Ecological risks of pesticides in freshwater ecosystems

Part 2: Insecticides

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Part 2: Insecticides

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ABSTRACT

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A literature review of freshwater model ecosystem studies with insecticides was performed 1) to assess the $NOEC_{ecosystem}$ for individual compounds, 2) to compare these threshold levels with water quality standards and 3) to evaluate the ecological consequences of exceeding these standards. Studies were judged appropriate for this purpose when 1) the test systems simulated a realistic freshwater community, 2) the experimental design was generally sound (ANOVA or regression design; exposure concentrations described) and 3) when published in 1980 and later. Most studies dealt with organophosphates (predominantly single applications) and synthetic pyrethroids (mostly repeated applications) in standing waters. Structural endpoints were more sensitive than functional ones. The most sensitive taxa were representatives of the crustaceans, insects and fish. Most studies tested relatively high concentrations, with even lowest concentrations showing effects. A $NOEC_{eco}$ could therefore be established for a limited number of compounds only. Based on toxic units, safe threshold values were more or less the same for compounds with a similar mode of action. This also accounted for the nature and magnitude of direct effects at higher concentrations. Usually, indirect effects were reported at higher concentrations than those for direct effects. Although laboratory single species toxicity tests may not allow predictions on (exact) ecological effects, some generalisations on direct effects and recovery can be made with respect to the acute EC_{50} of the most sensitive standard test species. Safe concentrations, as set for water quality standards, appear to be protective. Depending on exposure regime, the $NOEC_{eco}$ is generally in the range of (0.1-0.01) x EC_{50} of the most sensitive standard test species. Recovery of sensitive endpoints usually takes place within two months after the last application when peak concentrations stay lower than (0.1-1) x EC_{50} of the most sensitive standard test species.

Keywords: ecological risk assessment, insecticides, freshwater ecosystems, microcosms, mesocosms, ecotoxicology, aquatic ecology, water quality

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Contents

LIST OF ABBREVIATIONS

CAB	Chemical Abstracts
DLO	Agricultural Research Department, Wageningen, The Netherlands
DT_{50}	half-life value for degradation
EC_{50}	concentration at which effects occur in 50% of the number of test organisms
EPA	Environmental Protection Agency (USA)
EU	European Union, Brussels
gm-EC_{50}	geometric mean of different EC_{50} values for the same standard test species
LOEC	lowest concentration at which an effect is observed
$LOEC_{eco}$	LOEC for the most sensitive endpoint studied in the ecosystem
LC_{50}	concentration at which mortality of 50% of the number of test organisms occurs
LNV	Ministry of Agriculture, Nature Management and Fisheries, The Netherlands
MPC	Maximum Permissible Concentration
Nefyto	Dutch Association of Agrochemical Industries, The Hague, The Netherlands
NOEC	highest concentration at which no effect is observed
$NOEC_{eco}$	NOEC for the most sensitive endpoint studied in the ecosystem
NW4	Fourth Memorandum Water Management
OECD	Organisation for Economic Co-operation and Development, Paris
PD	Plant Protection Service, Wageningen, The Netherlands
PEC	Predicted Environmental Concentration
POM	Particulate Organic Matter
RIKZ	National Institute for Coastal and Marine Management, Middelburg, The Netherlands
RIVM	National Institute of Public Health and the Environment, Bilthoven, The Netherlands
RIZA	National Institute for Inland Water Management and Waste Water Management, Lelystad, The Netherlands
STOWA	Foundation for Applied Water Research, Utrecht, The Netherlands
TU_{gst}	Toxic Units on the basis of the most sensitive standard test organism; concentration active ingredient in the water (C_w) divided by the gm-EC_{50} of the most sensitive standard test organism
UP	Uniform Principles (registration criteria for crop protection products according to the EU)
WU	Wageningen University, The Netherlands

PREFACE

This report on the ecological risks of insecticides in freshwater ecosystems is a translation of a STOWA / SC-DLO report from Dutch. The reference of the original report is:

> Van Wijngaarden, R.P.A., Van Geest, G.J. & Brock, T.C.M. (1998): Ecologische risico's van bestrijdingmiddelen in zoetwater ecosystemen, deel 2: insecticiden. STOWA publicatie 98-31, Utrecht.

The translation of the report from Dutch into English was sponsored by the European Crop Protection Association. Theo Brock (Alterra) was responsible for the final editing of the English version.

During the past years, various experiments have been conducted in artificial ecosystems with the objective to validate the water quality criteria for pesticides. Many of these experiments have been published in the scientific literature so that this information can be used to establish ecological threshold values for pesticides in surface water.

This report is the second of the project “Ecological risks of pesticides in surface water” and deals with the insecticides. The herbicides are discussed in the first report of the project (Lahr *et al.*, 1998 (in Dutch); Brock *et al*., 2000 (in English)). The research project - which was financially supported by STOWA and The Netherlands Ministry of Agriculture, Nature Management and Fisheries - aims to provide insight into the correctness of the applied water quality criteria and the ecological consequences of criteria being exceeded. For this, the results of experiments with individual insecticides in aquatic (semi) field situations have been collected and evaluated. The project results allow a better estimation of the ecological risks of calculated and measured pesticide concentrations. This knowledge is also useful for the interpretation of (semi) field studies in the context of the registration policy of pesticides.

The library staff of SC-DLO (now Alterra) made an important contribution through their attentive assistance in collecting the literature. From STOWA, the project was initiated by Sjoerd Klapwijk and guided by Bas van der Wal, and from LNV by Her de Heer. Progress of the report was also discussed with Gertie Arts (Alterra), Margriet Beek (RIZA), Jolande de Jonge (RIZA), Jos Notenboom (RIVM), Erik van de Plassche (RIVM), Thomas Ietswaart (RIVM), and Dick Vethaak (RIKZ) and Paul van den Brink (Alterra). Their constructive criticism has gratefully been used.

1 INTRODUCTION

This report presents an analysis of the actual ecological risks of insecticides in freshwater ecosystems. In The Netherlands, the sales volume of insecticides/acaricides amounted to about 497000 kg active ingredient in 1995. This is about 5% of the total sales of pesticides (Nefyto, 1996). Some important groups of active ingredients for the control of insects, and which are also discussed in this report, are the organophosphorous insecticides (sales in 1995 about 253000 kg active ingredient), the carbamates (sales about 113000 kg active ingredient), chlorinated hydrocarbons (sales about 25000 kg active ingredient), synthetic pyrethroids (sales about 8000 kg active ingredient) and acyl-urea compounds (sales about 5000 kg active ingredient).

In The Netherlands, various reports have been published in which the aquatic ecotoxicology of insecticides is discussed (Teunissen-Ordelman & Schrap, 1996; Teunissen-Ordelman *et al.*, 1996; Crommentuijn *et al.*, 1997; Beek & Knoben, 1997). These reports present information on physico-chemical properties, presence in surface water, toxicity to aquatic organisms, and setting of water quality criteria. Results of controlled (semi) field experiments with insecticides have, however, hardly been included in these reports. For a limited number of insecticides, a comparison of the sensitivity of aquatic species between laboratory tests and mesocosm experiments has been presented by Emans *et al*. (1992) and Jak *et al.* (1994). A recent overview of the ecological impact of insecticides in freshwater ecosystems, however, is lacking.

The available literature shows that descriptive hydrobiological field research into effects of insecticides is scarce. This is the reason why the data presented in this report are mainly based on experiments in aquatic model ecosystems, depending on their dimensions also called microcosms (relatively small) or mesocosms (relatively large). An advantage of these experimental ecosystems constructed by the researcher is that they can be replicated. This allows research at the ecosystem level under conditions at which only a part of the systems is treated. These systems also have the advantage that several concentrations of a pollutant can be tested at the same time. For a discussion of the advantages and shortcomings of such systems in comparison with natural aquatic ecosystems we refer to Brock *et al.* (1993a; 1995a).

The objectives of the literature review presented in this report are:

a) listing the $NOEC_{eco}$ and $LOEC_{eco}$ values for individual insecticides as established experimentally by means of freshwater model ecosystems (microcosms, mesocosms) or adequate field studies. The $NOEC_{eco}$ is the highest tested concentration at which no, or hardly, effects on the structure and the functioning of the studied (model) ecosystem are observed; the $LOEC_{eco}$ is the lowest tested concentration at which clear effects occur;
b) comparison of these $NOEC_{eco}$'s with established criteria for insecticides in surface water;
c) assessment of the ecological consequences of criteria being exceeded, including indirect (secondary) effects and recovery time.

2 MATERIAL AND METHODS

2.1 Collected literature

The literature database present at Alterra served as basis for the study. This database has been formed over the years and is kept up-to-date by means of the literature bulletins ‘Chemical Abstracts’ and ‘Currents Contents’. The existing database was checked for possible gaps through a specific literature search, for which the programme ‘Winspirs’ (version 2.0) was used. This programme was used to search the databases of ‘Agris Current’ (1980 - today), ‘Biological Abstracts’ (December 1989 – today), and ‘CAB-Abstracts’ (1980 – today). Publications up to and including June 1997 have been included in this literature search.

2.2 Criteria for the selection of suitable (semi) field studies

The following criteria were applied in the selection of the studies:

1. The test system represents a realistic freshwater community (organisms of various trophic levels are present).
2. The description of the experimental set-up is adequate and unambiguous.
3. The exposure concentrations that are relevant for the study can be derived (at least the nominal concentrations are known).
4. The investigated ‘endpoints’ (parameters selected as measuring target) are sensitive to the substance and the effects can reasonably be expected to be related to the working mechanisms of insecticides. Especially Arthropoda and fish are considered as sensitive endpoints for insecticides (Hill *et al*., 1994a; Graney *et al*., 1994; see also Tables 4 and 5).
5. The effects are statistically significant and show an unambiguous dose-effect relationship, or the observed effects are in agreement with a dose-effect relationship from additional studies.
6. For the establishment of a $NOEC_{eco}$, at least the lowest test concentration within a study should not show a consistent effect that can be attributed to the treatment; the concentration above the $NOEC_{eco}$ shows a clear effect ($LOEC_{eco}$).
7. Toxicity data of standard test organisms (at least *Daphnia* and fish) and/or water quality criteria (MPC’s) should be known for the comparison of field concentrations with target concentrations.
8. The study was published in 1980 or later.

Subsequently, the selected studies were classified according to exposure regime (single, multiple, or continuous exposure), the type of test system (stagnant or running), and the working mechanism of the insecticides. First, a distinction was made between the three main groups: 1) acetylcholinesterase inhibitors (organophosphorous compounds and carbamates), 2) synthetic pyrethroids, and 3) other insecticides (organochlor and acyl-urea insecticides).

2.3 Criteria for the classification of effect classes

The treatment-related ecological effects described in the evaluated studies were classified according to sensitivity of the response of the studied endpoints, for which a distinction was made between eight categories:

a) microcrustaceans (incl. Cladocera, Copepoda, Ostracoda),
b) macrocrustaceans (incl. Amphipoda, Isopoda),
c) insects,
d) fish,
e) rotifers (Rotifera),
f) other macro-invertebrates,
g) algae and macrophytes,
h) community metabolism.

The effects reported on these endpoints are classified into five effect classes that are based on the following criteria:

Class 1: 'effect could not be demonstrated'

- no effects observed as result of the treatment (primarily, statistical significance plays an important role for this criterion), and
- observed differences between treatment and controls show no clear causal relationship.

Class 2: 'slight effect'

- effects reported in terms of 'slight'; 'transient', and
- short-term and / or quantitatively restricted response of sensitive endpoints, and
- effects only observed at individual samplings.

Class 3: 'pronounced short-term effect'

- clear response of sensitive endpoints, but total recovery within 8 weeks after the last application, and
- effects reported as 'temporary effects on several sensitive species'; 'temporary elimination sensitive species'; 'temporary effects on less sensitive species / endpoints', and
- effects observed at some subsequent sampling instances.

Class 4: 'pronounced effect in short-term study'

- clear effects (such as strong reductions in densities of sensitive species) observed, but the study is too short to demonstrate complete recovery within 8 weeks after (the last) application of the insecticide.

Class 5: 'pronounced long-term effect'

- clear response of sensitive endpoints and recovery time of sensitive endpoints is longer than 8 weeks after the last application, and

- effects reported as 'long-term effects on many sensitive species/endpoints'; 'elimination sensitive species'; 'effects on less sensitive species/endpoints' and/or other similar descriptions, and
- effects observed at various subsequent samplings.

For practical reasons, a recovery period of 8 weeks was applied in the above-mentioned criteria because in most (semi) field studies certain endpoints (macrocrustaceans, fish, other macro-invertebrates, macrophytes) are at most sampled two-weekly or monthly.

For all eight categories of endpoints, it was established for each studied concentration of each study into which effect class the response could be classified. Plotting these results against the tested (nominal) concentrations expressed in 'toxic units' (see Section 2.4) yields a picture of the reported effects and at which concentrations these occur (see Figure 1 as example).

To present a summary of all obtained results (and their variation), the data have also been analysed with logistic regression; a distinction was made between studies with a single and with multiple/chronic applications, and the effect-classes were reduced to a binary variable (yes/no; 0/1). The effect classes were classified in three different ways: no versus slight and clear effect (Class 1 versus 2,3,4,5); no and slight versus clear effect (Class 1,2 versus 3,4,5) and recovery versus no recovery within 8 weeks (class 1,2,3 versus 5). The first classification can be considered as a 'worst case'; all effects, however small, are included. The second classification is somewhat more liberal, slight effects occurring at a single sampling are considered as less important. The third classification determines whether or not the endpoint has been able to recover within 8 weeks. Class 4 effects are not taken into consideration in this classification because the duration of these studies was too short to determine whether or not the studied endpoints did recover within 8 weeks. The following logistic model was used for these calculations:

$$y = \frac{1}{1 + e^{-b(Ln(x)-a)}},$$

in which *y* is the response variable (yes/no effect; yes/no recovery), *x* is the concentration expressed in 'toxic units' (TU_{gst}; for calculation see Section 2.4), *a* is the concentration at which for 50% of the studies an effect or no recovery has been reported, and *b* is the slope of the sigmoid curve at this concentration. The 10-, 50- and 90-percentile has been calculated by means of this function; i.e., those fitted concentrations (expressed in TU_{gst}) for which it is predicted that at 10, 50 and 90% of the studies a certain effect occurs. The 95% confidence intervals for these percentiles have also been calculated. The calculations have been performed with the GENSTAT statistical programme (Payne & Lane, 1993).

2.4 Comparison between insecticides

To enable a good comparison between studies with different insecticides, the reported field concentrations have been 'normalised' by dividing these by the EC_{50} of the aquatic standard test species *Daphnia magna* or by the LC_{50} of a more sensitive standard fish (such as *Oncorhynchus mykiss (= Salmo gairdneri), Lepomis macrochirus* and *Pimephalus promelas).* In most cases *Daphnia magna* is found to be the most sensitive standard test organism for the evaluated insecticides. For some pyrethroids *Daphnia* as well as fish are a representative sensitive standard test organism. Only for lindane, an organochlor insecticide evaluated in this report, fish are much more sensitive than *Daphnia*. The effects of a treatment are expressed in terms of 'toxic units' by dividing the field concentrations by the acute 48-96 h $L(E)C_{50}$ of a representative standard test organism.

The publications by Crommentuijn *et al.* (1997), Mayer & Ellersieck (1986) and the references from the papers about the evaluated (semi) field studies have been used as first information source of the toxicity data. Subsequently, for some substances further research has been conducted in on-line literature databases. In case several EC_{50}'s were available for the same standard test organism, the geometric mean of these values was calculated for the most sensitive species. This procedure was followed because deviating EC_{50} values, if any, are thus given less weight. The geometric mean of available EC_{50} values for the selected standard test species is in the following of this report referred to as 'gm-EC_{50}'.

One of the objectives of this report is the mutual comparison of experiments with different insecticides, for which the tested concentrations have been normalised for their toxicity to the most sensitive standard test organism; the water concentration as tested in the different experiments has been divided by the gm-EC_{50} of the most sensitive standard test species (mostly *Daphnia magna*, sometimes fish). The unit of the resulting variable is defined as TU_{gst}: Toxic Unit of the most sensitive standard test organism.

All collected toxicity data for standard test organisms are included in the appendices. Only the gm-EC_{50}'s and the water concentrations expressed in TU_{gst} are presented in the following of the main report.

2.5 Comparison of ecological threshold values with criteria

The ecological threshold values ($NOEC_{eco}$'s) obtained from (semi) field studies are compared with the criteria applied in The Netherlands. For pesticides in surface water, a distinction can be made between registration criteria and water quality criteria. The water quality criteria are based on the Maximum Permissible Concentration (MPC). In case more than 4 adequate chronic toxicity data for aquatic organisms are available, the MPC is established according to the method described by Aldenberg & Slob (1993). In case less than 4 chronic NOEC's are available, the

MPC is determined according to the modified EPA-method as described by Crommentuijn *et al.* (1997).

Registration criteria are based on the criteria described in the Uniform Principles (EU, 1997). According to the Uniform Principles, in the first tier of the risk assessment, the concentration of a pesticide in surface water should not be higher than 0.01 x the acute $L(E)C_{50}$ for fish or *Daphnia* and 0.1 x the EC_{50} for algae. In addition, the average exposure concentration may not be higher than 0.1 x the chronic NOEC of *Daphnia* (21 days) and fish (28 days) in case of prolonged exposure. Within the Dutch legal framework, however, for algae the criterion of 0.1 x NOEC is applied.

In the second tier there may be a deviation from the above-mentioned registration criteria if it is demonstrated by means of an adequate risk assessment that the actual risk to aquatic organisms is acceptable.

Criteria according to the Uniform Principles (UP criterion) are in this report established on the basis of toxicity data for test organisms according to OECD protocols. The following standard test organisms are mentioned in OECD protocols: the algae *Selenastrum capricornutus*, *Scenedesmus subspicatus* and *Chlorella vulgaris*, the crustaceans *Daphnia magna* and *Daphnia* sp, and the fish *Brachydanio rerio*, *Pimephales promelas*, *Cyprinus carpio*, *Oryzias latipes*, *Poecilia reticulata*, *Lepomis macrochirus*, *Gasterosteus aculeatus* and *Oncorhynchus mykiss*.

In this report the UP criteria are based on the acute toxicity data because:

- adequate chronic toxicity data for the substances studied in (semi) field experiments are not always available whereas acute toxicity data are,
- in (semi) field studies usually nominal or measured peak concentrations of the studied pesticide are reported but not the average exposure concentrations for 21 and 28 days, respectively,
- 0.01 x the acute $L(E)C_{50}$ for the peak concentration is usually a more stringent criterion than 0.1 x the chronic NOEC for the average concentration.

In this report we define a liberal and a conservative UP criterion. The lowest $L(E)C_{50}$ value reported in the literature for one of the above-mentioned standard test organisms is taken as a basis for the calculation of the conservative UP criterion by dividing this value by a factor 100. The liberal UP criterion is established by dividing the ‘gm-$L(E)C_{50}$’ described in the preceding section by a factor 100. The liberal UP criterion is thus usually based on the geometric mean of the available acute $L(E)C_{50}$ values for *Daphnia magna*. Only in case of lindane and some pyrethroids, the liberal UP criterion is based on the geometric mean of the available acute $L(E)C_{50}$ values of the most sensitive standard fish.

3 AVAILABLE INFORMATION

3.1 Used studies

First, extensive summaries have been made of the selected studies. A concise version of these summaries is presented in Appendices I – XXI of this report, where the substances are arranged alphabetically.

3.2 Acetylcholinesterase inhibitors

This group includes the organophosphorous and carbamate insecticides. They inhibit the activity of the enzyme acetylcholinesterase. The inhibition of the enzyme results in accumulation of acetylcholine at the choline receptors and consequently in disturbance (over-stimulation) of the nerve impulses.

The following of the about 35 organophosphorous insecticides registered in The Netherlands are the most frequently used active ingredients in agriculture (consumption > 10 000 kg active ingredient), in decreasing order, dimethoate, dichlorvos, parathion-ethyl, oxydemeton-methyl, ethoprophos (also nematicide), chlorpyrifos, acephate, and chlorfenvinphos. The organophosphorous insecticides that are according to Phernambucq *et al.* (1996) most frequently found in fresh surface water in The Netherlands are the active ingredients dichlorvos, malathion, parathion-ethyl, mevinphos, ethoprophos, and diazinon. (Semi) field experiments have only been conducted with a small number of the more than 40 organophosphorous compounds we know. After testing against the assessment criteria, 24 studies remain that yield adequate information on ecological risks of 7 active ingredients (Table 1). The selected studies have mainly been conducted with chlorpyrifos (10 studies), fenitrothion (5 studies), and azinphos-methyl (4 studies).

Of the about 9 carbamate insecticides that are registered in The Netherlands, methiocarb, pirimicarb, propoxur, carbaryl, and carbofuran are the most frequently used active ingredients in agriculture. According to Phernambucq *et al.* (1996) pirimicarb and, to a lesser extent, carbofuran were found in fresh surface water during monitoring programmes. Five (semi) field studies give adequate information on the active ingredients bendiocarb, carbaryl, and carbofuran.

Table 1. Experiments with acetylcholinesterase inhibitors included in this report. E-stag = single application in a stagnant system; E-stream = single application in a running system; M-stag = multiple applications in a stagnant system; M-stream = multiple applications in a running system; L-stag = prolonged constant exposure in a stagnant system; L-stream = prolonged constant exposure in a running system.
(*) studies do not meet all criteria but yield information on low exposure concentrations.

Active ingredient	Experiment	Location	Authors
Organophosphorous insecticides			
Azinphos-methyl	E-stag	USA (lab)	Stay & Jarvinen 1995
-	E-stag	USA (Minnesota)	Tanner & Knuth 1995
-	M-stag	USA (Kansas)	Giddings *et al.* 1994
-	E-stag	USA (Minnesota)	Knuth *et al.* 1992
Chlorpyrifos	E-stream	Australia	Pusey *et al.* 1994
-	L-stag	NL (lab)	Van den Brink *et al.* 1995
-	L-stream	Australia	Ward *et al.* 1995
-	E-stag	USA (Kansas)	Biever *et al.* 1994
-	E-stag	NL	Van Wijngaarden *et al.* 1996; Van den Brink *et al.* 1996; Kersting & Van den Brink 1997
-	E-stag	NL (lab)	Brock *et al.* 1992 a,b; 1993b
-	E-stag	NL (lab)	Van Donck *et al.* 1995; Brock et al 1995; Cuppen et al 1995
-	E-stag	USA (Minnesota)	Siefert *et al.* 1989; Brazner *et al.* 1989; Brazner & Kline 1990
-	E-stag	USA (lab)	Stay *et al.* 1989
-	E-stag	Canada	Hughes *et al.* 1980
Diazinon	M-stag	USA (Kansas)	Giddings *et al.* 1996
Fenitrothion	E-stag	Senegal	Lahr & Diallo 1993
-	M-stag	Canada	Fairchild & Eidt 1993
- (*)	E-stream	UK	Morrison & Wells 1981
- (*)	E-stream	Canada	Poirier & Surgeoner 1988
- (*)	E-stream	Japan	Yasuno 1981
Parathion-ethyl	L-stag	NL	Dortland 1980
Parathion-methyl	E-stag	UK	Crossland 1984
-	E-stag	UK	Crossland 1988
Phorate	E-stag	USA (S. Dakota)	Dieter *et al.* 1996
Carbamates			
Bendiocarb	E-stag	Senegal	Lahr *et al.*1995
Carbaryl	E-stag	USA (Ohio)	Havens 1994; 1995
-	E-stream	Canada (Maine)	Courtemanch & Gibbs 1980
Carbofuran	E-stag	Canada (Alberta)	Wayland 1991

(*) study does not meet all selection criteria

3.3 Synthetic pyrethroids

Like other large groups of insecticides (e.g., organophosphorous compounds, carbamates and organochlorides) pyrethroids affect the functioning of the nerve system. There probably is an interaction of the pyrethroid with sodium channels in the nerve membranes as a result of which the nerve system is completely disordered by a continuous series of nerve pulses. Finally, this results in the death of the organism in question (Van Rijn *et al.*, 1995).

The most important emissions of synthetic pyrethroids to surface water are caused by the use in agriculture. Of the about 12 pyrethroids registered in The Netherlands, permethrin, deltamethrin, esfenvalerate and lambda-cyhalothrin are the most used products in agriculture (sales > 1 000 kg active ingredient). Of the pyrethroids registered in The Netherlands, the active ingredients cypermethrin, deltamethrin, esfenvalerate, and permethrin were found in surface water during chemical monitoring programmes (Teunissen-Ordelman *et al.*, 1996). They conclude that few monitoring data are available on the synthetic pyrethroids applied in The Netherlands.

Sixteen (semi) field studies that yield adequate information on the active ingredients cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenvalerate, lambda-cyhalothrin, permethrin, and tralomethrin (Table 2) remain after testing against the assessment criteria. These mainly are North-American studies in stagnant systems.

Table 2. Experiments with synthetic pyrethroids included in this report. E-stag = single application in a stagnant system; E-stream = single application in a running system; M-stag = multiple applications in a stagnant system; M-stream = multiple applications in a running system; L-stag = prolonged constant exposure in a stagnant system; L-stream = prolonged constant exposure in a running system.

Active ingredient	Experiment	Location	Authors
Cyfluthrin	M-stag	USA (Texas)	Johnson *et al.* 1994; Morris *et al.* 1994
Cypermethrin	M-stag	UK	Farmer *et al.* 1995
-	M-stag1	USA (North Carolina)	Hill 1985
-	M-stag2	USA (North Carolina)	Hill 1985
Deltamethrin	E-stag	Senegal	Lahr *et al.* 1995
-	E-stag	Canada	Morill & Neal 1990
Esfenvalerate	M-stag	USA (Alabama)	Webber *et al.* 1992
-	M-stag	USA (Missouri)	Fairchild *et al.* 1992b
-	M-stag	USA (Minnesota)	Lozano *et al.* 1992; Tanner & Knuth 1996
-	M-stag	USA (Missouri)	Fairchild *et al.* 1994
-	E-stag	USA (lab)	Stay & Jarvinen 1995
Fenvalerate	E-stag	Canada (Ontario)	Day *et al.* 1987
-	L-stream	USA (Iowa)	Breneman & Pontasch 1994
Lambda-cyhalothrin	M-stag	UK	Farmer *et al.* 1995
-	M-stag	USA (North Carolina)	Hill *et al.* 1994b
Permethrin	E-stag	Canada (Ontario)	Kaushik *et al.* 1985
Tralomethrin	M-stag	USA (Texas)	Mayasich *et al.* 1994

3.4 Other insecticides

In the context of this report the group ‘other insecticides’ includes all products that do not have a direct acetylcholinesterase-inhibiting effect or that do not belong to the synthetic pyrethroids. These are organochlor and acyl-urea insecticides.

Three chlorinated hydrocarbons are still permitted as insecticide/acaricide in The Netherlands. The most frequently applied substance is lindane with a sales volume of 19 000 kg active ingredient in 1995 (Nefyto, 1996). Lindane delays the functioning of the sodium/-potassium channels in the nerve cells as a result of which the impulses of the nerves are no longer transmitted to the muscles (Van Rijn *et al.* 1995). The studies with chlorinated hydrocarbons that were considered adequate have been conducted with lindane and methoxychlor (Table 3). Methoxychlor has been banned in The Netherlands since 1990.

The acyl-urea compounds diflubenzuron and teflubenzuron are applied as insecticide in The Netherlands. These insecticides disrupt the production of chitin (Van Rijn *et al.*

1995). The external skeleton of crustaceans and insects consists for a large part of chitin, as a result of which, in case of exposure to these compounds, problems arise in particular with the metamorphosis and molting of Arthropods. The studies with acyl-urea insecticides that were considered adequate had all been conducted with the active ingredient diflubenzuron (Table 3).

Table 3. Experiments with chlorinated hydrocarbons and acyl-urea insecticides included in this report. E-stag = single application in a stagnant system; E-stream = single application in a running system; M-stag = multiple applications in a stagnant system; M-stream = multiple applications in a running system; L-stag = prolonged constant exposure in a stagnant system; L-stream = prolonged constant exposure in a running system.

Active ingredient	Experiment	Location	Authors
Chlorinated hydrocarbons			
Lindane	L-stream	UK	Mitchell *et al.* 1993
-	L-stag	Germany	Peither *et al.* 1996
Methoxychlor	E-stag	Canada (Ontario)	Stephenson *et al.* 1986
-	E-stag	Canada (Ontario)	Solomon *et al.* 1989
Acyl-urea insecticides			
Diflubenzuron	E-stag	USA (lab)	Moffett *et al.* 1995
-	E-stag	USA (Wisconsin)	Moffett *et al.* 1995
-	E-stag	USA (Wisconsin)	Tanner & Moffett 1995
-	E-stag	Senegal	Lahr & Diallo 1993
-	L-stream	USA (lab)	Hansen & Garton 1982

4 APPLICATION METHOD AND ENVIRONMENTAL BEHAVIOUR

The exposure of aquatic organisms to insecticides and the observed effects during (semi) field studies are strongly related to the method of application and the behaviour of the substances in the test systems. Pollution of watercourses with insecticides is often the result of spray drift. It is therefore not surprising that in most studies that aim to study acute risks, the insecticide is applied by spraying of the water surface. In studies with running water and in stagnant systems with a chronic exposure regime, the insecticides are usually directly mixed in the water column.

In the selected studies with organophosphorous compounds, carbamates, lindane, methoxychlor, and diflubenzuron, the active ingredients are always applied in dissolved form via the aqueous phase (spray drift or direct mixing in the water column). In most studies with pyrethroids, the active ingredients are also applied by spraying on, or injection below, the water surface. In three studies, drift as well as runoff applications are performed in the same test system. In case of runoff applications, the pyrethroid is brought into the systems bound to soil material (simulation of runoff). It is not always clear in these studies, where both applications take place at different instances in the same mesocosms (lambda-cyhalothrin (Hill *et al*., 1994b); tralomethrin (Mayasich *et al*., 1994); cyfluthrin (Johnson *et al.*, 1994)) whether the observed effects are caused by the drift or by the runoff application. Reported measured concentrations do not always give hold in view of the high disappearance rate of pyrethroids from the water and the variation in the first sampling instance after spraying (< 1 hour to 24 hours). The evaluation of the effects in these studies is therefore based on the nominal concentration by drift. This is in all cases a worst case approach. It should be noted that the contaminated soil material of the runoff application does relatively soon disappear from the water column by sedimentation. The bio-availability of the soil-bound pyrethroids is also lower.

In stagnant water, clear concentration gradients over the first hours after application of insecticides in stagnant water are in particular found in case of drift application (Fairchild & Eidt, 1993; Crum & Brock, 1994; Farmer *et al.*, 1995; Van Wijngaarden *et al.*, 1996). Especially in case of drift application, most of the active ingredient is then found in the surface water layer so that here the initial concentration may be considerably higher than the intended nominal concentration. The exposure concentration in deeper water layers is then considerably lower than the nominal concentration.

After some days the insecticides are usually well mixed in the water column and often a considerable part of the applied amount can no longer be detected in the water. The relatively high disappearance rate from the water during the first days after application is not only caused by degradation but partly also by the distribution of the active ingredient over different environment compartments such as sediment and aquatic plants (see e.g. Brock *et al.*, 1993a; Crum & Brock, 1994) and volatilisation from the water.

Initial half-life values of dissolved organophosphorous compounds, carbamates and diflubenzuron in the water of stagnant (model) ecosystems are in the order of less than 1 day to about 10 days (Crossland & Bennett, 1984; Hanazato & Yasuno, 1990; Heinis & Knuth, 1992; Lahr & Diallo, 1993; Crum & Brock, 1994; Tanner & Knuth, 1995; Wayland & Boag, 1995; Giddings *et al.*, 1996). The half-life value of the organophosphorous insecticide chlorpyrifos in the sediment of microcosms is 2 to 4 times higher than in the water column on top (Brock *et al.*, 1997).

The initial half-life values of pyrethroids in the water column are in the order of less than 1 hour to 2 days (Heinis & Knuth, 1992; Fairchild *et al.*, 1992a; Johnson *et al.*, 1994; Farmer *et al.*, 1995). Values of 2 to 3 days are only reported for permethrin (Solomon *et al.*, 1985). Generally, the half-life value of the sediment-sorbed pyrethroids is much longer (days to weeks) in the above-mentioned studies.

Of the studied insecticides the reported initial disappearance rate is lowest for the chlorinated hydrocarbons. The initial half-life value of lindane in the water column is about 22 days in the microcosm study of Peither *et al.* (1996). The half-life value of methoxychlor ranges from 6 to 13 days in the water column of an enclosure study with this substance (Solomon *et al.*, 1986).

The above shows that the reported nominal concentrations cannot directly be converted into actual exposure concentrations for aquatic organisms. The observed initial stratification of insecticides in the water column in case of simulation of spray drift makes it likely that organisms close to the bottom and between dense vegetations of aquatic plants are initially exposed to lower concentrations than a organisms that are in particular present close to the water surface. Nevertheless, in the description of the effects as a result of peak exposures we have usually nevertheless taken the nominal concentration as starting point because:

- the applied nominal dose is given in almost all studies,
- the measured initial concentrations are not always comparable/reliable due to large differences in first sampling instance after treatment (hours to days) and the relatively high initial disappearance rate of most insecticides.
- in the registration policy the short-term exposure as a result of drift is calculated by assuming instantaneous mixing of the dose over the water column (PEC_{max}).

5 SENSITIVE ENDPOINTS

5.1 Reported effects

A distinction between direct and indirect effects is frequently made in the reported effects of insecticides in (semi) field experiments. Direct (primary) effects of an insecticide are the toxicological effects of the substance that affect growth, survival and/or reproduction of organisms. Indirect (secondary) effects are the ecological effects that are the result of the reduction in activity and/or density of organisms that are sensitive to the toxic substance. Without direct effects, indirect effects in an ecosystem do not occur. A significant decrease in population density of a certain species after application of an insecticide cannot beforehand be considered as a direct effect but can also be attributed to an indirect effect.
Supporting laboratory experiments with the insecticide and the species in question are required for a definite establishment of direct effects in ecosystems.

Table 4. Reported negative effects on various taxonomic groups as a result of single applications of acetylcholinesterase-inhibiting insecticides in aquatic micro- and mesocosms. The effects are arranged according to toxic units (TU_{gst}) and are expressed as percentage of the cases in which a reduction in numbers or biomass of one or more taxa within a taxonomic group was reported.

	TU_{gst}			
	0.01-0.1	**0.1-1**	**1-10**	**10-100**
Amphipoda	0% (n=4)	43% (n=7)	100% (n=7)	100% (n=7)
Cladocera	0% (n=5)	83% (n=12)	100% (n=17)	100% (n=11)
Copepoda	0-20% (n=5)	30% (n=10)	38% (n=13)	63% (n=8)
Isopoda	-	-	100% (n=1)	100% (n=2)
Ostracoda	0% (n=3)	14% (n=7)	38% (n=8)	67% (n=6)
Trichoptera	?** (n=1)	100% (n=1)	100% (n=1)	100% (n=1)
Ephemeroptera	0% (n=2)	75% (n=4)	100% (n=3)	100% (n=3)
Diptera	0% (n=3)	57-71% (n=7)	100% (n=7)	100% (n=8)
Hemiptera	-	-	100% (n=1)	80-100% (n=5)
Odonata	0% (n=1)	0% (n=2)	75% (n=4)	83-100% (n=6)
Coleoptera	-	-	100% (n=1)	67% (n=3)
Hydracarina	0% (n=1)	0% (n=2)	50% (n=4)	33% (n=3)
Pisces	0% (n=3)	67%* (n=3)	83% *(n=6)	100%* (n=3)
Rotifera	0% (n=3)	0% (n=6)	0% (n=7)	0% (n=4)
Mollusca	0% (n=2)	0% (n=5)	0% (n=6)	13% ***(n=8)
Annelida	0% (n=2)	0% (n=3)	0% (n=6)	13%*** (n=8)
Turbellaria	-	0% (n=1)	50% (n=2)	33%*** (n=3)
Plants	0% (n=2)	0% (n=5)	0% (n=9)	50%*** (n=6)

* direct as well as indirect effects reported
** data do not allow clear conclusions as to whether or no effect
*** reported as indirect effects

The reported negative effects of the studied insecticides, arranged according to toxic units (TU_{gst}; exposure concentration in water divided by the geometric mean of the $L(E)C_{50}$ values of the most sensitive standard test organism), are presented in Tables 4 and 5, and in the Appendices. In the evaluated (semi) field studies reductions in population densities at relatively low exposure concentrations are in particular found in populations of crustaceans (cluster Amphipoda – Ostracoda in Tables 4 and 5), insects (cluster Trichoptera – Coleoptera) and fish (Pisces). Negative effects in these animal groups were already observed below 1 TU_{gst} after single applications (Table 4) and below 0.1 TU_{gst} after repeated application (Table 5).

Table 5. Reported negative effects on various taxonomic groups as a result of repeated application of pyrethroids in aquatic micro- and mesocosms. The effects are arranged according to toxic units (TU_{gst}) and are expressed as percentage of the cases in which a reduction in numbers or biomass of one or more taxa within a taxonomic group was reported.

	TU_{gst}			
	0.001-0.01	**0.01-0.1**	**0.1-1**	**1-10**
Amphipoda	-	100% (n=1)	100% (n=11)	100% (n=7)
Isopoda	-	-	80% (n=5)	100% (n=2)
Copepoda	0% (n=1)	60% (n=5)	56% (n=16)	73% (n=11)
Cladocera	0% (n=1)	0% (n=2)	50% (n=10)	86% (n=7)
Ostracoda	0% (n=1)	0% (n=1)	50% (n=2)	-
Trichoptera	0% (n=1)	67% (n=3)	86% (n=7)	83% (n=6)
Ephemeroptera	0% (n=1)	50% (n=6)	82% (n=17)	85% (n=13)
Diptera	0% (n=1)	33% (n=6)	82% (n=17)	100% (n=13)
Hemiptera	0% (n=1)	50% (n=2)	67% (n=6)	100% (n=2)
Odonata	0% (n=1)	33% (n=3)	36% (n=11)	50% (n=10)
Coleoptera	0% (n=1)	0% (n=2)	64% (n=11)	60% (n=10)
Hydracarina	0% (n=1)	100% (n=1)	100% (n=1)	-
Pisces	0% (n=1)	0% (n=5)	33% (n=6)	83% (n=6)
Rotifera	0% (n=1)	0% (n=3)	0% (n=13)	0% (n=11)
Mollusca	0% (n=1)	0% (n=3)	0% (n=12)	0% (n=10)
Annelida	0% (n=1)	0% (n=2)	0% (n=11)	0% (n=6)
Turbellaria	0% (n=1)	0% (n=1)	0% (n=7)	0% (n=3)
Plants	0% (n=1)	0% (n=5)	0% (n=13)	8% (n=12)

Reductions in numbers of other animal groups such as Rotifera, Mollusca, and Annelida and Turbellaria are only observed at relatively high exposure concentrations and in a limited number of studies. Negative effects on plants are only reported at exposure concentrations higher than 1-10 TU_{gst}.

The data above correspond well with data from laboratory single-species toxicity tests with aquatic organisms (see e.g. Crommentuijn *et al*., 1997). In a limited number of studies, besides micro- and mesocosm experiments, laboratory toxicity tests have also been conducted with characteristic organisms from these model ecosystems. It is generally found that after a similar exposure to insecticides the same species shows a more or less similar sensitivity in the laboratory and under (semi) field conditions (e.g. Dortland, 1980; Crossland, 1984; Van Wijngaarden *et al*., 1996). This makes it likely that the observed reductions in densities of crustaceans, insects and fish can often be ascribed to the direct toxic effects of the studied insecticides. One should, however, be aware that insects, crustaceans and fish may also include relatively insensitive representatives (e.g. Dortland, 1980; Brock *et al*., 1992a; Lahr & Diallo, 1993; Giddings *et al*., 1996). It is even found to be difficult to name a most sensitive indicator species, genus or order that is representative of all insecticides and/or all (semi) field studies.

The measured endpoints in the studies are for the purpose of this report divided into eight groups, whereby a distinction is made between seven structural categories and one functional category. For the structural categories this usually concerns densities (numbers) and biomass of aquatic populations. For the functional category these are endpoints concerning community metabolism (oxygen balance, water chemistry, decomposition). The first four structural categories – Microcrustaceans, Macrocrustaceans, Insects and Fish – include the organisms that will most probably be directly affected. The three following structural categories – Rotifers, Other macro-invertebrates and Algae & macrophytes – often include organisms that are indirectly affected but where the occurrence of direct effects cannot always be excluded.

In this chapter there will be no further distinction between direct and indirect effects of the insecticides on the studied endpoints. More information on the routes of indirect effects that may occur after application of insecticides is given Chapter 6.

5.2 Acetylcholinesterase inhibitors

Figure 1 presents a picture of the effects (expressed in classes) as these were found in (semi) field studies with acetylcholinesterase inhibitors and at which normalised concentrations (expressed in TU_{gst}) and in which categories these occur. Clear effects are observed in the categories Microcrustaceans, Macrocrustaceans, Insects and Fish from about 0.1 TU_{gst} (Figure 1 A-D). Effects are hardly ever observed at water concentrations below 0.1 TU_{gst}, with the exception of one study (Van den Brink *et al.*, 1995) with a chronic exposure to the insecticide chlorpyrifos. Figure 1 A-D shows a more or less clear dose-effect relationship. The number of studies in which effects are reported as well as the class of the effects increase with higher concentrations. Figure 1 also shows that in particular single doses have been studied in the (semi) field experiments with acetylcholinesterase inhibitors. In comparison with single applications, more pronounced effects (class 5) in microcrustaceans and insects do occur at similar exposure concentrations in studies with repeated or chronic applications.

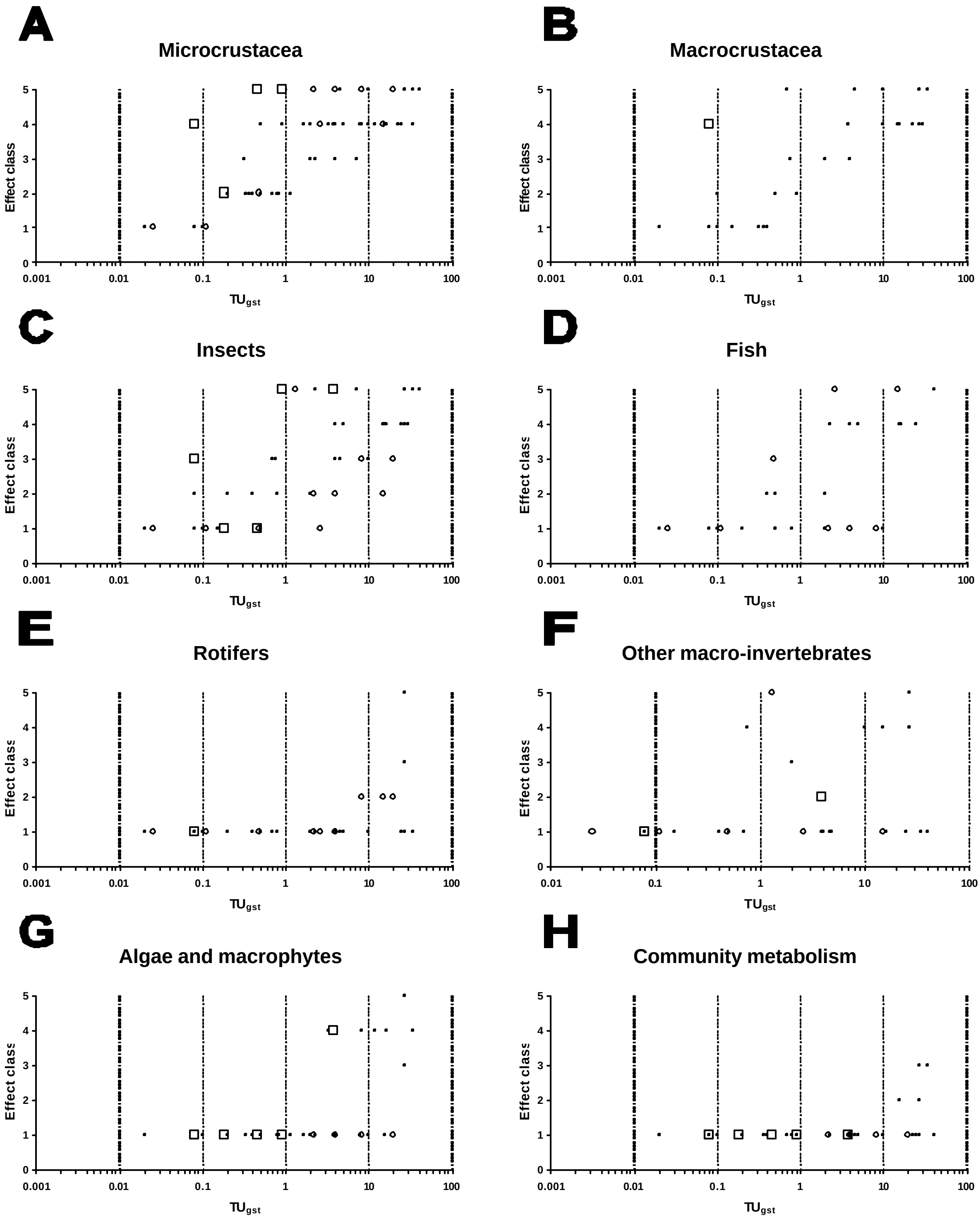

Figure 1. Classified effects of insecticides with an acetylcholinesterase-inhibiting effect in (semi) field studies. The figure includes observations of studies with single and multiple application of an insecticide in test systems with stagnant water and of chronic application in stagnant as well as running test systems. The effects are classified into several categories structural endpoints (A – G) and a functional category (community metabolism; H). The effects are also classified according to magnitude and duration. 1 = no significant effect, 2 = slight effect, 3 = clear short-term effect (< 8 weeks), 4 = clear effect in short-term study (recovery moment unknown), 5 = clear long-term effect (> 8 weeks). The small closed circles (•) indicate the experiments with a single application. The large open circles (○) and squares (□) indicate the experiments with multiple applications or chronic exposure, respectively.

Clear effects of acetylcholinesterase inhibitors in the structural categories Rotifers, Other macro-invertebrates, and Algae & macrophytes (Figure 1 E-H) do generally only occur from 1 TU_{gst}. Effects in the category Community metabolism were observed at concentrations higher than 10 TU_{gst}. This indicates that the structure of the aquatic community is more sensitive to acetylcholinesterase inhibitors than functional characteristics of the ecosystem.

Results of studies with acetylcholinesterase inhibitors that were pulse-applied in running systems are not presented in Figure 1 due to the deviating and usually short-term exposure regime. A pulse of 6 hours with a concentration of 0.08 TU_{gst} chlorpyrifos had no effect on the fauna of experimental streams (Pusey *et al.*, 1994). A clear effect on insect populations was observed in the same study at an equally long application of 3.85 TU_{gst}, after which recovery of the reduced populations occurred within 8 weeks (Appendix Table V b). Courtemanch & Gibbs (1980) found a clear decrease of Plecoptera and Ephemeroptera for carbaryl in streams at a nominal pulse concentration of 5.7 TU_{gst} (Appendix Table III b). Morrison & Wells (1981) studied pulse applications of fenitrothion in streams. At 0.1 TU_{gst} they found no, and at 1.7 TU_{gst} a slight effect, especially in the form of drift of insects (Appendix Table XII b).

5.3 Synthetic pyrethroids

Figure 2 presents a picture of the effects as these were found in (semi) field studies with pyrethroids. The (semi) field studies with pyrethroids in particular concern effects of repeated applications.

Effects are observed in the categories Microcrustaceans and Insects from about 0.01 TU_{gst}. In the range 0.01-0.1 TU_{gst} they especially concern Effect class 2 (slight effect). At higher exposure concentrations, in the range 01-1 TU_{gst}, clear effects (Effect classes 3, 4 and 5) in the categories Microcrustaceans, Macrocrustaceans and Insects are regularly reported, while for fish slight effects (class 2) are then reported in a limited number of studies (Figure 2 A-D). In some studies, clear effects at concentrations lower than 1 TU_{gst} are also reported for the category Rotifers (Figure 2 E). At concentrations higher than 1 TU_{gst}, effects can be observed in all categories of structural endpoints (Figure 2 A-G).

Figure 2 clearly illustrates that after repeated exposure to pyrethroids and at final peak concentrations higher than 0.1 TU_{gst}, long-term (> 8 weeks after last application) effects on – in particular – crustaceans and insects cannot be excluded. The pyrethroid studies also confirm the picture that the structure of the aquatic community is more sensitive to insecticides than functional characteristics of the ecosystem (Figure 2 A-G versus 2 H).

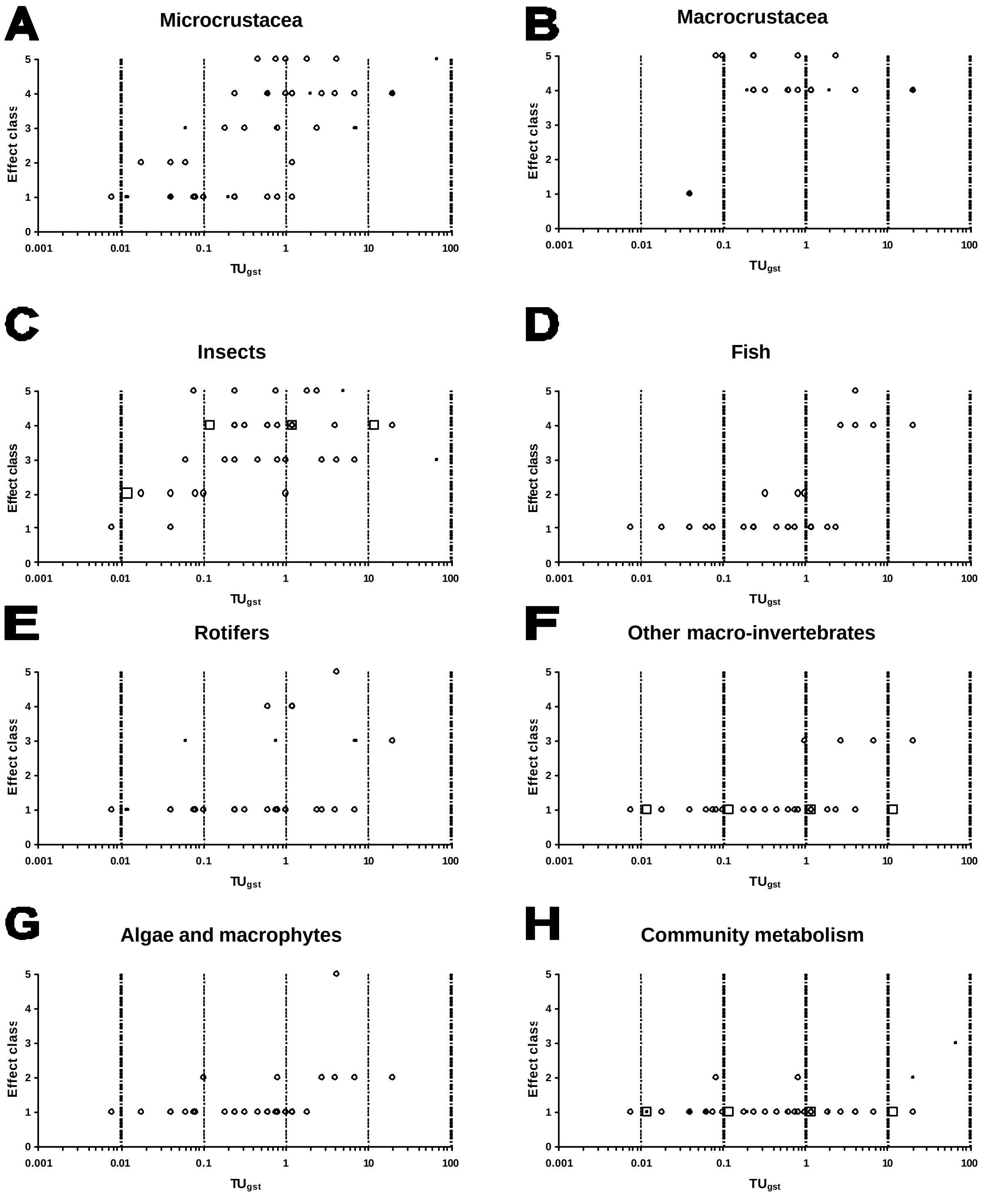

Figure 2. Classified effects of synthetic pyrethroids in (semi) field studies. The figure includes observations of studies with single and multiple application of an insecticide in test systems with stagnant water and of chronic application in stagnant as well as running test systems. The effects are classified into several categories structural endpoints (A – G) and a functional category (community metabolism; H). The effects are also classified according to magnitude and duration. 1 = no significant effect, 2 = slight effect, 3 = clear short-term effect (< 8 weeks), 4 = clear effect in short-term study (recovery moment unknown), 5 = clear long-term effect (> 8 weeks). The small closed circles (•) indicate the experiments with a single application. The large open circles (○) and squares (□) indicate the experiments with multiple applications or chronic exposure, respectively.

The effects of pyrethroids in the field are closely related to the transport route and fate of these compounds. This means that species that hardly differ in sensitivity in the laboratory may show a totally different response in the field. A film layer is formed on the water when the pyrethroid reaches the surface water via drift. This means that species that are especially found on the water surface have an increased risk. This appears from a study with lambda-cyhalothrin (Hill *et al.*, 1994b) in which surface bugs (Gerridae and Veliidae) react more sensitive than 'submerging' bugs and beetles such as Notonectidae and Haliplidae. Once in the water, pyrethroids sorb rapidly to sediment, suspended matter and macrophytes. Sorption considerably reduces the biological availability (Hill, 1985), as a result of which organisms that live freely in the water are initially exposed to higher concentrations than sediment-inhabiting organisms. A striking example of this is found in the Chironomidae family, where in (semi) field studies the subfamilies Tanypodinae and Orthocladiinae decrease in numbers at much lower concentrations than the Chironominae. This is because many species of the last subfamily live in the sediment whereas the Tanypodinae and also the Orthocladiinae usually live in open water. Not all water inhabitants, however, react more sensitive than sediment dwellers. Two sensitive groups (Isopoda and Amphipoda) are, on the contrary, found on or in the direct vicinity of sediment. Also within the Ephemeroptera, the 'sediment dwelling' family Caenidae reacts somewhat more sensitive than representatives of the Baetidae which mainly live in open water. The above shows that pyrethroids tend to move to interfaces (air-water, water-soil). Consequently, organisms that live there may be exposed to higher concentrations (e.g. Hill, 1985).

A (semi) field study with cypermethrin (Hill, 1985) also suggests that sediment-adsorbed pyrethroids cause fewer effects on water-inhabiting organisms. In this study, the same nominal concentration is applied separately as drift and as soil slurry (simulation runoff) in a comparable test system. The drift application had a stronger effect on Cladocera, Copepoda and Insecta than the application of soil slurry.

The above-described results of (semi) field studies raise the question to what extent sediment-bound pyrethroids present a risk to aquatic ecosystems because the residence time of sediment-bound pyrethroids is usually some weeks and in case of repeated applications in particular benthic organisms may be chronically exposed. Judging by the results of (semi) field studies with repeated applications, there are no clear indications that adsorbed pyrethroids do in the long term cause chronic toxicity. The observed effects can all be related to the acute toxicity and repeated applications do not result in further reductions of (benthic) organisms.

Pyrethroids may, however, in a different way cause long-term effects. There are indications that a short-term exposure to realistic field concentrations may retard the growth of insect larvae. Liess (1994) exposed *Limnephilus lunatus* larvae in Petri dishes for one hour to a concentration range of fenvalerate, ranging from 0.001 to 10 µg/L, after which the animals were raised for 90 days in an outdoor artificial stream system. This one-hour treatment resulted already in a significant retardation in growth and emergence period from 0.01 µg/L. First, one may wonder to what extent a constant exposure for one hour without the presence of sorbing surfaces (macrophytes, sediment etc.) is a realistic field situation. A (semi) field study with esfenvalerate of which the fate is extensively described by Heinis & Knuth (1992), however, shows that such an exposure may also occur in the field. In this study, the average concentration in the water column during the first eight hours was fairly constant at 55 to 115% of the nominal value.

A delay in the development of insects may have far-reaching consequences, e.g. by missing one generation per year. Despite the results of Liess (1996), there are no patently obvious examples of growth retardation in the evaluated (semi) field studies. Only two studies with lambda-cyhalothrin yield possible indications of such effects. Farmer *et al.* (1995) found a delay in flying-out period of the Baetidae at 0.017 µg/L while Hill *et al.* (1994b) found a significant increase in Libellulidae nymphs at 0.016 µg/L. This last increase may, however, also be explained as an indirect effect, caused by the disappearance of the competition with more sensitive predators. This shows that there is on this point still insufficient knowledge to arrive at a proper risk assessment. There are only a limited number of studies in which emergence traps have been used. In addition, organisms that run the possible risk of retarded development, such as caddis flies and mayflies, are usually caught in (too) low numbers to allow a reliable judgement.

The evaluated studies with pyrethroids did not include adequate studies that describe the effects of repeated applications (pulsed exposure) in running test systems.

5.4 Other insecticides

Figure 3 presents a picture of the reported effects of the heterogeneous group of other insecticides. Included are observations in (semi) field studies with the acyl-urea insecticide diflubenzuron (represented by the symbol *) and with the chlorinated hydrocarbons lindane and methoxychlor (represented by the other symbols and in correspondence with Figures 1 and 2).

Significant effects are not observed in studies with diflubenzuron at exposure concentrations lower than 0.1 TU_{gst} (Figure 3). Pronounced effects (Effect class 3 and 4) can for this substance, however, already be found within the concentration range of 0.1-1 TU_{gst}, in particular in Microcrustaceans and Insects. Within this concentration range a pronounced effect is in a number of cases also observed in Macrocrustaceans, Fish, Rotifers, and Algae & macrophytes.

In studies with chlorinated hydrocarbons Effect class 2 was reported once within the concentration range 0.01-0.1 TU_{gst}; this concerns the response of insects (Figure 3 C) in an artificial stream with a chronic exposure to lindane. Pronounced effects in the concentration range 0.1 - 1 TU_{gst} (mainly Effect class 4) are frequently reported in case of chronic or repeated exposure to chlorinated hydrocarbons. The evaluated studies with other insecticides did not include adequate studies that describe the effects of a pulsed exposure regime in running test systems.

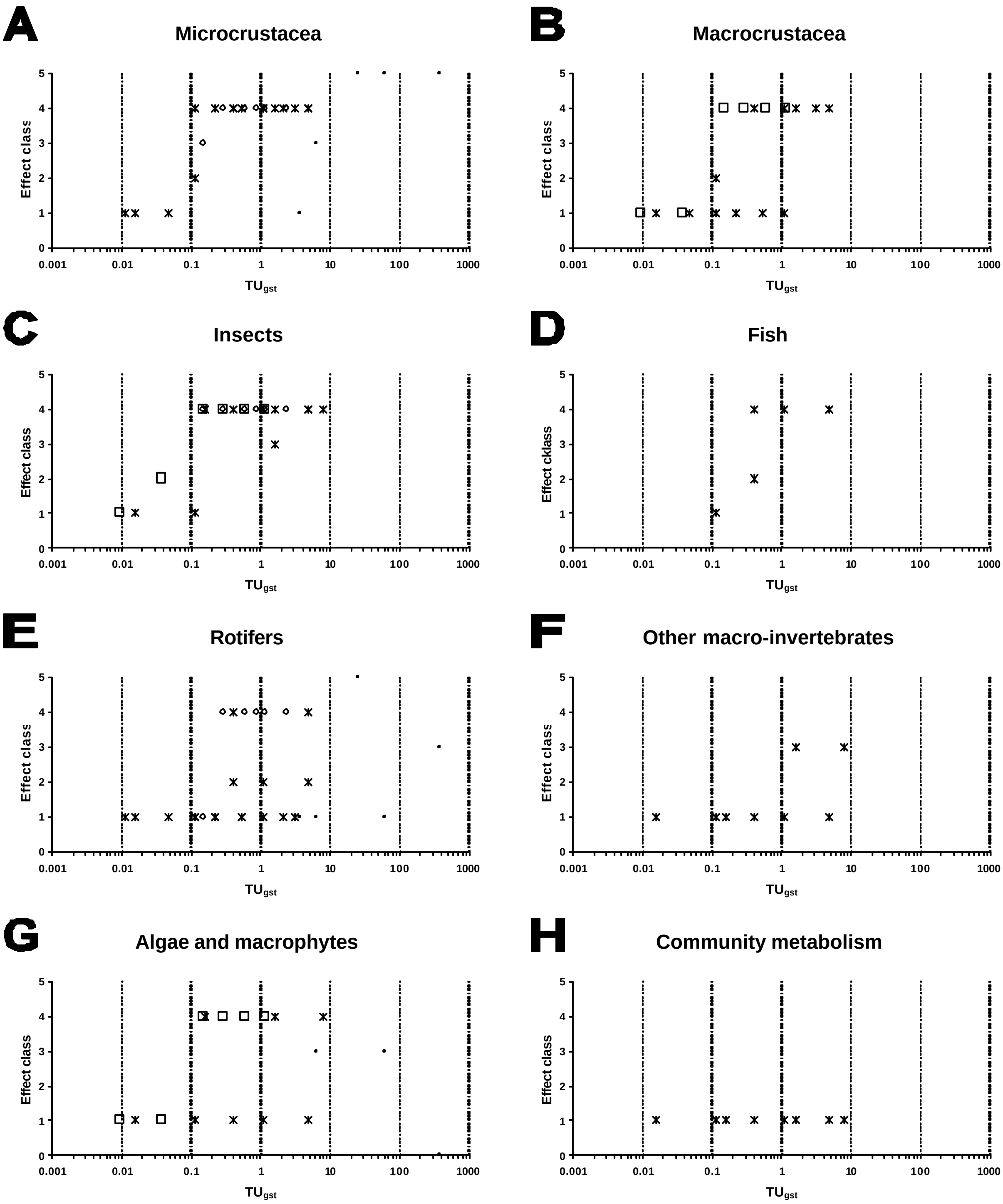

Figure 3. Classified effects of other insecticides in (semi) field studies. The figure includes observations of studies with single and multiple application of an insecticide in test systems with stagnant water and of chronic application in stagnant as well as running test systems. The effects are classified into several categories structural endpoints (A – G) and a functional category (community metabolism; H). The effects are also classified according to magnitude and duration. 1 = no significant effect, 2 = slight effect, 3 = clear short-term effect (< 8 weeks), 4 = clear effect in short-term study (recovery moment unknown), 5 = clear long-term effect (> 8 weeks). The asterisks (*) indicate the experiments with diflubenzuron. The small closed circles (•) indicate the experiments with a single application of chlorinated hydrocarbons (lindane and methoxychlor). The large open circles (O) and squares (☐) indicate the experiments with multiple applications or chronic exposure of chlorinated hydrocarbons, respectively.

5.5 Response most sensitive endpoints

Figures 4 and 5 present a general picture of the results of all insecticide studies. The Effect classes for the most sensitive endpoints of the different studies are presented in Figure 4. This confirms the picture that effects on the sensitive endpoints are usually not observed in case of a single application of insecticides up to 0.1 TU_{gst} (Figure 4 A). At higher doses, slight to clear effects may be expected after single applications. At single doses of 1 TU_{gst} and higher, there is a good chance that the recovery of sensitive endpoints takes longer than 8 weeks (Effect class 5) in microcosms and mesocosms.

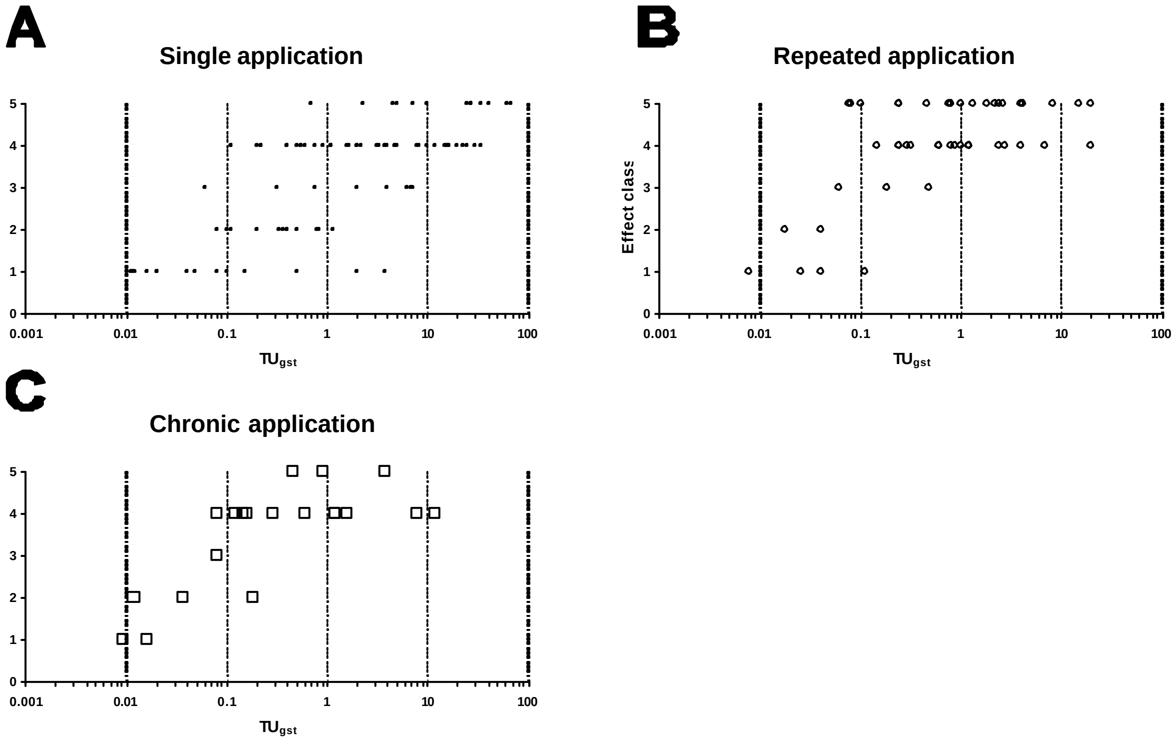

Figure 4. Effect observations for the most sensitive endpoint in the separate (semi) field studies with all insecticides, based on the data presented in Figures 1-3. The effects on the most sensitive endpoints are presented for a single application (A), multiple applications (B), and chronic exposure (C). The effects are also classified according to magnitude and duration. 1 = no significant effect, 2 = slight effect, 3 = clear short-term effect (< 8 weeks), 4 = clear effect in short-term study (recovery moment unknown), 5 = clear long-term effect (> 8 weeks).

It should be noted that Figure 4 B-C shows far fewer observations in class 1 for repeated and chronic exposure. Concentrations below 0.01 TU_{gst} hardly have been studied. Nevertheless, the results make it likely that, in case of repeated and chronic application of insecticides, below 0.01 TU_{gst} no clear effects are to be expected. Within the concentration range 0.01-0.1 TU_{gst} mainly slight (class 2) to short-term clear effects (class 3) are reported for the most sensitive endpoints in case of repeated and chronic application. Above 0.1 TU_{gst}, clear effects are most probably to be expected in test systems that are repeatedly or chronically stressed with insecticides. In that case there is even a relatively good chance of the occurrence of prolonged effects (class 5), at least for the most sensitive endpoint.

Figure 5 presents the 10-, 50- and 90-percentile values (in TU_{gst}) for the various effect classes of the data that are presented in Figure 4 (most sensitive endpoint per study). The confidence intervals of the 10-percentile values are generally considerably higher than those of the 50-percentile values and are therefore less suitable for comparison of exposure regimes. Comparison of the 50- and 90-percentile values for the exposure regimes ‘Single’, ‘Multiple’, and ‘Chronic’ shows that these values are considerably higher for a single application of an insecticide. The differences between multiple and chronic exposure are smaller. This means that for an adequate risk analysis it is at least desirable to make a distinction between the exposure regime as a result of a single application on the one hand and that of a multiple / chronic application on the other. It should be noted that the category multiple applications is rather heterogeneous because the number of applications between studies and substances varies strongly.

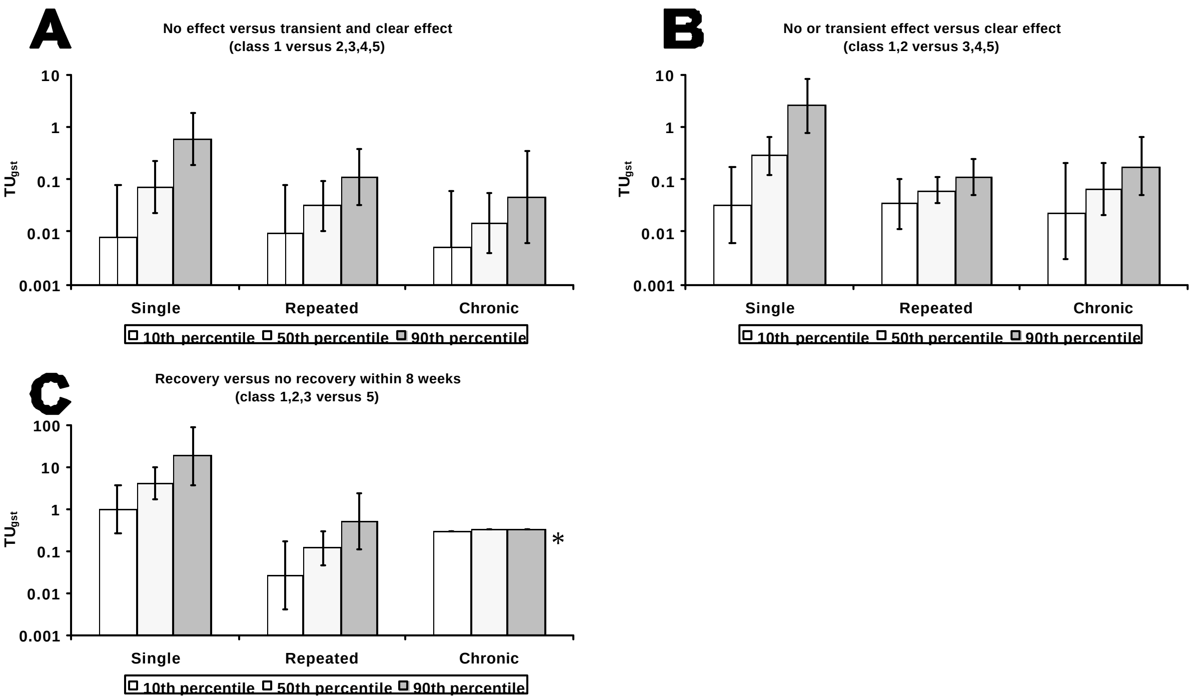

Figure 5. 10-, 50- and 90-percentile values (expressed in TU_{gst} with 95% confidence intervals) as calculated by means of logistic regression for the most sensitive endpoints of the studies for single, multiple and chronic exposure of an insecticide. The values are calculated for 3 classifications, A: no versus slight and clear effect, B: no and slight versus clear effect, and C: recovery versus no recovery within 8 weeks. When the classification ‘no and slight’ versus ‘clear’ effect is taken (5 B), a 50-percentile value of 0.28 TU_{gst} is calculated for a single application of an insecticide. This means that it is predicted that the most sensitive endpoints show a clear response (classes 3, 4 and 5) at a concentration of 0.28 TU_{gst} in 50% of the studies
* confidence intervals cannot be calculated because the number of observations is too low.

The dose response model - generated on the basis of the reported effects and corresponding concentrations - with which the percentile values are calculated (Figure 5) has the potential to make risk assessments for measured or calculated insecticide concentrations in aquatic ecosystems. It can therefore in a more general sense be indicated that there is at a certain concentration a certain chance that there are no, negligible, or serious effects on the most sensitive endpoints due to insecticide stress.

Predictions for single applications are – relatively speaking – the most accurate. Until now, especially observations are lacking in the concentration range 0.001 – 0.01 TU_{gst} (Figure 4) for multiple and chronic applications; this means that a threshold value for the presence or absence of an effect is still ill-founded.

The three scenarios, sketched on the basis of the regression model (Figure 5), can be interpreted as a model for a strict (Figure 5 A), a milder (Figure 5 B), and a liberal (Figure 5C) risk assessment. A consideration in the choice of a mild or liberal scenario may, e.g., be that water courses are usually interconnected so that sensitive populations will usually recover sooner than those in stagnant, isolated micro / mesocosms.

6 INDIRECT EFFECTS

In ecosystems, direct toxic effects of pesticides may cause changes in biological interactions and processes which also involve species and processes that are less sensitive to the toxic mode of action of the pesticide. Such ecological changes are called indirect or secondary effects (Hurlbert, 1975).

Figures 6 and 7 schematically show the indirect effects of insecticides as these were observed in stagnant test systems. Because the exact mechanism for indirect effects is difficult to establish in most (semi) field studies, the routes that are shown are in fact based on appraisals of the best explanations, in which the discussions by the various authors of the papers as well as our own perceptions have played an important role.

A wide range of structural and functional endpoints are studied in the various experiments. These endpoints are often monitored and elaborated at different taxonomical levels (e.g., sometimes at species level and sometimes at order level). In addition, the course of the effect chain is also governed by the specific biotic and abiotic characteristics of the studied test systems and the concentrations that have been used. Two examples:

- The review by Hanazato (1998) shows that within the crustaceans the larger zooplankton species are generally more sensitive to insecticides. In a competitive way, however, they are dominant over the smaller species. Consequently, exposure to relatively low insecticide concentrations may have a different effect on the competitive relationships between zooplankton populations than exposure to higher concentrations.
- At corresponding concentrations of the insecticide chlorpyrifos, other indirect effects were observed in macrophyte-dominated microcosms than in similar plankton-dominated test systems (Brock *et al.*, 1992a & b, 1993b)

The examples above show that reported indirect effects often are of a study-specific (and thus also anecdotal) nature. A number of effect chains have, however, so frequently been reported that it may be expected that they will regularly occur in insecticide-stressed aquatic ecosystems.

As already described in Chapter 5, in particular representatives of crustaceans, insects and fish are directly sensitive to insecticides. These animal groups may represent various trophic levels. This means that indirect effects may show up at the various trophic levels within the community (primary producers, herbivores, predators, detritivores) via shifts in competitive relationships (see Figures 6 and 7). Indirect effects are less frequently reported for functional endpoints in insecticide-stressed test systems. Oxygen concentrations in the water are, e.g., measured in many studies but the number of studies with significant effects on the oxygen concentration is limited.

The studies performed with multiple applications (Figure 7) show, in as far as the ecological interactions are concerned, no different picture than the studies conducted with a single insecticide application (Figure 6). Certain components, however, received more or less attention. In repeatedly stressed test systems there was more attention for effects on fish and their prey. An ecological interaction specifically mentioned in this respect is the suppression of the recovery of sensitive arthropod populations (e.g. cladocerans, insects) by the predation pressure of the (surviving) fish (Figure 7). Barron & Woodburn (1995), however, state in their review on chlorpyrifos that reduced fish populations in aquatic test systems may in the longer term lead to an increase in zooplankton and a decrease in phytoplankton populations. An increase in phytoplankton is in particular found in chlorpyrifos-stressed systems where the arthropods are the top predators.

The occurrence of algal blooms in the form of phytoplankton or periphyton and the increase in population density of Rotifera and Gastropoda is frequently reported as indirect effect in insecticide-stressed aquatic systems (Figures 6 and 7). The increase in algae can be explained by a decrease in grazing by crustaceans and insects. The increase in Rotifera and Gastropoda can be explained by the decreased competition with crustaceans and insects on the one hand, and by the increase in food in the form of algae on the other. The increase in algae as indirect effect of insecticide stress may possibly also lead to other symptoms of eutrophication such as the decrease in biomass of macrophytes by shading (Figure 6).

In the longer term, herbivores that are less sensitive to the insecticide (such as Rotifera and Gastropoda) may again suppress algal blooms. The phenomenon in stressed ecosystems that less sensitive species take over the role of disappeared species is called functional redundancy (Levine, 1989). In plankton-dominated test systems that are regularly stressed with insecticides, the increase in phytoplankton biomass may, incidentally, be a longer lasting phenomenon because less sensitive zooplankton species (such as Rotifers) are usually less efficient grazers of phytoplankton than Cladocera (e.g. Jak *et al.*, 1998).

Besides the above-described effects on the grazer-food chain of aquatic ecosystems, insecticides may also directly and indirectly affect the detritus-food chain. Representatives of the functional group ‘shredders’ are in particular the macrocrustaceans (such as freshwater shrimps and water isopods) and insects (certain caddis larvae) that are sensitive to insecticides. Shredders play an important role in the degradation of coarse organic matter (POM). It is therefore not surprising that a reduced decomposition of POM has been observed in insecticide-treated test systems. Functional redundancy may be restricted in the shredders because this group contains relatively few detrivores that are insensitive to insecticides.

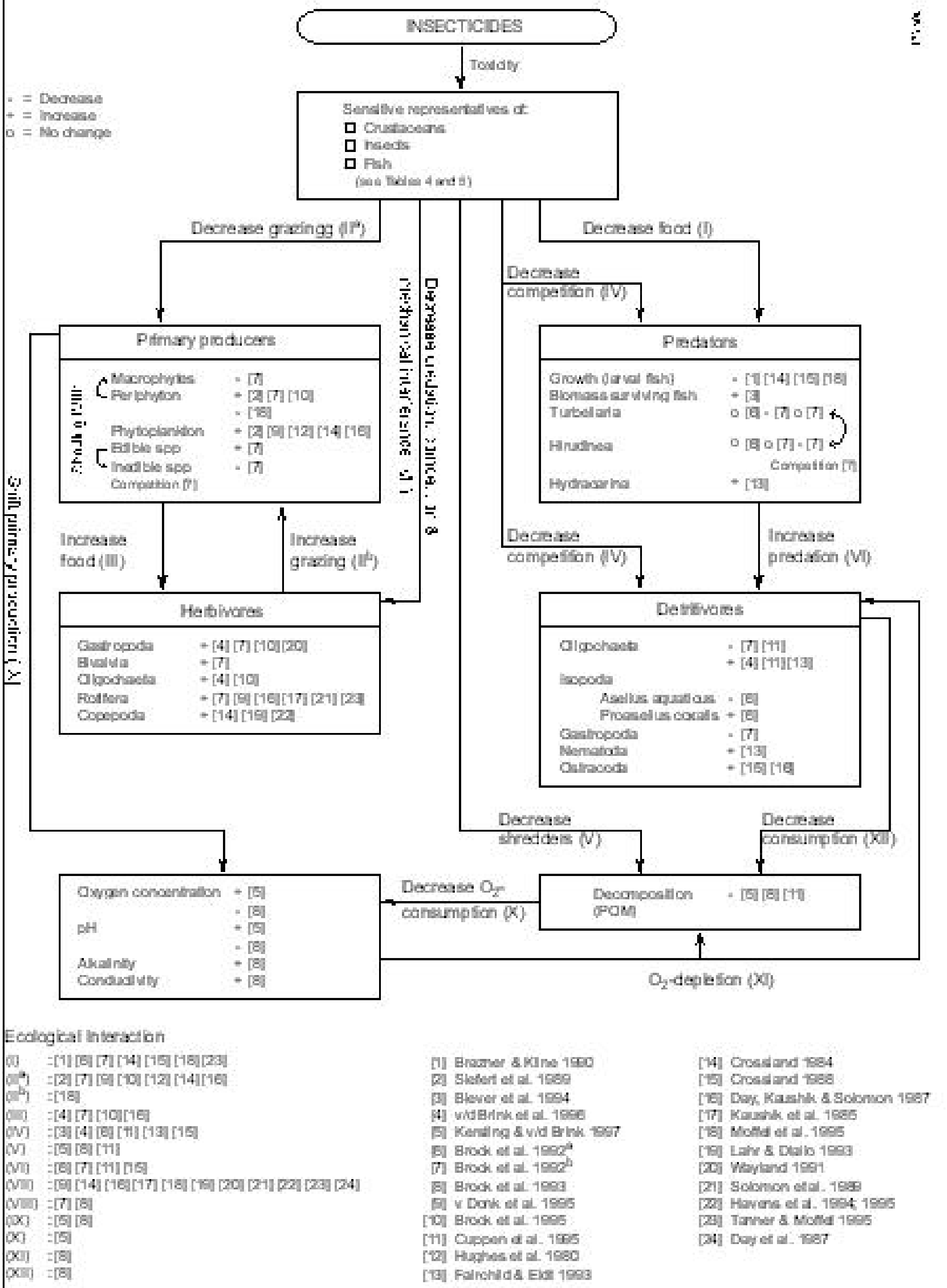

Figure 6. Schematic presentation of the indirect effects of insecticides in aquatic (semi) field experiments in which a single dose of the pesticide was applied in stagnant test systems. The Roman numerals denote the different types of ecological interactions and the Arabic numerals refer to the literature references.

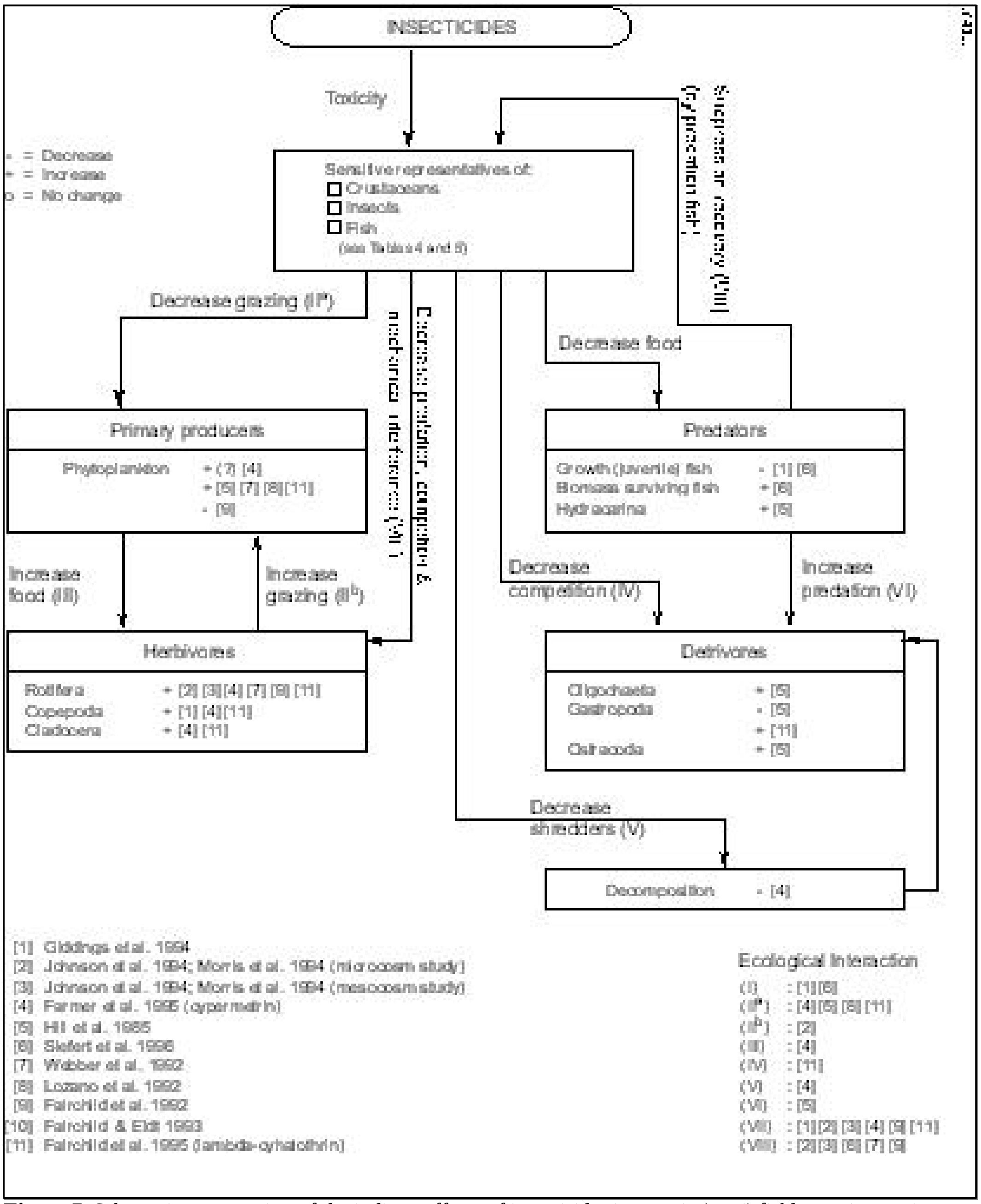

Figure 7. Schematic presentation of the indirect effects of insecticides in aquatic (semi) field experiments in which a repeated dose of the pesticide was applied in stagnant test systems. The Roman numerals denote the different types of ecological interactions and the Arabic numerals refer to the literature references.

Reductions as well as increases in population densities of less sensitive detrivores are reported for insecticide-stressed systems. The increase of e.g. oligochaete worms is usually explained by reduced competition with sensitive crustaceans and insects. For populations of detritivorous Oligochaeta, however, a significant decrease is also reported in some studies. This decrease can be explained by an increase in predation pressure by, e.g., Turbellaria and Hirudinea. In insecticide-stressed systems these predators change their diet after the disappearance of preferred food in the form of sensitive insects and crustaceans.

To gain insight into the concentration levels at which follow-up chains of indirect effects start to occur, data of adequate studies performed in stagnant water and with a single insecticide application are summarised in Table 6. These studies show that the frequency of reported indirect effects increases with increasing concentrations of the studied insecticides (Table 6). Indirect effects on functional endpoints were only measured in the highest concentration range. This again shows that functional aspects of the ecosystem are less sensitive to toxic stress by insecticides than structural aspects.

The general picture in Table 6 is that indirect effects on structural endpoints are certainly to be expected from exposure concentrations in the range of 0.1-1 TU_{gst}. An indirect effect within the concentration range 0.01-0.1 TU_{gst} was only reported once after single application of insecticides (Table 6). This was a study with the pyrethroid fenvalerate (Day *et al*., 1987). All reported secondary effects clearly exceed the 0.01 TU_{gst} level in case of multiple and chronic exposures.

Table 6. Indirect effects summarised from studies in stagnant waters after a single application of an organo-phosphorous insecticide, a carbamate, or a pyrethroid. The nominal concentrations reported in the studies are expressed in TU_{gst} (see Section 2.4). Subsequently, indirect effects are classified into the corresponding concentration ranges. **X**: at least one study reports one concentration in the particular class where a secondary effect occurs.

Range TU_{gst}	Structural aspects		Functional aspects	
	shifts in animal populations	shifts in algae populations and higher plants	decrease in decomposition	shifts in community metabolism
10-100	**X**[1,3,4,5,8,9,10]	**X**[4,5,8,10]	**X**[3,4,5]	**X**[3,4]
1-10	**X**[1,2,3,4,6,7,10,13]	**X**[1,10]		
0.1-1	**X**[1,2,11,13]	**X**[1]		
0.01-0.1	**X**[12]			

Organophophorous compounds
[1] Siefert *et al.* '89; Brazner & Kline '90
[2] Biever *et al.* '94
[3] V.d. Brink *et al.* '96; Kersting & V.d. Brink '97
[4] Brock *et al.* '92[a]; '92[b]; '93[b]
[5] V. Donk *et al.* '95; Brock *et al.* '95; Cuppen *et al.* '95
[6] Hughes *et al.* '80
[7] Fairchild & Eidt '80
[8] Crossland '84
[9] Crossland '88

Carbamates
[10] Havens '95
[11] Wayland '91

Pyrethroids
[12] Day *et al.* '87
[13] Kaushik *et al.* '85

7 RECOVERY

An advantage of (semi) field studies, in particular when these are conducted in the open air, is that information can be obtained about recovery of disturbed populations and ecosystem functions. It has already been discussed in Chapter 4 that insecticides may disappear relatively rapidly from the water column, e.g., by degradation and sorption to sediment and aquatic plants. In case the insecticides degrade rapidly or adsorb strongly, so that the bio-availability decreases, critical threshold values for sensitive aquatic organisms may soon be ‘underceeded’ after the last application. The recovery rate of affected populations then largely depends on the life cycle of the species in question, for which the number of generations per year, the possession of relatively insensitive life stages, and the capacity to actively migrate to other aquatic systems are of importance.

The relationship between life cycle and recovery of species is clearly illustrated in the paper by Van den Brink *et al.* (1996) in which the long-term response of various aquatic organisms after a single application of the insecticide chlorpyrifos in experimental ditches is described. In this study Cladocera show a relatively rapid recovery as a result of their short generation time and the possession of a relatively insensitive life stage in the form of winter eggs. Insect populations with several flying-out periods per year (such as *Cloeon, Chaoborus)* also show a relatively rapid recovery, in contrast to affected insect populations with only one or two generations per year (such as *Caenis horaria).* Insects do usually not have aquatic life stages that are insensitive to insecticides but the winged adult stage offers the possibility to recolonise isolated aquatic systems. In case a species cannot easily reach an isolated system and neither has insensitive aquatic life stages, there is a good chance that the species disappears for a longer period as a result of insecticide stress; *Gammarus pulex* is a good example of this. This species died-out locally in isolated chlorpyrifos-treated test systems (Van den Brink *et al*., 1996) and recovery only occurred after this species was reintroduced by the researchers. In non-isolated water courses, however, *Gammarus pulex* may, after local elimination by insecticides, show a relatively rapid recovery as a result of successful recolonisation by active swimming behaviour (Liess, 1993).

It follows from the above that recovery of insecticide-affected populations does not only depend on the "underceeding" of the critical threshold values of the toxic substance. Hanazato (1998), e.g., found that -in the interactions between insecticides and zooplankton- the structure of the community (presence or absence of predators) did have a co-effect on the recovery process. Other factors such as the moment of contamination in relation to the stage of the life cycle of the organisms, the length of the life cycle, and the frequency of the contamination are also important. A connection with (or proximity of) non-disturbed aquatic ecosystems, from which recolonisation may take place, may also be a determining factor in the rate of recovery. It should be noted that in (semi) field experiments into the effects of pesticides most attention focuses on dominant populations that are usually characterised by a relatively short life cycle (most invertebrates). Microcosm and mesocosm studies are generally less suitable to study the recovery of populations of larger organisms with a long life span (such as vertebrates). In addition, the duration of many published microcosm and mesocosm studies is too short to be able to derive the recovery period of sensitive populations. Many observations are therefore classified into Effect class 4 (see Figures 1-4). Another point of attention in the interpretation of (semi) field studies is that most experiments concern isolated test systems. This means that eliminated populations with a limited dispersion capacity cannot rapidly recolonise these test systems. In as far as the recovery of dominant populations is concerned, studies in stagnant microcosms and mesocosms should therefore be considered as a worst case approach.

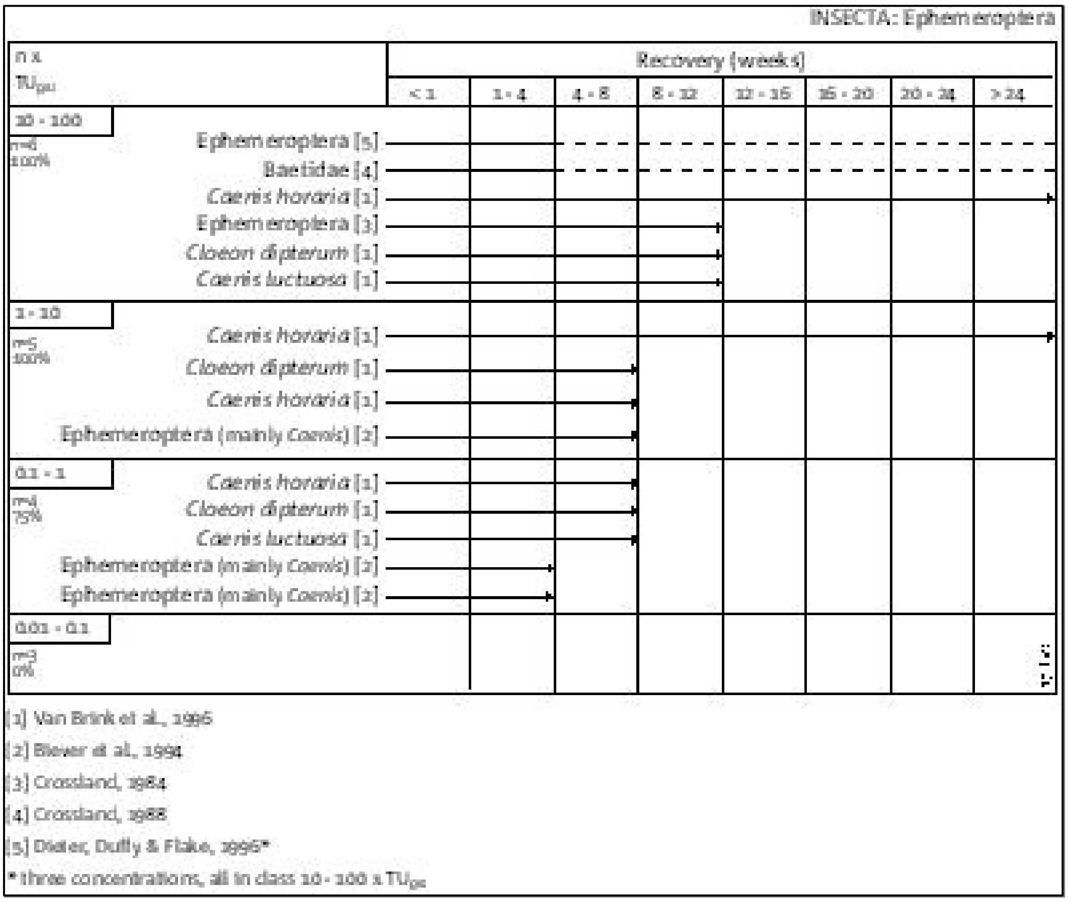

Figure 8. Recovery of Ephemeroptera after a single application of an organophosphorous insecticide in stagnant open-air systems. The arrows indicate over which period of 4 weeks a taxon has recovered. Broken lines indicate that the taxon has not recovered at the end of the study. The number of observations available within the concentration range (in TU_{gst}) (n = x) and the percentage of these observations that show a recovery are given directly underneath the box with the concentration class.

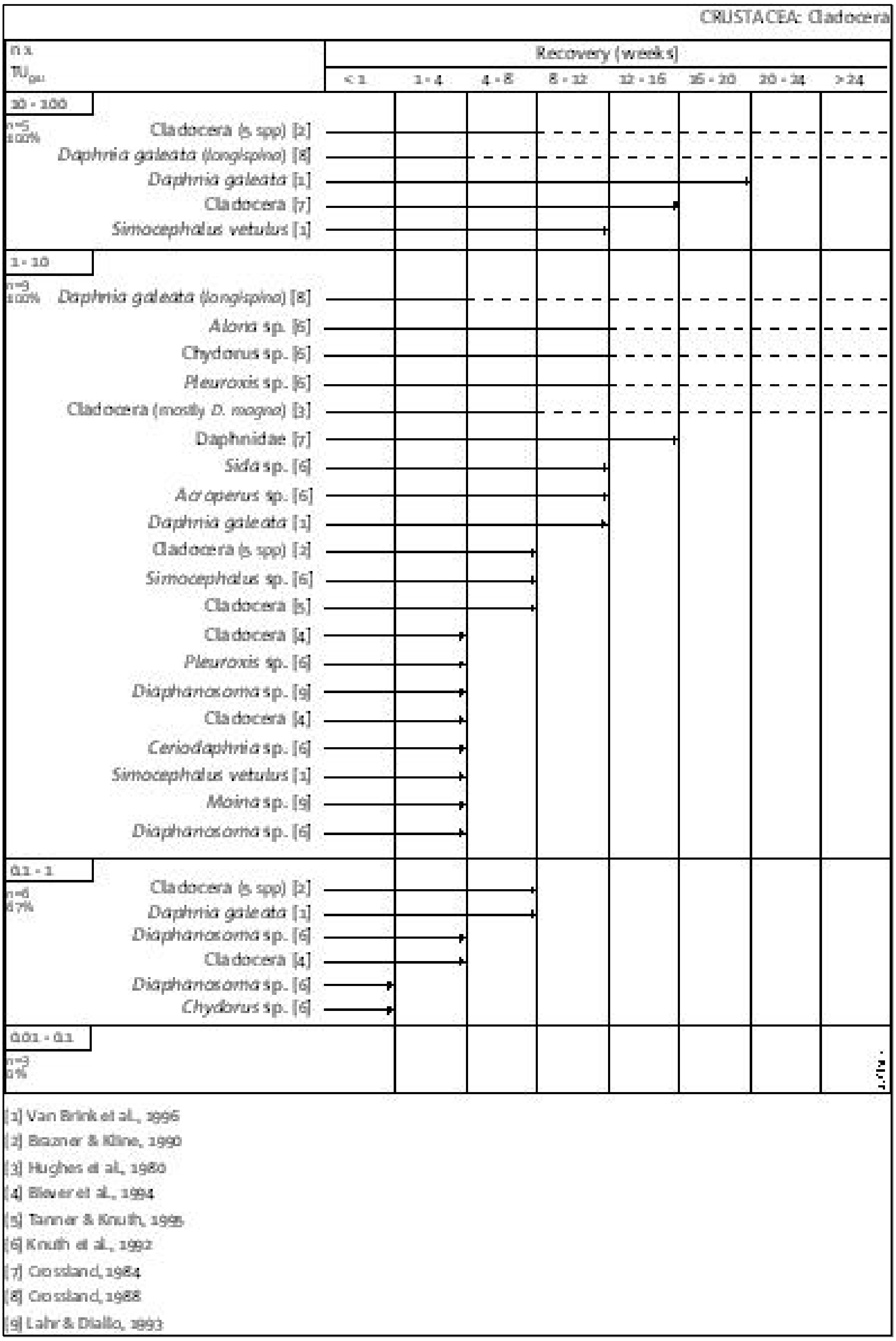

Figure 9. Recovery of Cladocera after a single application of an organophosphorous insecticide in stagnant open-air systems. The arrows indicate over which period of 4 weeks a taxon has recovered. Broken lines indicate that the taxon has not recovered at the end of the study. The number of observations available within the concentration range (in TU_{gst}) (n = x) and the percentage of these observations that show a recovery are given directly underneath the box with the concentration class.

In the assessed (semi) field studies, statements on recovery mainly concern sensitive populations of crustaceans and insects. The recovery moment of two relatively well studied groups within the crustaceans and insects in systems treated with organophosphorous insecticides is given as an illustration (Figures 8 and 9). The general trend is: the higher the exposure concentrations are, or the longer the duration of exposure is, the longer it takes before recovery is observed. Also when the other insecticides are taken into consideration, it appears that, in case of a single application, populations of sensitive crustaceans and insects do usually recover within 8 weeks if the exposure concentration does not exceed 1 TU_{gst}. In case of repeated and chronic exposure to an insecticide, recovery within 8 weeks after termination of the application is usually the case if the final exposure concentration in the water does not exceed 0.1 TU_{gst} (Figures 1-3). Also when we consider the most sensitive ecological endpoint for each study, it can be concluded that these threshold values usually guarantee recovery within 8 weeks after the last application (Figure 5). The few exceptions to the rule concern studies characterized by some populations of macrocrustaceans (such as *Gammarus)* which are unable to actively migrate over land to recolonise isolated test systems, or studies characterised by population of univoltine insects that have a limited number of flying-out periods per year.

Table 7. $NOEC_{eco}$ and $LOEC_{eco}$ values for (semi) field studies with single or multiple application of an insecticide with an acetylcholinesterase-inhibiting effect.

Active ingredient	Dose	$NOEC_{eco}$ (µg/L)	$LOEC_{eco}$ (µg/L)	Reference
Stagnant systems				
Azinphos-methyl	single	0.2	0.72	Stay & Jarvinen, 1995
	single	0.2	1	Knuth *et al.*, 1992
	single	-	≤ 1	Tanner & Knuth, 1995
	multiple	0.22	0.95	Giddings *et al.*, 1994
Chlorpyrifos	single	0.1	0.3	Biever *et al.*, 1994
	single	0.1	0.9	Van den Brink *et al.*, 1996
	single	-	≤ 0.5	Brazner *et al.*, 1989; Siefert *et al.*, 1989; Brazner & Kline, 1990
	single	-	≤ 0.5	Stay *et al.*, 1989
	single	-	≤ 5	Brock *et al.*, 1992a, b, 1993b
	single	-	≤ 10	Hughes *et al.*, 1980
	single	-	≤ 35	Van Donk *et al.*, 1995; Brock *et al.*, 1995; Cuppen *et al.*, 1995
	continuous	-	≤ 0.1	Van den Brink *et al.*, 1995
Diazinon	multiple	-	≤ 2.4	Giddings *et al.*, 1996
Fenitrothion	single	-	≤ 80	Lahr & Diallo, 1993
	multiple	-	≤ 14.3	Fairchild & Eidt, 1993
Parathion-ethyl	continuous	-	≤ 0.2	Dortland, 1980
Parathion-methyl	single	-	≤ 10	Crossland, 1988
	single	-	≤ 100	Crossland, 1984
Phorate	single	-	≤ 23	Dieter *et al.*, 1996
Bendiocarb	single	-	≤ 24	Lahr *et al.*, 1993
Carbaryl	single	-	≤ 2	Havens, 1994, 1995
Carbofuran	single	5	25	Wayland, 1991
Running systems				
Chlorpyrifos	single	0.1	5	Pusey *et al.*, 1994
	continuous		≤ 0.1	Ward *et al.*, 1995
Fenitrothion	single	1.1	18.7	Morrison & Wells, 1981
	single	-	≤ 30.8	Poirier & Surgeoner, 1988
	single	-	≤ 460	Yasuno *et al.*, 1981
Carbaryl	single	-	≤ 34	Courtemanch & Gibbs, 1980

8 EVALUATION OF THE SETTING OF CRITERIA

8.1 Acetylcholinesterase inhibitors

Table 7 presents a review of the $NOEC_{eco}$ and $LOEC_{eco}$ values for organophosphorous and carbamate insecticides which can be derived from the evaluated (semi) field studies. Next, summarising $NOEC_{eco}$ and $LOEC_{eco}$ have been derived for each substance (Table 8). The summarising $NOEC_{eco}$ is the highest found $NOEC_{eco}$ that is lower or equal to the lowest found $LOEC_{eco}$.

In Table 8 these threshold levels are compared with the Maximum Permissible Concentration (MPC) for freshwater ecosystems, and the liberal and conservative standard based on the criteria described in the Uniform Principles. The standards for the acetylcholinesterase inhibitors are in all cases below the $LOEC_{eco}$ that could be derived from the (semi) field experiments. The standards are also lower than the $NOEC_{eco}$, sometimes even more than a factor 10. A $NOEC_{eco}$ could, however, only be derived for 5 acetylcholinesterase inhibitors and these usually concerned an exposure regime as a result of a single application in a stagnant system.

Table 8. Summarised $NOEC_{eco}$ and $LOEC_{eco}$ values for acetylcholinesterase-inhibiting insecticides in (semi) field studies compared with various standards.

Active ingredient	Exposure regime	$NOEC_{eco}$ (µg/L)	$LOEC_{eco}$ (µg/L)	MPC NW4 criterion (µg/L)	Liberal UP criterion (µg/L)	Conservative UP criterion (µg/L)
Stagnant systems						
Azinphos-methyl	single	0.2	0.72	0.012	0.02	0.011
	multiple	0.22	0.95	0.012	0.02	0.011
Chlorpyrifos	single	0.1	0.9	0.003	0.01	0.002
	continuous	-	≤ 0.1	0.003	0.01	0.002
Diazinon	multiple	-	≤ 2.4	0.037	0.01	0.007
Fenitrothion	single	-	≤ 80	0.009	0.11	0.016
	multiple	-	≤ 14.3	0.009	0.11	0.016
Parathion(-ethyl)	continuous	0.2	0.5	0.011	0.011	0.0037
Parathion-methyl	single	-	≤ 10	0.012	0.014	0.0014
Phorate	single	-	≤ 23	-	0.015	0.006
Bendiocarb	single	-	≤ 24	-	0.74	0.32
Carbaryl	single	-	≤ 2	0.23	0.056	0.056
Carbofuran	single	5	25	0.91	0.33	0.23
Running systems						
Chlorpyrifos	single	0.1	5	0.003	0.01	0.002
	continuous		≤ 0.1	0.003	0.01	0.002
Fenitrothion	single	1.1	18.7	0.009	0.11	0.016
Carbaryl	single	-	≤ 34	0.23	0.06	0.056

8.2 Synthetic pyrethroids

The $NOEC_{eco}$ and $LOEC_{eco}$ values for pyrethroids are per study presented in Table 9. Table 10 compares the summarising $NOEC_{eco}$ and $LOEC_{eco}$ values from these studies with the standards. A $NOEC_{eco}$ could be derived for three of the eight pyrethroids with which adequate (semi) field experiments have been conducted. The $NOEC_{eco}$ values for these substances (esfenvalerate, fenvalerate, lambda-cyhalothrin) are slightly higher than the UP criterion. The criteria for aquatic organisms as described in the Uniform Principles do therefore seem to be satisfactory. A comparison with the MPC is not possible because these data are lacking. It also appears that the available knowledge on critical threshold values of synthetic pyrethroids in running waters is very scanty.

Table 9. $NOEC_{eco}$ and $LOEC_{eco}$ values for (semi) field studies with single or multiple application of an insecticide from the group of pyrethroids.

Active ingredient	Dose	$NOEC_{eco}$ (µg/L)	$LOEC_{eco}$ (µg/L)	Reference
Stagnant systems				
Cyfluthrin	multiple	-	≤ 0.036	Johnson *et al.*, 1994; Morris *et al.*, 1994
Cypermethrin	multiple	-	≤ 0.07	Farmer *et al.*, 1995
	multiple	-	≤ 0.16	Hill, 1995
Deltamethrin	single	-	≤ 0.2	Morrill & Neal, 1990
	single	-	≤ 2.7	Lahr *et al.*, 1995
Esfenvalerate	single	0.01	0.05	Stay & Jarvinen, 1995
	multiple	0.01	0.01	Webber *et al.*, 1992
	multiple	-	≤ 0.01	Lozano *et al.*, 1992
	multiple	-	≤ 0.25	Fairchild *et al.*, 1992b
Fenvalerate	single	0.01	0.05	Day *et al.*, 1987
Lambda-cyhalothrin	multiple	0.0016	0.016	Hill *et al.*, 1994b
	multiple	-	≤ 0.017	Farmer *et al.*, 1995
Permethrin	single	-	≤ 0.5	Kaushik *et al.*, 1985
Tralomethrin	multiple	-	≤ 0.0027	Mayasich *et al.*, 1994
Running systems				
Fenvalerate	continuous	-	≤ 0.01	Breneman & Pontasch, 1994

Table 10. Summarised $NOEC_{eco}$ and $LOEC_{eco}$ values from studies with pyrethroids in (semi) field experiments compared with various standards.

Active ingredient	Exposure regime	$NOEC_{eco}$ (µg/L)	$LOEC_{eco}$ (µg/L)	MPC NW4 criterion (µg/L)	Liberal UB criterion (µg/L)	Conservative UB criterion (µg/L)
Stagnant systems						
Cyfluthrin	multiple	-	≤ 0.036	-	0.0015	0.0014
Cypermethrin	multiple	-	≤ 0.07	0.0001	0.0068	0.005
Deltamethrin	single	-	≤ 0.2	0.0004	0.0004	0.00029
Esfenvalerate	single	0.01	0.05	-	0.0025	0.0022
	multiple	0.01	0.01	-	0.0025	0.0022
Fenvalerate	single	0.01	0.05	-	0.008	0.003
Lambda-cyhalothrin	multiple	0.0016	0.016	-	0.0021	0.0021
Permethrin	single	-	≤ 0.5	0.0003	0.0065	0.002
Tralomethrin	multiple	-	≤ 0.0027	-	0.0015	0.0015
Running systems						
Fenvalerate	continuous	-	≤ 0.01	-	0.008	0.003

8.3 Other insecticides

The $NOEC_{eco}$ and $LOEC_{eco}$ values for diflubenzuron, lindane and methoxychlor are presented in Table 11. Table 12 compares the summarising $NOEC_{eco}$ and $LOEC_{eco}$ values from these studies with the standards. The $NOEC_{eco}$ value of diflubenzuron is slightly higher than the UP criterion. The $NOEC_{eco}$ for lindane corresponds with the UP criterion but the MPC has a somewhat higher value. The UP criterion for methoxychlor is considerably lower than the $NOEC_{eco}$. A chronic $NOEC_{eco}$ is available for diflubenzuron and lindane (running systems). These studies suggest that the established standards also seem quite satisfactory in case of continuous exposure.

Table 11. $NOEC_{eco}$ and $LOEC_{eco}$ values for studies with the other insecticides in (semi) field studies.

Active ingredient	Dose	$NOEC_{eco}$ (μg/L)	$LOEC_{eco}$ (μg/L)	Reference
Stagnant systems				
Diflubenzuron	single	0.07	0.7	Moffet *et al.*, 1995 (exp. 1A)
	single	0.3	0.7	Moffet *et al.*, 1995 (exp. 1B)
	single	-	≤ 0.7	Moffet *et al.*, 1995 (exp. 2)
	single	-	≤ 30	Tanner & Moffet, 1995
Lindane	continuous	-	≤ 4	Peither *et al.*, 1996
Methoxychlor	single	3	5	Stephenson *et al.*, 1986
	single	-	≤ 20	Solomon *et al.*, 1989
Running systems				
Diflubenzuron	continuous	0.1	1	Hansen & Garton, 1982
Lindane	continuous	0.25	1	Mitchell *et al.*, 1993

Table 12. Summarised $NOEC_{eco}$ and $LOEC_{eco}$ values from studies with the group other insecticides in (semi) field studies compared with various standards.

Active ingredient	Exposure regime	$NOEC_{eco}$ (μg/L)	$LOEC_{eco}$ (μg/L)	MPC NW4 criterion (μg/L)	Liberal UP criterion (μg/L)	Conservative UP criterion (μg/L)
Stagnant systems						
Diflubenzuron	single	0.3	0.7	-	0.063	0.015
Lindane	continuous	-	≤ 4	0.92	0.27	0.16
Methoxychlor	single	3	5	-	0.008	0.008
Running systems						
Diflubenzuron	continuous	0.1	1	-	0.063	0.05
Lindane	continuous	0.25	1	0.92	0.27	0.16

9 DISCUSSION ECOLOGICAL RISKS INSECTICIDES

The ecological risks of 21 insecticides in freshwater ecosystems are discussed in this report. Most of these substances are (still) registered in The Netherlands. No adequate studies, however, have been found for various insecticides that are relevant in The Netherlands. Examples of insecticides used in The Netherlands with which adequate (semi) field experiments have not (yet) been conducted (or reported in the scientific literature), include the organophosphorous compounds dimethoate, dichlorvos, chlorfenvinphos, oxydemeton-methyl and acephate, the carbamates methiocarb, pirimicarb and propoxur, and the acyl-urea compound teflubenzuron. It can nevertheless be concluded on the basis of the insecticides assessed in this report that the selected (semi) field experiments give, after normalisation of the test concentrations, a well-interpretable picture of the ecological effects of groups of insecticides with a similar working mechanism. This is in particular true for the acetylcholinesterase inhibitors and synthetic pyrethroids. As for the herbicides (Lahr *et al.*, 1998; Brock *et al*., 2000), the ‘normalisation’ of the reported field concentrations, by dividing these (nominal) concentrations by the $L(E)C_{50}$ of the most sensitive standard test organism, appears to facilitate the comparison between studies with different insecticides. It is notable that –at corresponding exposure concentrations of substances with the same working mechanism- the reported direct effects show much agreement between studies that have been conducted in Europe, North America and Australia, and between studies that have been conducted in model ecosystems in the laboratory and outdoors.

Figures 1-2, in which the reported ecological effects of various insecticides with a similar working mechanism are classified into effect classes, show that the observations of frequently studied endpoints (such as Microcrustaceans, Insects) show a wide range per effect class. This may –on the one hand- be explained by the fact that the literature is rather variable when considering duration of the study, properties of the test systems, climatological conditions, studied aquatic organisms, and the taxonomic level of the identifications. Differences in environmental behaviour (dissipation rate) of the various insecticides could be another cause of the observed variation. We could, however, not establish a clear relationship between the reported degradation rate of insecticides in standardised water-sediment studies and the extent of the observed effect in (semi) field studies.

After testing of available (semi) field studies against evaluation criteria, only a limited number of these studies appear to be suitable for the validation of criteria. A $NOEC_{eco}$ could only be established for 11 substances because the lowest tested concentration showed a clear effect in the studies with the other substances. Nevertheless, the MPC’s derived for surface water (VROM, 1997), which are also included in the Fourth Memorandum Water Management, for almost all insecticides described in this report are lower than the lowest $NOEC_{eco}$. The only exception is lindane, for which an MPC of 0.92 µg/L has been derived and for which the lowest observed chronic $NOEC_{eco}$ is 0.25 µg/L. In addition, the UB criteria calculated in this report do generally offer aquatic organisms and ecosystem functions sufficient protection against insecticides. On the basis of the data presented in this report it seems meaningful to make a distinction between various exposure regimes for the water quality criteria. For a single application of non-persistent insecticides it seems to be possible to be a factor 10 more lenient than for repeated and chronic exposure.

Sensitive endpoints for direct effects of the studied insecticides are in particular structural ecosystem characteristics and they usually concern population densities of crustaceans and insects. These direct effects in (semi) field studies can generally be well-predicted on the basis of laboratory toxicity tests with similar species. Different (semi) field studies conducted with the same insecticide (e.g., chlorpyrifos) also show similar effects at comparable exposure concentrations. If the most sensitive endpoints of the studies are taken into consideration, different studies conducted with the same insecticide also yield a similar critical threshold value (see $NOEC_{eco}$ values in Table 7).

Indirect effects of insecticides may show large differences in different types of test systems at similar exposure concentrations of the same product. Nevertheless, general trends can be observed if we consider the response on the basis of functional groups. This makes it likely that food web modelling will in due course offer possibilities as instrument for ecological risk assessment of stress by insecticides (Gezondheidsraad, 1997; Traas *et al*., 1998).

Although a large part of the evaluated studies was terminated too early to be able to judge about recovery of sensitive populations, it seems possible to paint, on basis of the remaining studies, a general picture of the recovery of –in particular- sensitive populations of invertebrates. Sensitive crustaceans and insects with a short life cycle usually recovered within 8 weeks after a single peak burdening lower than the $L(E)C_{50}$ of the most sensitive standard test organism in stagnant surface water. For pragmatic reasons we have in our study chosen a recovery period of 8 weeks as criterion for a classification into Effect class 3 or Effect class 5 (a short-term or long-term effect, respectively). In view of the life cycles of macro-evertebrate populations it is common in microcosm and mesocosm experiments to sample macro-evertebrate populations two-weekly or monthly. The time scale at which recovery moments can be assessed are determined by the chosen time intervals between the observations. Practically speaking this means that macro-invertebrate populations 'get the time' of 4 to 2 observations to reach the control level or not. For the short-cyclic zooplankton (sampling frequency usually weekly) there are in principle sufficient measuring points in a period of 8 weeks after the (last) application to assess recovery within this period.

Fewer data are available on the recovery rate of sensitive invertebrates in systems that are repeatedly exposed (see Figures 1-4). When the most sensitive endpoint is taken into consideration, recovery within 8 weeks after the last application seems possible if the final concentration does not exceed 0.1 of the $L(E)C_{50}$ of the most sensitive standard test organism. Species with a long, complex life cycle, however, usually get insufficient attention in these (semi) field studies because they are often not dominantly present in the test systems. An adequate risk assessment for such species will probably improve by the development of recovery models for such populations.

The results described in this report only relate to individual insecticides. There are hardly adequate (semi) field studies in the literature that provide insight into the possible effects of mixtures of pesticides on aquatic organisms and ecosystem functions. The consequences of a possible simultaneous presence of several compounds is neither taken into consideration in the registration and assessment of the risks of pesticides to aquatic organisms. The simultaneous presence of several products around the criterion concentration may possibly lead to ecological risks to aquatic organisms. A literature study into the combination effect of pesticides on aquatic organisms seems to justify the conclusion that concentration addition can be considered as most realistic worst case approach (Deneer, 2000). Concentration addition means that the concentration of the present substances is expressed as fraction of the $L(E)C_{50}$ of standard test organisms after which these fractions per organism are added up for all substances. On the basis of such a calculation we can for insecticides still use the data in this report because we describe the effects of insecticides in ‘toxic units’ (TU_{gst}).

10 CONCLUSIONS

- After testing against the assessment criteria, only a limited number of the available (semi) field studies was found to be suitable for the establishment of ecological threshold values for insecticides in surface water.
- Most (semi) field studies with insecticides are aimed at short-term effects of relatively high exposure concentrations.
- Most knowledge on ecological effects of insecticides in surface water is available for a limited number of organophosphorous compounds (incl. azinphos-methyl, chlorpyrifos, and parathion-methyl) and synthetic pyrethroids (incl. esfenvalerate, fenvalerate and lambda-cyhalothrin), and for lindane and diflubenzuron.
- In comparison with stagnant water, relatively little suitable information has been published on the ecological effects of insecticides in running water.
- The application method to the water and the aquatic fate of insecticides may strongly affect the exposure concentrations and the ecological effects in the field. This was particularly clear in the (semi) field studies with pyrethroids.
- After normalisation of the test concentrations (TU_{gst}), the results of the selected (semi) field studies yield a better interpretable picture of the ecological effects of groups of insecticides with a similar working mechanism.
- In the (semi) field studies with acetylcholinesterase inhibitors the most sensitive organisms are found in the classes of crustaceans and insects. This is in agreement with the observation that *Daphnia magna* is the most sensitive standard test organism for these insecticides.
- Also in the (semi) field studies with synthetic pyrethroids and other insecticides (incl. lindane, diflubenzuron), the most sensitive organisms are found in the classes of crustaceans and insects. Although fish are also reported as most sensitive standard test organism besides *Daphnia magna,* the fish are under (semi) field conditions usually less sensitive than representatives of crustaceans or insects.
- In aquatic ecosystems no specific indicator species can be named which are indicative for stress by a group of insecticides with a similar working mechanism. Generally, representatives of Cladocera, Amphipoda, Ephemeroptera and Diptera are most sensitive to cholinesterase inhibitors, and Amphipoda, Isopoda, Diptera, Ephemeroptera and Hemoptera to synthetic pyrethroids.
- The MPC's for insecticides and standard concentrations according to the Uniform Principles seem sufficiently protective for communities of aquatic microcosms and mesocosms, even in case of repeated or chronic exposure.
- No, or only a small, ecological effect is usually observed in (semi) field studies after a single peak burdening lower than 0.1 x $L(E)C_{50}$ of the most sensitive standard test organism.
- In stagnant test systems, sensitive crustaceans and insects usually recovered within 8 weeks after a single peak burdening lower than the $L(E)C_{50}$ of the most sensitive standard test organism.

- In stagnant test systems, sensitive crustaceans and insects with a short life cycle usually recovered within 8 weeks after the last application in case of repeated application and a final exposure concentration lower than 0.1 x $L(E)C_{50}$ of the most sensitive standard test organism.
- Information on the recovery of species with a relatively long life cycle (e.g. vertebrates) is hardly available.
- Besides the exposure concentration and the life cycle of sensitive populations, the ecological infrastructure (such as degree of isolation of the test system) also determines the extent and rate of recovery.
- Indirect effects of insecticides in (semi) field studies are only observed at relatively high exposure concentrations (> 0.1-1 TU_{gst}). Regularly reported indirect effects of insecticide stress are the increase of algae in the periphyton and phytoplankton (symptoms of eutrophication!) and the increase of less sensitive herbivores (incl. Rotifera and Gastropoda).

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APPENDICES