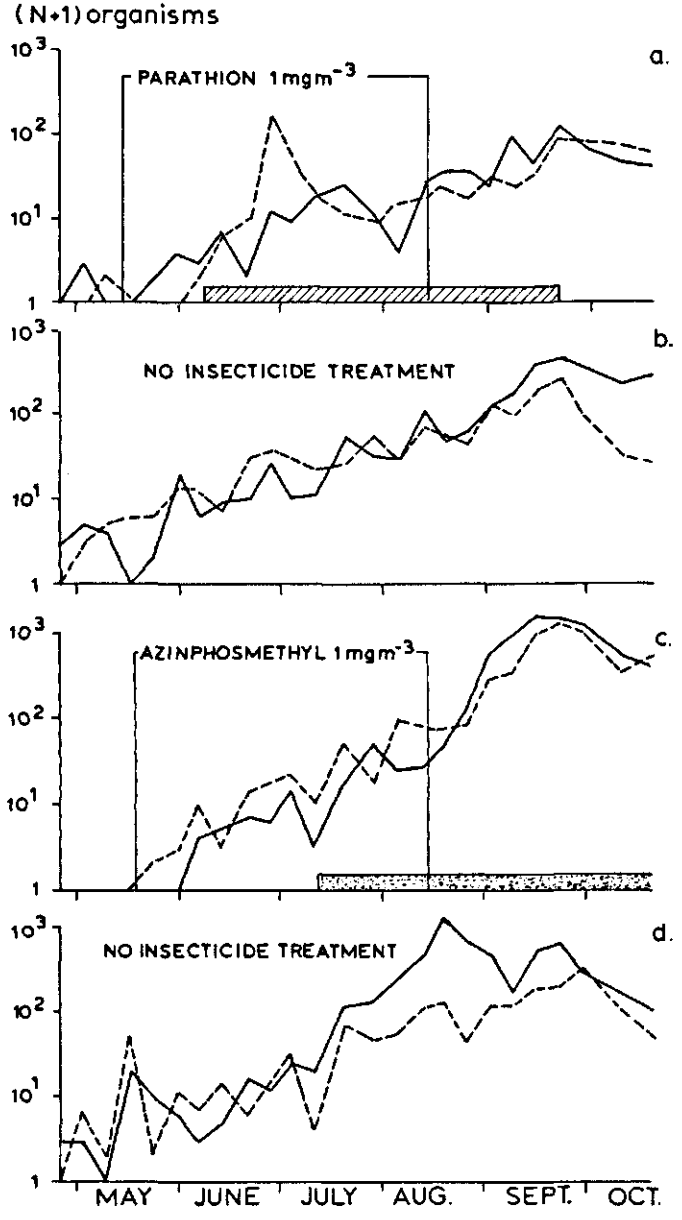



Figure 30. Numbers of Ostracoda present in the zooplankton samples from MES I/IV (Figs a/d, respectively) in 1977. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. —— treated subsystem (except MES II, IV) --- control.

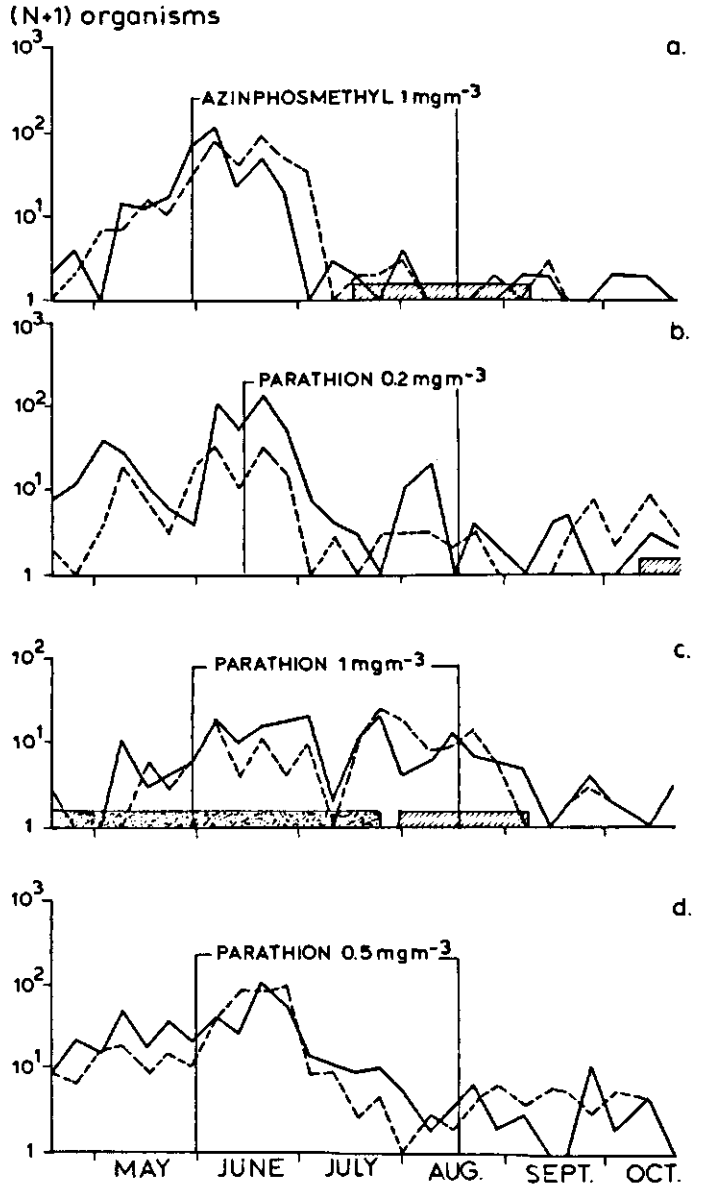



Figure 31. Numbers of Ostracoda present in the zooplankton samples from MES I/IV (Figs. a/d, respectively) in 1978. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. — treated subsystem --- control.

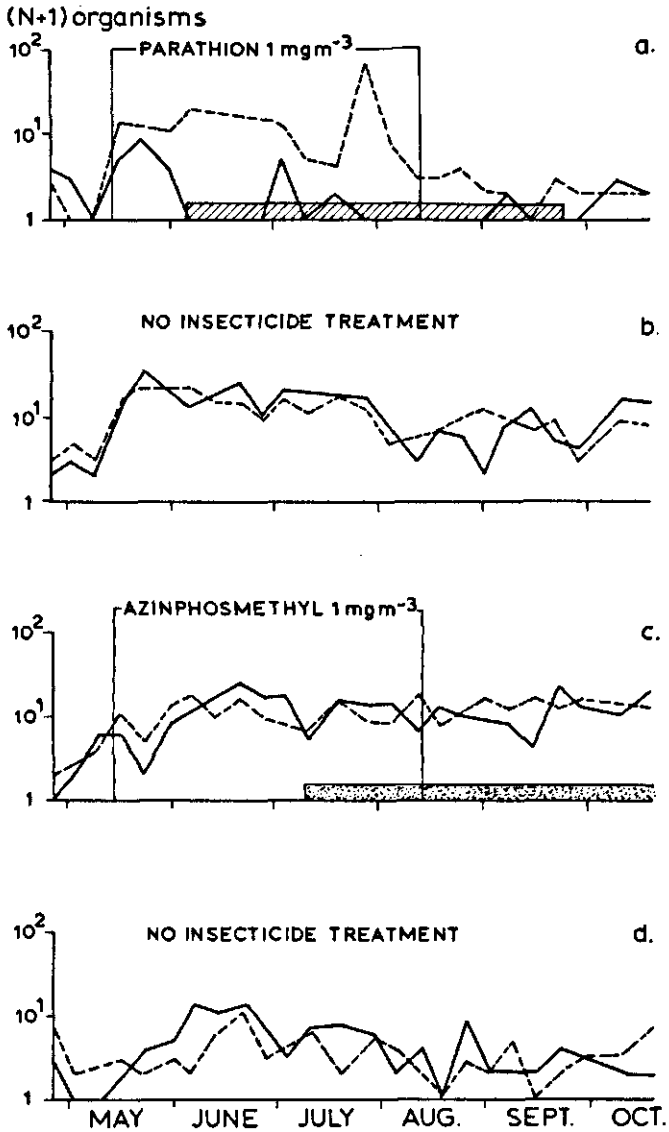



Figure 32. Numbers of Chaoborus crystallinus present in the zooplankton samples from MES I/IV (Figs. a/d, respectively) in 1977. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. —— treated subsystem (except MES II, IV) --- control.

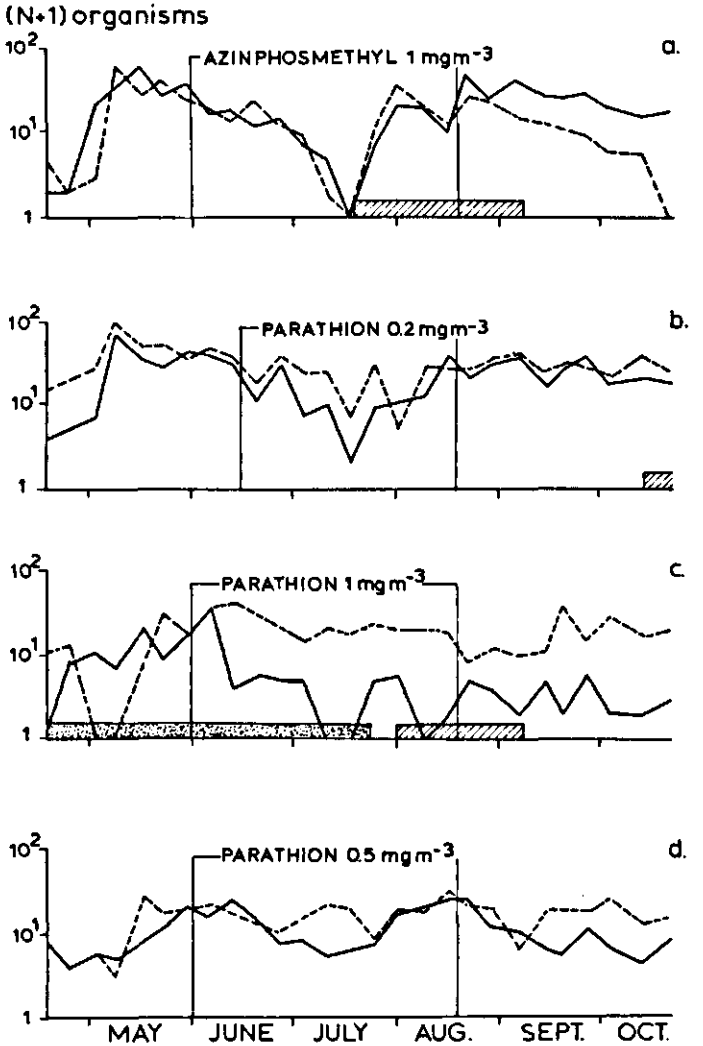



Figure 33. Numbers of Chaoborus crystallinus present in the zooplankton samples from MES I/IV (Figs. a/d, respectively) in 1978. Period of treatment is indicated by vertical lines. Shaded bars indicate periods of algal bloom, dotted bars of ciliate bloom. —— treated subsystem --- control.

Appendix F. Number of macrofauna collected from the water plants that were removed from the MES in November 1977 and 1978.

| Organisms | Sub system | MES I | | MES II | | MES III | | MES IV | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1977 | 1978 | 1977 | 1978 | 1977 | 1978 | 1977 | 1978 |
| *Tricladida* | A | 15 | 99 | 28 | 218 | 159 | 26 | 53 | 64 |
| | B | 4 | 58 | 18 | 323 | 216 | 18 | 42 | 56 |
| Dendrocoelum lacteum | A | 4 | 63 | 17 | 167 | 30 | 4 | 9 | 33 |
| | B | 2 | 42 | 9 | 291 | 55 | 1 | 4 | 17 |
| Dugesia lugubris | A | 11 | 36 | 2 | 41 | 129 | 22 | 5 | 4 |
| | B | 2 | 16 | 1 | 117 | 161 | 17 | 5 | 5 |
| Polycelis spp. | A | - | - | 9 | 10 | - | - | 39 | 27 |
| | B | - | - | 8 | 5 | - | - | 33 | 34 |
| *Hirudinea* | A | 141 | 26 | 127 | 123 | 161 | 38 | 5 | 71 |
| | B | 57 | 27 | 68 | 131 | 195 | 54 | 6 | 64 |
| Herpobdella spp. | A | 119 | 19 | 97 | 88 | 36 | 30 | 54 | 69 |
| | B | 49 | 12 | 46 | 123 | 126 | 50 | 25 | 59 |
| Glossiphonia spp. | A | 11 | 7 | 10 | 3 | 14 | 2 | - | 2 |
| | B | 1 | 4 | 5 | 5 | 6 | 1 | 1 | 5 |
| Helobdella stagnalis | A | 11 | - | 20 | 24 | 18 | 6 | - | - |
| | B | 7 | 9 | 17 | - | 28 | 3 | - | - |
| *Crustacea* | A | 243 | 54 | 664 | 11 | 367 | 38 | 176 | 20 |
| | B | 60 | 28 | 412 | 1 | 1039 | 84 | 188 | 164 |
| Asellus sp. | A | 200 | 54 | 430 | 11 | 307 | 38 | 122 | 20 |
| >4 mm | B | 58 | 28 | 410 | 1 | 633 | 84 | 163 | 164 |
| Asellus sp. | A | 43 | - | 234 | - | 60 | - | 54 | - |
| <4 mm | B | 2 | - | 2 | - | 406 | - | 25 | - |
| *Oligochaeta* | | | | | | | | | |
| Lumbriculus | A | - | - | - | - | 142 | - | - | - |
| variegatus | B | - | - | - | - | 134 | - | - | - |
| *Mollusca* | A | 42 | 11 | 163 | 232 | 92 | 448 | 130 | 64 |
| | B | 91 | 1 | 111 | 67 | 30 | 111 | 127 | 42 |
| Gastropoda | A | 42 | 11 | 163 | 232 | 63 | 441 | 130 | 64 |
| | B | 91 | 1 | 111 | 65 | 29 | 109 | 127 | 41 |
| Bivalvia | A | - | - | - | - | 29 | 47 | - | - |
| | B | - | - | - | 2 | 1 | 2 | - | 1 |

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