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Mesocosm experiments in the approval procedure for pesticides

- a litterature study on effects of mesocosm characteristic and validity of extrapolation methods to protect sensitive species

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Content

Foreword

Existing knowledge indicates that agriculture can contribute to deterioration of water quality through the release of pesticides into surface water either directly by wind drift or indirectly through runoff. To evaluate the potential hazard of various pesticides to aquatic life several approaches are available to managers and regulatory authorities. For “problematic” pesticides extended risk evaluations often are based on tests carried out under near-natural conditions in mesocosms. However, the most important limitations of mesocosm experiments are the lack of a standardised design and ambiguous interpretation of results.

This report is intended to provide guidance to managers how to interpret results from mesocosm studies and in specific to identify ”good” experiments encompassing sensitive organismic groups, presence of sediment and macrophytes etc.

The guidance was developed from a critical analysis of already published results of mesocosm experiments. In addition to analysis of sensitivities of different taxonomic groups and comparison of effects in mesocosms to extrapolated hazard concentrations the influences of mesocosm size, location (latitude) and season were quantified. The main results are summarised in chapter 8 and the appendices provide detailed information on the mesocosm experiments included in the analysis.

Summary and conclusions

Objective
Based on a critical analysis of already published results of mesocosm experiments, the objective of the project was to elaborate a checklist for evaluating mesocosms in connection with the approval procedure.

Methods
The checklist has been elaborated on the basis of
1. a thorough examination of existing literature,
2. a critical review of investigations based on objective criteria,
3. construction of a database containing all relevant data,
4. statistical analyses elucidating the effects of pesticides on the various groups of organisms, the influence of mesocosm system characteristics on pesticide impact, etc.

Two different approaches have been applied in the study. We have used a multivariate statistical method (PLS, partial least squares) to examine relationships between toxic effects of pesticides and system characteristics such as mesocosm design, season and location of study. This analysis has been carried out at a rather high level of taxonomy and organism functionality. These analyses have been supplemented by more detailed analysis using traditional statistics to examine differences in sensitivity, potentials of recovery etc. within taxonomic groups.

Database
Selected studies were entered into a database provided that they met certain criteria for documentation and quality. The generated database is based on 112 publications and includes 91 experiments covering 3,635 effect concentrations for 31 different pesticides. Of a total of 3,635 effect concentrations 410 focus on flow-through systems. The majority of the effect concentrations are on zooplankton, followed by effects on macroinvertebrates, phytoplankton and periphyton.

The database encompasses mesocosm studies with 8 different *herbicides* (2,4-D, Alachlor, Atrazine, Glufosinate-ammonium, Glyphosate, Hexazinone, Linuron, Triclopyr-ester), 22 *insecticides* (Aminocarb, Azinphos-methyl, Bifenthrin, Carbaryl, Carbofuran, Chlorpyrifos, Cyfluthrin, Deltamethrin, Diazonon, Diflubenzuron, Dimethoate, Endosulfan, Esfenvalerate, Fenitrothioun, Fenvalerate, Lambda-cyhalothrin, Lindan, Methoxychlor, Mexacarbate, Permethrin, Tebufenozide, Tralomethrin) and 1 *fungicide*: Propiconazole.

Relationships between toxic effect of pesticides and system characteristics - PLS (partial least squares)
PLS is a regression technique that is used to describe the relationship between two sets of variables, X: system characteristics (season, mesocosm size, single species toxicity, log K_{ow}) and Y: toxic effect to each group in the mesocosm. Each substance thus makes up an observation, and the various physical-

chemical characteristics and the toxic effects on the various test organisms function as individual variables.

Separate PLS models were developed for macroinvertebrates, zooplankton and micro algae (periphyton and phytoplankton). For all communities the *lowest effect concentrations* observed for each functional or taxonomic group in each mesocosm experiment were used as Y variables, expressing the toxic response of the organisms in the mesocosms.

When appropriate PLS models are developed it is possible to use the models for prediction of effect concentrations for the organisms in the mesocosms and to associate the predicted effect concentrations with for instance a 95 % confidence interval. *The PLS models even allow the effect concentrations with associated confidence interval to be predicted for experiments where toxicity data for certain groups of organisms were missing*. Since the PLS models are based on all the appropriate data in the database it is thus possible to develop en evaluation procedure taking all the available information into account, rather than basing the evaluation on a restricted use of a single or a few mesocosm experiments for each pesticide. Thus with the aid of the PLS models it is possible to evaluate all mesocosm experiments with pesticides on a common basis.

The amount of data available for the different communities was quite variable, and a direct comparison of PLS models should therefore be conducted with caution. However, important conclusions are

Macroinvertebrates
To obtain a PLS model with a reasonable predictability of the toxic effects to various macroinvertebrate groups, mesocosms should contain sediment and preferably macrophytes in the test system. Overall, the model developed was able to predict 63 % of the observed effects among macroinvertebrates.

In summary, the PLS analysis showed that

1. All macroinvertebrate groups in the mesocosms seem to be most sensitive when the experiments are conducted at high latitudes. Therefore, toxic effects at lower concentrations are expected with increasing distance from Equator, which may be due to a slower turn-over of populations at high latitudes, i.e. fewer generations each year at lower temperatures. Therefore, recovery of populations affected by pesticide exposure takes longer time at northern latitudes.
2. Macroinvertebrates living within the sediment (i.e. infauna) were less sensitive the pesticides than macroinvertebrates living on the sediment surface.
3. At a given total dose the effect of pesticides decreases with number of pesticide additions. Therefore, a low but persistent pesticide concentration will have a lower effect on the macroinvertebrates than a high but temporary pesticide concentration.
4. The toxic effects of pesticides are most pronounced in shallow mesocosms. At decreasing mesocosm depth a larger fraction of the pesticides will end up in the sediment compartment and thus increase the exposure to the sediment living macroinvertebrates. This interpretation is further reinforced by the inverse relation between Log K_D of pesticides and toxicity to invertebrates.

Zooplankton
The PLS model with the highest predictability for zooplankton was obtained when *the pesticides were applied as single addition* and the analysis was restricted to *insecticides* only.

The PLS analysis showed that
1. Hydrophobic *insecticides* with high single species toxicity were the most toxic to the zooplankters in the mesocosmos.
2. Cladocerans were the most sensitive group to *insecticides* followed by copepods and rotifers.
1. The effect of climate zone (latitude) and season was contradictory, as the highest sensitivity was obtained at low latitudes but outside the summer months.

Microalgae
The highest predictability of pesticide effects to microalgae was obtained when only field mesocosm experiments were included in the analysis.

The PLS analysis showed that
1. Hydrophobic and adsorpable pesticides with high single species toxicity were the most toxic to the micro algae in the mesoscosmos.
2. At a given total dose pesticides added over a short period were more toxic to the algae in mesocosmos than pesticides dosed at longer intervals. Frequent dosings will prevent microalgae to recover, while microalgae characterised by short generation times will be able to recover in between dosings applied at longer intervals.

Comparison of sensitivity among different groups of organisms
Zooplankton
Direct effects of *insecticides* on zooplankton were examined and quantified by relating the dosing of *insecticides* to changes in abundance relative to corresponding controls (without *insecticide* dosing). For comparison the average decrease in abundance within the period 3-14 days after the first application of *insecticide* was used.

Zooplankters are very sensitive to *insecticide* exposure. At the group level:

1. Cladocerans and Chaoborus are the most sensitive followed by copepod nauplii and adult copepods.
2. At a given concentration the cladoceran population on average will show larger reductions (20 %) than the copepod population.
3. Copepod nauplii on average will show 10 % larger reductions than the adult population. Observed reductions in one group are a very good predictor of the reductions the other group.

The variation in sensitivity within each zooplankton group as demonstrated in mesocosm studies is considerable. For esfenvalerate LOEC varied 2.5 orders of magnitude for the different species among cladocerans. This variation is probably related to the size of the different species, their habitat and/or feeding mode.

Recovery within zooplankton was dependent on the maximal impact by *insecticides* on the population. For cladocerans the time elapsed for full

recovery after the *insecticide* dosage varied between 10 and 120 days. In mesocosm experiments where cladocerans had been *reduced severely (i.e. > 95 %) it took more than 12-15 weeks* for full recovery. At reductions below 80 % of the initial population size recovery was fast, less than 20 days. Still, even at population reductions close to 100 % full recovery of cladocerans was observed in all mesocosms (where the length of observation period was sufficient). For copepods an almost identical relation between initial decrease and recovery was obtained.

Indirect effects of insecticides on plankton communities
The most prominent indirect effect of *insecticides* in the plankton community includes increases in phytoplankton and rotifers. Following a decrease in population size of crustacean zooplankton, phytoplankton biomass generally will increase due to relaxation of grazing control. In addition, planktonic rotifers that are less sensitive to *insecticides* will increase in abundance due to increased food availability and reduced competition from crustacean zooplankton. Generally, low impacts on the crustacean zooplankton will not result in increased growth of phytoplankton. If however, zooplankton becomes reduced by more than 50 % dramatic increases in phytoplankton (>100 %) must be expected.

The indirect effects of *insecticides* on the plankton community are at least as sensitive as direct effects, e.g. a 75 % reduction in crustacean zooplankton on average will be followed by a 500 % increase in rotifer abundance and a 200 % increase in phytoplankton biomass. However, indirect effects are very variable in both magnitude and direction and thus *less robust compared to direct effects*.

Macroinvertebrates
In the data base direct effects of *insecticides* on macroinvertebrates were examined and quantified by relating the dosing of *insecticides* to changes in abundance relative to corresponding controls (without *insecticide* dosing). For comparison the average decrease in abundance within *the period 28-56 days* after the first application of *insecticide* was used. The sensitivity of alternative end-points such as increase in drift in artificial streams and emergence of imago insects was compared to sensitivity of abundance.

The analysis showed that:
1. The sublethal effects drift in stream macroinvertebrates generally appears to be a more sensitive endpoint than changes in as abundance.
2. The endpoint emergence of adult insects generally is as sensitive as changes in abundance of larvae. *Insecticides* may increase the mortality of larvae and reduce growth rate. In effect, emergence will decrease or be delayed.
3. The insect order Tricoptera consistently was the most sensitive macroinvertebrate group to *insecticides,* followed by Plecoptera/Hemiptera/Ephemeroptera/Coleoptera/Amfipoda/Isopoda (no particular order). Chironomidae as a very diverse group (individual size, mode of feeding etc.) showed a rather large variation in sensitivity (1-2 orders of magnitude). Odonata and Gastropoda consistently were the groups with the lowest sensitivity to *insecticides*.

In macroinvertebrates recovery may take place by invasion from non-affected populations (e.g. by drift in streams, reproduction from insects) and reproduction by surviving individuals. In order to evaluate recovery mesocosm studies need to be carried out in the field (to allow flying insects to

lay eggs) and should at the minimum extend a full life cycle length of the organisms studied after *insecticide* dosage. Very few studies in the database fulfilled the criteria. Chironomids and Isopoda were the most important taxonomic groups in the "slight recovery group" whereas Chironomids and Ephemeropterans dominated the "moderate recovery group". Both Chironomids and Ephemeropterans in general are considered as good colonisers with short life cycles and this probably explains why they show the most rapid recovery.

Comparison of extrapolated Hazard Concentrations and Observed Effects in mesososms

Only a limited number of "high quality" mesocosm experiments examining the effects of pesticides in freshwater systems have been reported. As a consequence, an alternative approach using the results from numerous standardised single species tests has been developed. Hazard concentrations for ecosystems may be calculated from distribution-based extrapolation of single species toxicity data (EC50, LC50) using (slightly) different statistical methods. The mostly used calculation of hazard concentration, $HC_{5,50\%}$ aim to protect 95% of the organisms in an ecosystem with a 50% probability. A alternative approach adopted by the OECD procedure by multiplying the lowest LC(EC)50 observed among all standardised tests by 0.1 (application factor of 10).

To compare the "validity" of extrapolated Hazard Concentrations in protecting complex ecosystems we used the ratio $HC_{5,50}$/LOEC or OECD/LOEC. In 14 out of 66 experiments the widely used approach failed to protect all organisms in the ecosystem. Even using the more conservative OECD approach the hazard concentration failed to protect the organisms in 6 experiments. In about half of the experiments where $HC_{5,50}$/LOEC exceeded 1, NOEC could not be established for the most sensitive parameter, hence the ratio $HC_{5,50}$/LOEC calculated for these experiments represent a minimum.

The vast majority of examples of "failures" of extrapolated hazard concentrations were found in experiments, where LOECs were recorded for macroinvertebrates and insects, while LOECs for phytoplankton and zooplankton except for two occasions occurred in experiments with ratios $HC_{5,50}$/LOEC well below 1. Therefore, extrapolated hazard concentrations generally will protect the plankton environment in ecosystems, which hardly is surprising as the extrapolated values primarily rely on standardised tests with cladocerans and phytoplankton. On the other hand, extrapolated hazard concentrations are much less successful in protecting the macroinvertebrate community.
The importance of including macroinvertebrates in mesocosm experiments was further demonstrated by an ANOVA. If macroinvertebrates were monitored in mesocosms the risk that extrapolated hazard concentrations would fail to protect the whole ecosystem would be substantial.

1 Objective

When evaluating the effects of pesticides on the aquatic environment, system level analyses (mesocosm experiments) are undertaken when lower tier tests (1) indicate a risk for effects in the emvironment, (2) to increase the confidence in the risk assessment, (3) to elucidate effects on organism interactions and (4) to quantify indirect effects of pesticides. When used for risk evaluation, the most important limitations of mesocosm experiments are the lack of a standardised design and ambiguous interpretation of results. The primary objective of mesocosm investigations is to demonstrate whether a given pesticide is toxic or not under near-natural conditions. Based on a critical analysis of already published results of mesocosm experiments, the objective of the present project is to elaborate guidance for evaluating mesocosms in connection with the approval procedure.

2 Introduction

The regulation of pesticide use and protection of non-target species primarily rely on evaluations based on single species tests with organisms belonging to different trophic and taxonomic groups. If specific pesticides are evaluated to constitute a potential hazard to aquatic life, further and extended analysis must be carried out to show that the pesticide does not constitute a risk to the aquatic environment (EU directive 91/414). In line with several other countries Denmark relies on extended risk evaluations based on tests carried out under near-natural conditions (i.e. mesocosms). Several guidelines (e.g. OECD 1996) describe how to carry out mesocosm experiment (experimental design) and what endpoints should be measured. The aim of such guidelines is primarily to define endpoint of regularly concern, which can effectively be addressed only from an appropriate experimental design. Ecological endpoints are those which are directly related to observable changes in the biotic and abiotic components of an aquatic ecosystem. Typically both structural and functional elements are included in the biotic component.

Hypothesis test (i.e. Anova design) is used for investigation of whether the response of a mesocosm unit is different from that of a control unit. Hypothesis tests are used for comparing means and are characterised by having *multiple replicates* in control and treatment groups. The greater number of replicates, the more accurately is the group variability defined and the greater the power of the test for resolving differences. Hypothesis tests are best for objectively determining if an identified difference between control and treatment groups is statistically significant.

Point estimate tests (i.e. regression design) are designed to evaluate *regression relationships and,* by using regression equations between pesticide concentration and observed effects, estimate an exposure concentration which will not cause an adverse effect (i.e. No Effect Concentration, NEC) or predict the intensity of an effect at a given exposure level. Regression analysis is used to iteratively fit observed data to theoretical equation. This requires multiple treatments at various concentrations related to a response. The greater number of treatment concentrations along the response gradient, the greater the confidence in the fitted concentration-response line.

Hybrid tests incorporate features of both hypothesis and point estimate tests. Employing both multiple replicates and multiple doses, one can determine if a given treatment level significantly differs from controls and may estimate how different another treatment level will be above or below the given treatment concentration. The dilemma facing an experimenter is, with a limited number of mesocosm units one can reduce the number of replicates to increase the number of exposure concentrations, or as an alternative, reduce the number of dose levels and increase replicates. Fewer replicates will reduce the power to resolve significant effects and fewer dose levels will reduce the confidence in estimating the fit and the NEC.

In this report we have focussed on how to interpret results from mesocosm experiments and subsequently identify “ideal” experimental condition, which

satisfy both realism (design of experiments) and regulatory needs, such as consistency of results.

3 Methods

The present guideline has been elaborated on the basis of

- a thorough examination of existing literature within the area,
- a critical review of investigations based on objective criteria,
- construction of a database containing all relevant data,
- statistical analyses elucidating the effects of pesticides on the various groups of organisms, the influence of mesocosm system characteristics on pesticide impact, etc.

In risk evaluation ecological, socio-economic and regulatory endpoints are typically included. In this report only endpoints derived directly from measurements or calculations of specific parameters within tests have been included.

Two different approaches have been applied in the study. We have used a multivariate statistical method to examine relationships between toxic effects of pesticides and system characteristics such as mesocosm design, season and location of study. This analysis has been carried out at a rather high level of taxonomy and organism functionality to satisfy the requirement of data within each group. These analyses have been supplemented by more detailed analysis using traditional statistics to examine differences in sensitivity, potentials of recovery etc. within taxonomic groups.

4 Database/mesocosm data

First, an extensive search in databases for literature about the effects of pesticides on mesocosms, streams and lakes was undertaken. Having eliminated all non-relevant papers, 1744 papers remained of which the number was reduced to comprise only papers containing available data on specific pesticides. Having scrutinised the abstracts, the papers on the selected pesticides were obtained. The papers were then thoroughly examined, and effect concentrations etc. were entered into a database provided that they met certain criteria for documentation and quality, which included:

- Effect concentrations were already established or could be estimated from tables and figures.
- True replicates were included in the experiment to allow evaluation of variability.
- Besides the control systems, two or more pesticide concentrations were analysed.
- The experimental conditions were described in detail.
- Pesticide exposure was described, including solvents, active or formulated products.
- In stagnant water mesocosms analysis of pesticide concentration were carried out at the minimum at the start of experiment.
- The mesocosms encompassed a complex ecosystem (at least two different functional groups present were present, e.g. phytoplankton and zooplankton).
- Type 1 Errors (e.g. spurious differences between treatments and controls) were not included in the data base (see below).

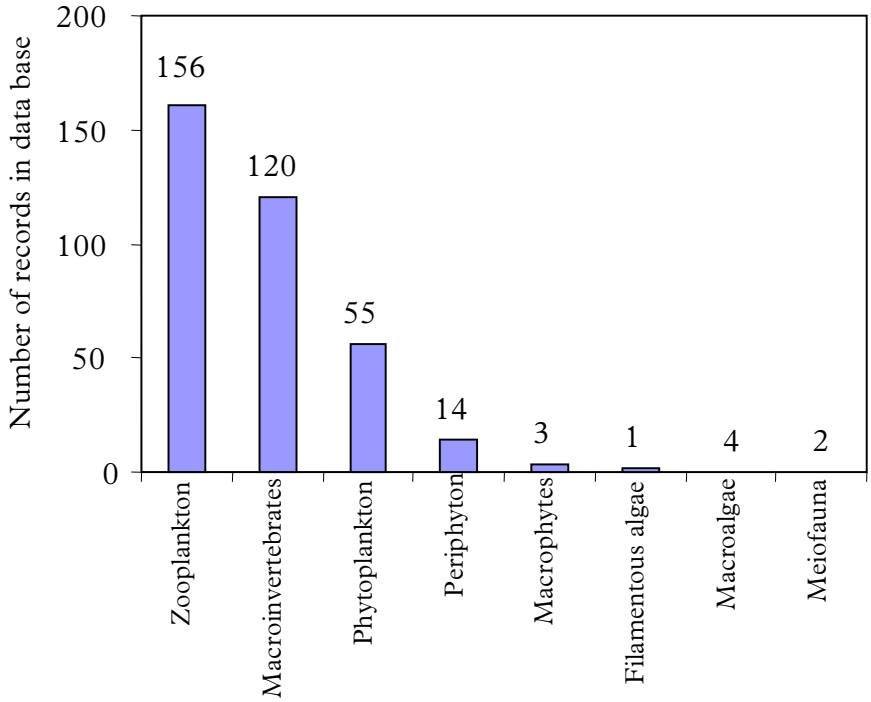
Several papers and reports in the scientific literature originate from the same experiment and may be based on the same data set. To avoid duplication a particular study was identified by the pesticide administration, dates of start and end, location (latitude & longitude, name of facility) etc during the evaluation process. In most studies the deviation in a response parameter (e.g. abundance) is referred to a corresponding control and tested for being significantly different. At increasing number of observations (dates and taxonomic groups) the risk for Type 1 Error increases accordingly, e.g. at a significance level of 5% every 20^{th} observation by chance will be different from the corresponding control. Prior to entering data into the data base we eliminated Type 1 Errors by a Bonforroni adjustment. In case a p-value in a study was not explicitly given, we assumed it to be 0.05. A measured difference was only considered significant if the number of observed significant differences was above a minimum number given by:

$(0.05/\text{total number of tests}) > (\text{p-value})^{MASF}$, where

total number of tests represent the total number of tests (i.e. number of observation days*number of taxonomic groups monitored), p-value given in the study and MASF the minimum number of significant differences recorded for a taxonomic group.

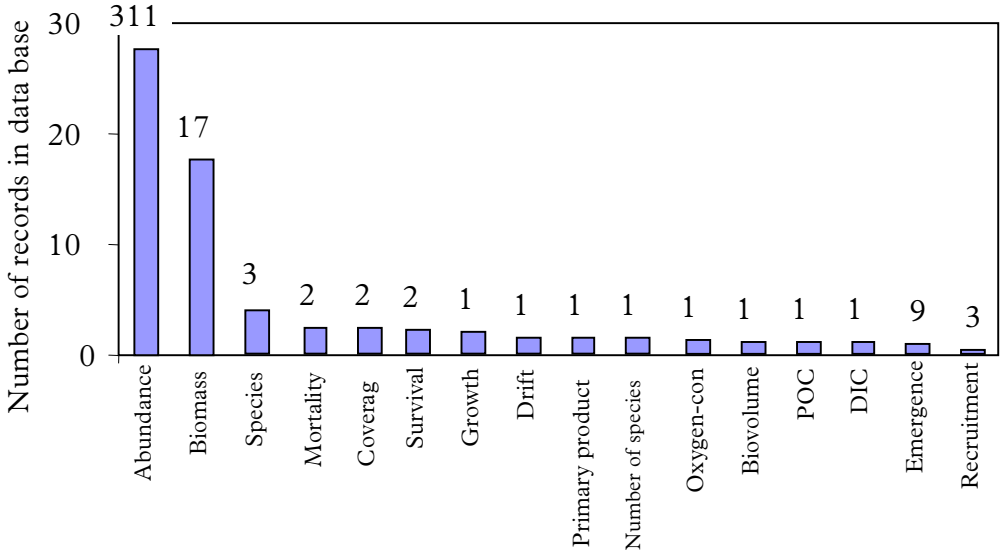
The generated database is based on 112 publications and includes 91 experiments covering 3,635 effect concentrations for 31 different pesticides. Of a total of 3,635 effect concentrations 410 focus on flow-through systems. The majority of the effect concentrations are on zooplankton (1,644 values), followed by effects on macroinvertebrates (1,191 values), phytoplankton (558 values) and periphyton (145 values) (see Fig. 1).

Figure 1. Number of effect concentrations in data base distributed among different taxonomic groups.



Abundance is the dominant effect parameter with 3,114 values out of a total of 3,635 followed by mortality with only 177 values (Fig. 2). Functional effect parameters such as production and growth have seldom been measured and are therefore represented only at a limited scale. The sensitivity of the different effect parameters is discussed in Chapter 6 (primarily covering macroinvertebrates).

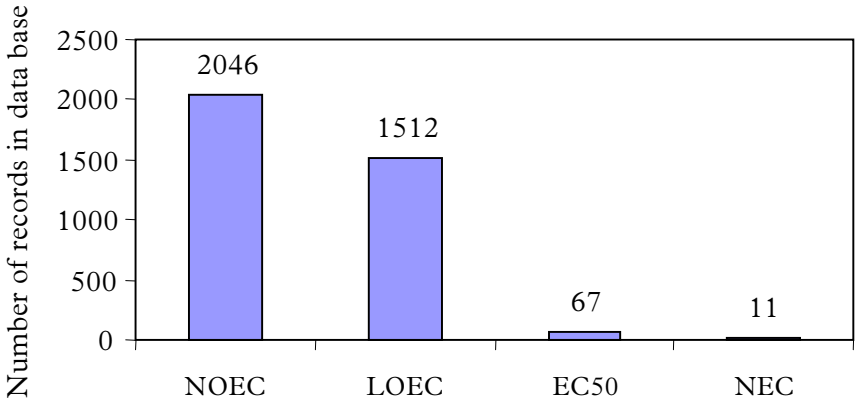
Figure 2. Number of different effect parameters contained in the data base.



The database encompassed mesocosm studies with 8 different herbicides (2,4-D, Alachlor, Atrazine, Glufosinate-ammonium, Glyphosate, Hexazinone, Linuron, Triclopyr ester), 22 *insecticides* (Aminocarb, Azinphos-methyl, Bifenthrin, Carbaryl, Carbofuran, Chlorpyrifos, Cyfluthrin, Deltamethrin, Diazinon, Diflubenzuron, Dimethoate, Endosulfan, Esfenvalerate, Fenitrothion, Fenvalerate, Lambda-cyhalothrin, Lindane, Methoxychlor, Mexacarbate, Permethrin, Tebufenozide, Tralomethrin) and 1 fungicide: Propiconazole.

The dominant end points in the studies of the database are NOEC and LOEC values (2,046 and 1,512 values, respectively). The database only includes 67 EC50 values and 11 NEC values (Fig. 3). For the majority of experiments both NOEC and LOEC values were stored in the database.

FIGURE 3. NUMBER OF DIFFERENT END-POINTS CONTAINED IN THE DATA BASE.



In the mesocosm data analysed 81 out of 90 experiments had multiple replicates allowing to identify LOEC's and NOEC's (i.e. Hypothesis test). More than 80% of the statistical tests were carried out using ANOVA. Eighteen experiments had a sufficient range of concentrations to calculate EC50 or NEC's (i.e Point estimate tests), while 9 experiments could be characterised as hybrid tests with calculated values of EC50 (NEC) in addition to NOEC and LOEC. For further details, see Chapter 5 and 7.

The mesocosm investigations typically lasted several months (Fig. 4). More than 75% of the effect values included measurements made more than 2 weeks after the first (or only) addition of pesticides. Generally, samplings within 1-2 weeks refer to experiments where effects on phyto- and zooplankton were studied while sampling schemes in excess of 1-2 months also included organisms with long life cycles (macroinvertebrates and macrophytes).

The size of the mesocosms varied widely (Fig. 5). Systems with a volume from 0.003 m^3 (3 litres) up to 1,100 m^3 (1,100,000 litres) are included in the database, investigations in small systems typically having been undertaken in flow-through environments.

Figure 4. Distribution of recorded effect concentrations after initial dosing of pesticide.

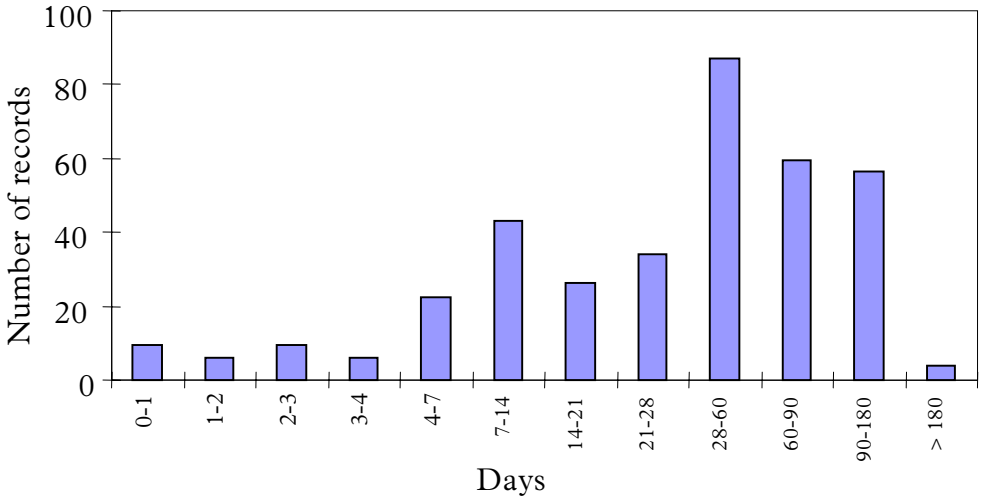
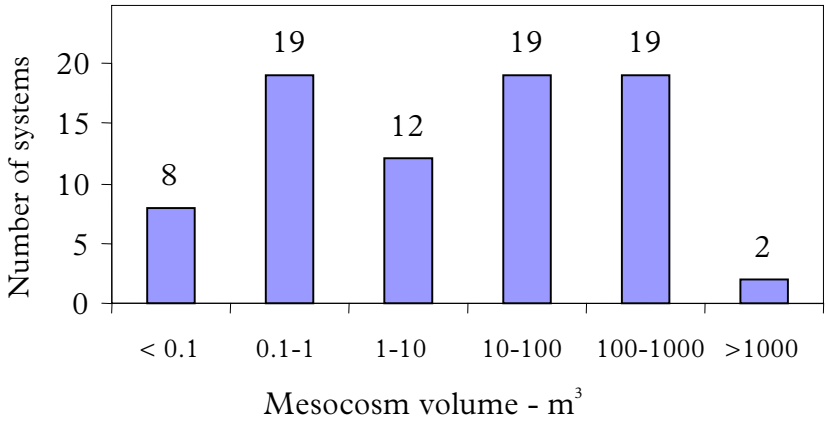
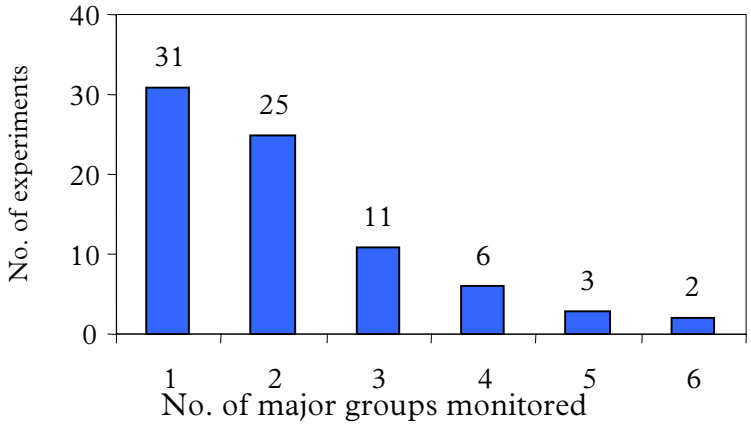


Figure 5. Distribution of system volume in mesocosm experiments contained in data base.



Depending on the objectives of the study mesocosm experiments are designed at various levels of complexity. Generally, systems should be as natural as possible and should contain different taxonomic groups at various trophic levels. Therefore, seen on this background it is highly conspicuous that the number of major groups (defined as macroalgae, phytoplankton, epibenthic microalgae, vascular plants, zooplankton, macroinvertebrates and vertebrates) monitored and included in the database mesocosm experiments is extremely low (Fig. 6). Thus, in more than 40% of the experiments only data from one major group is included and in approx. 30% of the experiments only two major groups are included. However, based on descriptions of experiments, mesocosms generally contained more major groups than were actually monitored during the study.

Figure 6. Number of major groups monitored (NOEC or LOEC) for effects of pesticides in mesocosm studies contained in data base. In 31 experiments only one major group (e.g. zooplankton) were followed.



The database contains a system description of the mesocosm experiments (i.e. size, time table, addition of pesticides, solvents in which the pesticide may have been dissolved, statistical design, construction and selection of materials for mesocosm systems, water quality, etc.) and of the effects (effect concentrations at various points of time, taxonomic groups, species, functional groups, life stages, effect parameters, end points, measurement methods, statistical tests, measurement programmes, etc.). Parameters for water quality (e.g. the concentration of inorganic nutrients) have only been briefly described and are therefore not combined in the analyses. Likewise, in mesocosms including sediment, properties of the sediment (e.g. grain size, organic content) are only described in sufficient detail in about 40% of the studies.

Most mesocosm experiments contained in the database included a sediment compartment (87 %), while macrophytes were present in at least 28 experiments (explicitly noted) and absent in 37 experiments. Based on the information given in analogous experiments (same laboratory and mesocosm set-up) we have assumed presence and absence of macrophytes in an additional 8 experiments. For every species and higher taxonomic groups a functional grouping according to habitat and feeding mode was conducted for the zooplankton and macroinvertebrates, provided that this information was available. The grouping was based on information obtained from various sources (Friberg, pers. comm.)

Single species data

The single species data encompassed by the database have primarily been gathered from the US-EPA (US- Environmental Protection Agency) 'Aquire' database. For some pesticides, data have been obtained either from the Danish EPA or the Pesticide Manual (1998). From the US-EPA database, only data with complete or moderate experimental documentation have been used (see Table 1). In Annex 0 is shown all primary toxicity data used for extrapolation

Table 1. Overview of pesticides contained in the mesocosm data base including calculated hazard concentrations. Primary data used for calculation of Hazard Concentrations is shown in Annex A.

Pesticide	CAS No.	Pesticide type	Hazard conc. ($HC_{5,50}$) µg l^{-1}	Tax Group in extra-polation	Hazard Conc. (OECD) µg l^{-1}	Most sensi-tive group*
Dimethoate	60515	Insect.	3.07	C & I	0.70	I
Carbaryl	63252	Insect.	0.58	F, C & I	0.07	I
Methoxychlor	72435	Insect.	0.09	F, C & I	0.08	C
Azinphos-met	86500	Insect.	0.05	F, C & I	0.02	C
2,4-D	94757	Herbicide	1200	F, C & A	240	C
Endosulfan	115297	Insect.	0.02	F, C & I	0.01	C
Fenitrothion	122145	Insect.	2.26	F, C & I	0.32	I
Mexacarbate	315184	Insect.	2.47	C & I	0.80	I
Linuron	330552	Herbicide	19.7	C & A	5.00	A
Diazinon	333415	Insect.	0.03	F, C, A & I	0.003	I
Lindan	608731	Insect.	2.93	F, C & I	1.80	I
Glyphosate	1071836	Herbicide	1417	F, C, A & I	720(160)	A(M)
Carbofuran	1563662	Insect.	0.05	F, C, A & I	0.02	I
Atrazine	1912249	Herbicide	19.9	F, C, A & I	2.60	A
Aminocarb	2032599	Insect.	5.40	F, C, A & I	2.40	I
Chlorpyrifos	2921882	Insect.	0.04	F, C & I	0.01	I
Alachlor	15972608	Herbicide	0.73	F, C & A	0.60	A
Diflubenzuron	35367385	Insect.	0.15	F, C & I	0.18	C
Hexazinone	51235042	Insect.	4.18	C & A	0.90	C
Fenvalerate	51630581	Insect.	0.05	F, C & I	0.01	C
Permethrin	52645531	Insect.	0.39	F, C & I	0.03	C
Deltamethrin	52918635	Insect.	0.01	F, C & I	0.00	C
Triclopyr ester	55335063	Herbicide	131	F, C & A	120	F
Propiconazole	60207901	Fungicide	11.5	F, C & I	0.32	C
Esfenvalerate	66230044	Insect.	0.18	F & C	0.02	C
Tralomethrin	66841256	Insect.	0.07	F, C & I	0.01	C
Cyfluthrin	68359375	Insect.	0.07	F, C & I	0.01	C
Glufosinate-am	77182822	Herbicide	415295	F & C	56000(370)	C(A)**
Bifenthrin	82657043	Insect.	0.04	F & C	0.01	C
Lambda-cyhaloth	91465086	Insect.	0.08	F & C	0.01	F
Tebufenozide	112410238	Insect.	87.3	F, C, A & I	16.00	C

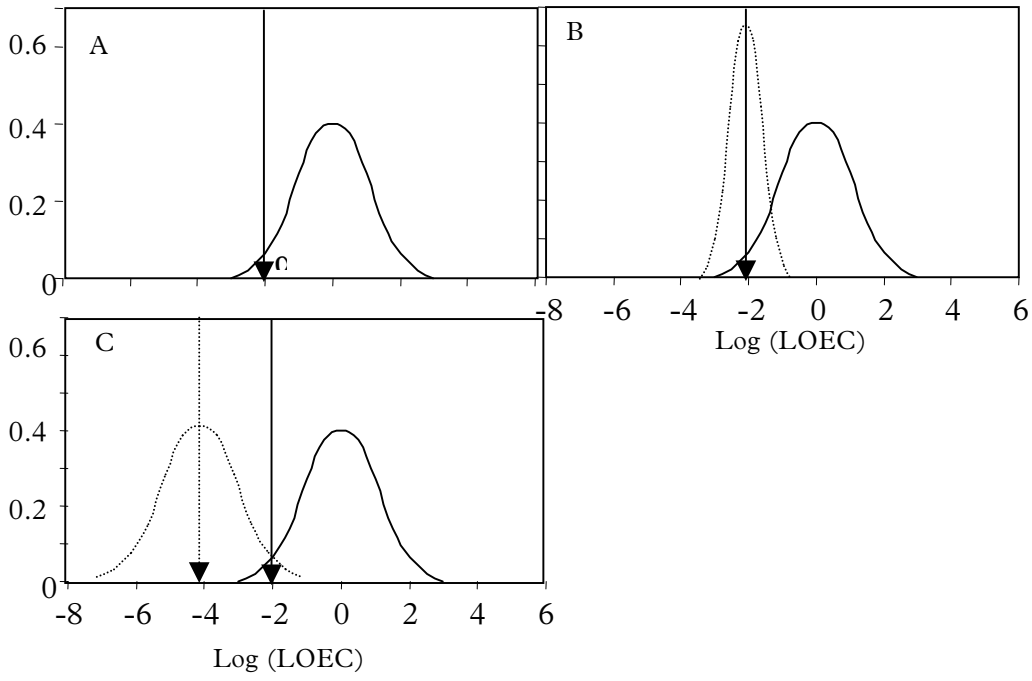
*F: Fish, I: Insect, C: Crustacea, A: Algae, M: macrophyte
** only 1 record for algae

The data collected are limited to freshwater organisms. As a minimum, we have endeavoured to procure toxicity data for fish (LC50-96h), crustaceans (LC50-48h) and algae (EC50-96h). For invertebrates both mortality and immobilisation were accepted as effect parameters. For a number of *insecticides*, (azinphos-methyl, mexacarbate, diazinon, lindane, chlorpyrifos, diflubenzuron, fenvalerate, tralomethrin, bifenthrin), however, we have not been able to obtain toxicity values for algae. However, most of these *insecticides* are probably of low toxicity to algae.

Hazard concentrations ($HC_{5,50\%}$) for ecosystems have been calculated from distribution-based extrapolation of single species toxicity data (EC50, LC50) as described in Miljøprojekt Nr. 250 (Miljøstyrelsen, 1994) and a statistical method (Wagner & Løkke, 1991). $HC_{5,50\%}$ denotes the pesticide concentration that with a 50% probability protects 95% of the ecosystem organisms. We

have chosen the 50% probability level instead of e.g. 95 % level for statistical reasons, as the uncertainty of the estimate will increase both at higher and lower probabilities (see Fig. 7).

Figure 7: Principles of probabilistic extrapolation methods adopted from Smith and Cairns (1993). **A:** α denotes the hazardous concentration to be estimated as a fraction of a log-normal distribution (Wagner and Løkke 1991) or a logistic distribution (Aldenberg and Slob 1993) of LOEC's estimated for different species. The fraction of species to the right of α is supposed to be protected. **B:** As the density function in figure A represents a sample of toxi-city data α must be considered as an estimate with an associated error. Consequently α is assumed to follow the distribution superimposed on the log- normal or the logistic distribution. α therefore denotes the pesticide concentration that with a 50% probability protects 95% of the ecosystem organisms . **C**: Based on the distribution of α a hazardous concentration providing a probability of exceeding α might be provided. The $HC_{5,95}$ concentration thus denotes a concentration protecting 95 % of the species with a probability of 95%; in this fictive example $HC_{5,95}$ is 2 orders of magnitude lower than $HC_{5,50}$.



Initially, hazard concentrations for the different pesticides were calculated within separate groups (algae, crustaceans, insects and fish). However, due to shortage of single species data for several pesticides taxons were grouped prior to extrapolation. In Table 1 is shown the calculated hazard concentrations and the taxonomic groups used for the extrapolation.

In practice, it is assumed that the 95% protection level protects the ecosystem against inadvertent effects (e.g. Emans et al. 1993). It must, however, be

emphasised that this percentage is based on a political-managemental decision, and in some instances a 90% protection of ecosystem species is considered adequate to avoid adverse effects on the natural ecosystem (Hall et al., 1998). In comparison, hazard calculations are based on the lowest effect concentration and an application factor of 10 (as shown in Table 1). In correspondence with the accepted guidelines (Emans et al. 1993), a geometrical average was calculated for the same species or the same genus. On average the EC50(LC50)/10 (i.e. OECD method) was 5.9 times lower than $HC_{5,50\%}$, however they were strongly linearly correlated ($r^2 = 0.96$, after log transformation).

Physico-chemical properties

Solubility in water, distribution coefficients between octanol and water ($logK_{ow}$), sorption coefficients and degradation half-life are all database parameters. Data have mainly been obtained from the Pesticide Manual (1998).

5 PLS (partial least squares)

PLS is a sort of a "regression technique" that is used to describe the relationship between two sets of variables (X and Y matrices). The method is widely used within QSAR (quantitative structure-activity relationships) to compare the toxic effects of different substances on different test organisms (Y matrix) with the physico-chemical characteristics of the substances (X matrix) (Eriksson et al., 1995). Each substance thus makes up an observation, and the various physical-chemical characteristics and the toxic effects on the various test organisms function as individual variables. Each observation thus includes several variables, and the PLS-technique is therefore a so-called multivariate technique.

The advantage of PLS and other multivariate techniques is that the complexity of a data set, consisting of several variables, often can be reduced to a much lower number of dimensions by which the essential relationships between the two matrices can be elucidated. Being a multivariate technique (*and not a multiple regression technique*) PLS can include variables that are either true independent (e.g. depth of the mesocosm and $\log K_{ow}$) or interrelated (e.g. $\log K_{ow}$ and $\log K_D$). For a number of pesticides, the PLS technique in this report has been used to predict the toxic response of various organisms (Y matrix) in model ecosystems on the basis of system volume, depth, location (latitude, longitude) and the toxicological and physico-chemical characteristics of the pesticide (X matrix). Initially, the "field half-life" was included in the X matrix. However, as we were only able to obtain data for 11 pesticides, this parameter was omitted.

Compared to other multivariate techniques the main advantages of the PLS analysis are, that it allows to analyse data sets consisting of more variables than observations, and that the method can handle observations where data on one or more variables are missing. The handling of such missing observations is based on an iterative procedure by which the missing values are estimated. As a rule of thumb the PLS method may thus handle data sets with up to 20% missing data, provided that these data are randomly distributed throughout the data set (Eriksson et al. 1995). In addition the PLS routine allows estimations of confidence intervals around the predicted values.

5.1 Development of PLS models

Due to the limited number of experiments analysing effects in more than 2 major groups of organisms (see Fig. 6) separate PLS models were developed for communities of macroinvertebrates, zooplankton and micro algae (periphyton and phytoplankton). For the remaining groups of organisms, such as micro-organisms (bacteria, ciliates) and macrophytes (vascular plants) it was not possible to develop PLS models due to shortage of data. The raw data, extracted from the database, for the PLS models is shown in Table 3, 8 and 11. For all communities the *lowest effect concentrations* observed (significant positive or negative deviations from controls) for each functional or taxonomic group in each mesocosm experiment were used as Y variables, expressing the toxic response of the organisms in the mesocosms. It should be

noticed that by including only LOECs (but tested for significance, see above) the PLS analysis do not explicitly take account of recovery of populations.

In less than 25 experiments were the pesticide concentration monitored and described in such a detail that an observed effect could be ascribed to a specific pesticide concentration. Therefore, nominal (added) effect concentrations were used throughout. In the case of several dosings, the total (accumulated) concentration was used as the nominal effect concentration. The effect of dosing mode of the pesticides (number of doses, interval between doses) was entered as independent variables (X-matrix) and thus accounted for specifically in the analysis. All analyses were based on log-transformed data, e.g. log Kow, log Depth.

Initially, a series of scenarios were defined and a PLS model was fitted using the auto-fit routine of the program SIMCA-P 8.1 and examined for each scenario. However, due to the limited amount of data it was not possible to examine all scenarios. The different scenarios selected are shown in Table 6. The final choice of PLS models were based on successively narrowing the mesocosm characteristics (e.g. including only mesocosms with a sediment compartment). For each PLS model possible outliers were identified using plots of residuals (normal distributed) and predicted values against observed values. For the models chosen the experimental set-up in the outliers was examined in detail to explain their deviance.

After removing outliers PLS models with the highest predictability were selected for further interpretation.

5.2 Interpretation of PLS models

To interpret the PLS analyses, the following procedure was used:

1. For each data set, the number of significant PLS axes were found.
2. For each of the significant PLS axes, the importance of the different variables was determined by means of so-called loadings or weights that are a measure of how much each variable contributes to the axis in question. The weights can be both positive and negative, and weights with opposite signs can be interpreted as being negatively correlated, whilst weights with identical signs can be interpreted as being positively correlated.
3. To reduce complexity the interpretations were limited to variables with an overall significant contribution to the PLS model (roughly equivalent to loadings larger then 0.2 or lower than -0.2; see Fig. 7).

To obtain an adequate amount of data for a PLS analysis, it was necessary to group the observations into different data sets. In the following section, the results of each of these analyses will be described followed by a general discussion including recommendations. An overview of the abbreviations used can be found in Table 2.

5.3 Using the PLS models in a standardised evaluation procedure of pesticides

When appropriate PLS models have been developed it is possible to use the models for prediction of effect concentrations for the organisms in the mesocosms and to associate the predicted effect concentrations with for

instance a 95 % confidence interval. *The PLS models do even allow to predict the effect concentrations with associated confidence interval for experiments where toxicity data for certain groups of organisms were missing.* Since the PLS models are based on all appropriate data in the database it is thus possible to develop an evaluation procedure taking all the available information into account, rather than on a restricted use of a single or a few mesocosms experiment for each pesticide. Effects of pesticides in nature will depend on a suite of factors including the direct toxic effects of a pesticide, the physical-chemical conditions in the environment and the biological structure and interactions within the environment. Several of these conditions not related to the direct toxic effects may be as important for the actual effects as the pesticide concentration and moreover they may modify the effect of different pesticides in a more or less uniformly way. Thus with the aid of the PLS models it is possible to evaluate all mesocosm experiments with pesticides on a common basis.

Throughout the following the lowest observed effect concentrations (LOEC) obtained for the various groups of organisms in the mesocosm experiments were used as Y-variables in the PLS analysis. Thus the predicted effect concentrations are conceptually comparable to $HC_{5,50}$ concentrations estimated on the basis of single species toxicity data and the extrapolation methods of Wagner and Løkke (1991). Similarly the lower limit of the confidence interval might be considered as equivalent to a $HC_{5,95}$ concentration estimated with the aid of the extrapolation procedure of Wagner and Løkke (1991).

However, with the aid of PLS models it is possible to take the information from other mesocosm experiments into account, whereby the critical extrapolation from lower levels of biological organisation (single species level) to higher levels of biological organisation (ecosystem community) is avoided. In effect, by applying the PLS technique in a steadily growing data base the hazards of new pesticides at ecosystem level can be evaluated by interpolation instead of by extrapolation. Furthermore, the importance of pesticide properties, such as log K_{ow} and log K_D, and system properties, such as the volume and size of the mesocosm, are taken into account.

Table 2. List of abbreviations used in PLS figures and in Tables 3, 8, 11.

Common X matrix for all PLS models	
Variable	Explanation/remark
Day number	Refers to julian day of first pesticide dosage. Sinusiodal distribution with mid summer (21 June) as the highest day number (log(183)).
Latitude	
Longitude	
Log K_D	Particle sorption coefficient
Log K_{OW}	Distribution coefficient between octanol and water
Dosing interval	Time in days between addition of pesticides
Number of additions	Number of additions of a pesticide
Volume	Volume in litre
Depth	Average depth in m.
$HC_{5,50}$	Extrapolated $HC_{5,50}$ concentration (Wagner and Løkke 1991)
OECD	EC50 (algae) or LC50 for the most sensitive organism divided by 10 (OECD 1991)
Y matrix for macroinvertebrates	
Variable	Explanation/remark
Non_pred	Lowest effect concentration for non predatory organisms
Pred	Lowest effect concentration for predatory organisms
Epi_fauna	Lowest effect concentration for epifauna organisms
In_fauna	Lowest effect concentration for infauna organisms
Y matrix for zooplankton communities	
Variable	Explanation/remark
Cladocea	Lowest effect concentration for Cladocera abundance
Copepod	Lowest effect concentration for Copepod abundance
Rotifer	Lowest effect concentration for rotifer abundance
Y matrix for microalgae	
Variable	Explanation/remark
Micr_algae	Lowest effect concentration for microalgae abundance

5.4 PLS models for macroinvertebrates

An overview of raw data from the database used in the PLS models developed for macroinvertebrates is shown in Table 3. The analyses are based on data from 17 different experiments with a total of 9 different pesticides.

Table 3. Overview of raw data from the database for the PLS models developed for macroinvertebrates. See Table 2 for abbreviations used. Sediment 1 refers to sediment present in mesocosm, 0 to no sediment; Macrophytes: 1 = present, 0 = no macrophytes; N= no information given on presence of marcophytes Field/lab: 1 = Field study, 0 = Laboratory study. For all macroinvertebrate groups the lowest effect concentrations observed for each functional group in each mesocosm experiment were used as Y variables, expressing the toxic response of the organisms in the mesocosms (values shown in bold). L = Laboratory study at controlled temperature and light availability (hence latitude and longitude not relevant); F = flow-through study; - = no data. See Annex B for literature references.

Exp.	Pesticide	CAS No.	Day number	Latitude	Longitude	Field half life	Log K_D	Log K_{OW}	Dosing interval	Residence time	Number of additions	Volume	Depth	Sediment	Macrophytes	Field/Lab
									d	*d*		m^3	*m*			
83tll	cyfluthr	68359375	174	33	100	-0.0100	5.00	6.00	14	500	11	634.7	1.3	1	N	1
84tll	cyfluthr	68359375	174	33	100	-0.0100	5.00	6.00	14	500	11	1.9	1.0	1	N	1
42flm	chlorpyr	2921882	167	51,58	5,4	-0.0100	3.78	4.60	0	500	1	55.0	0.5	1	1	1
57flm	esfenval	66230044	155	46,45	92,07	-0.0086	3.72	6.22	28	500	2	25.0	0.5	1	1	1
123flm	lamb_cyh	91465086	144	51	0	-0.0100	5.26	7.00	14	500	4	25.0	1.0	1	1	1
57tll	Diazinon	333415	134	38,58	95,14	-0.0075	3.00	3.30	70	500	4	11.2	1.3	1	1	1
53flm	chlorpyr	2921882	79	L	L	-0.0100	3.78	4.60	0	500	1	0.70	0.7	1	1	0
76tll	lamb_cyh	91465086	174	35,26	77,59	-0.0100	5.26	7.00	14	500	12	450.0	1.0	1	1	1
75mli	hexazin	51235042	L	L	L	-0.0033	1.73	1.04	0	0.1	1	F	F	0	0	1
50flm	chlorpyr	2921882	54	L	L	-0.0100	3.78	4.60	0	500	1	0.70	0.7	1	1	0
25tll	endsulfa	115297	L	L	L	-0.0060	4.09	4.74	21	500	2	0.003	0.1	1	1	0
110tll	carbofur	1563662	100	53,33	113,15	-0.0060	1.34	1.52	0	500	1	1.2	0.6	1	1	1
47flm	esfenval	66230044	139	31,5	89,5	-0.0086	3.72	6.22	17	500	5	700.0	1.5	1	1	1
51flm	chlorpyr	2921882	54	L	L	-0.0100	3.78	4.60	0	500	1	0.70	0.7	1	0	0
113tll	esfenval	66230044	68	56,5	10	-0.0086	3.72	6.22	0	500	1	36.0	0.5	1	N	1
125flm	trahalom	66841256	125	32	97	-0.0111	5.00	5.00	13	500	6	635.0	1.3	1	1	1
60flm	lamb_cyh	91465086	141	51,3	1,2	-0.0100	5.26	7.00	14	500	4	25.0	1.0	1	1	1

Table 3 cont.

Exp.	Single species toxicity µg l^{-1}					Mesocosm LOEC µg l^{-1}			
	$HC_{5,50}$	LC50/10 (OECD)	Lowest LC50 Insecta	Lowest LC50 oth.Arthopoda	Std. of LC50	Non-predatory	Predatory	Epi-fauna	In-fauna
83tll	0.070	0.014	0.458	0.14	2.20	**2.625**	**4.12**	**2.625**	**2.625**
84tll	0.070	0.014	0.458	0.14	2.20	**2.63**	**2.63**	**2.625**	**2.625**
42flm	0.0440	0.0077	1.80	0.077	4.96	**0.10**	**0.10**	**0.10**	**0.10**
57flm	0.178	0.024	-	0.24	1.27	**0.16**	**0.16**	**0.02**	**0.16**
123flm	0.0803	0.0117	-	0.30	2.25	**0.068**	**0.068**	**0.068**	**0.68**
57tll	0.0280	0.0027	0.027	0.451	26.38	**36.80**	**9.60**	**9.60**	**88.0**
53flm	0.0440	0.0077	1.80	0.077	4.96	**35.0**	**35.0**	**35.0**	**35.0**
76tll	0.0803	0.0117	-	0.30	2.25	**0.0012**	**0.0012**	**0.0012**	**0.115**
75mli	4.1839	0.90	-	442000	2.35	**2700**	**2700**	**2700**	**2700**
50flm	0.0440	0.0077	1.80	0.077	4.96	**5.0**	**-**	**5.0**	**5.0**
25tll	0.0220	0.010	2.30	0.10	16.54	**2.0**	**2.0**	**2.0**	**2.0**
110tll	0.0474	0.0193	0.193	12.50	15.06	**5**	**-**	**5.0**	**-**
47flm	0.1779	0.0240	-	0.24	1.27	**1.28**	**1.28**	**1.28**	**-**
51flm	0.0440	0.0077	1.80	0.077	4.96	**35.0**	**-**	**35.0**	**-**
113tll	0.1779	0.024	-	0.24	1.27	**0.035**	**-**	**0.035**	**-**
125flm	0.0746	0.015	0.58	0.15	1.97	**70.15**	**-**	**70.15**	**-**
60flm	0.0803	0.01173	-	0.30	2.26	**0.068**	**-**	**0.068**	**-**

The PLS models examined for macroinvertebrates are shown in Table 4.

Table 4 Predictability of the examined PLS models for macroinvertebrates. Q^2(cum) denotes the cumulative predictability (both significant axis included). See Annex B for literature references.

Data included	Outliers	Q^2(cum)
All available data for stagnant water with sediment	none	0.481
Mesocosm data for stagnant water with sediment	experiment 76tll[1)] and 125flm[2)]	0.599
Mesocosm data for experiments with macrophytes and sediment	experiment 76tll and 125flm	0.625

[1)] Mesocosm experiment 76tll consisted of 450 m^3 cosms dosed with Lambda-cyhalothrin every 14 days during a period of 147 days. Along with experiment 107tll (2,4-D) the exposure scheme was by far the most extensive in terms of length of exposure period and number of additions.

[2)] Mesocosm experiment 125flm consisted of 635 m^3 cosms pulse-exposed to Trahalomethrin 5 times during 65 days. Because of rather high through-flow 90% of the pecticide was washed-out within 24 h after each dosage. Hence, the calculated total exposure concentration (=sum of each dosage) inevitably will grossly overestimate the actual concentrations (i.e. the experiment may not qualify for a true stagnant water experiment).

As shown in Table 4 the highest predictability (Q^2(cum)) was obtained for the PLS model based on mesocosm experiments where both sediment and macrophytes were present in the test system. However, an almost as high Q^2(cum) were obtained for the PLS models for mesocosm experiments with sediment but without macrophytes in the test system. On the other hand a much lower predictability (Q^2 = 0.481) was obtained when the PLS model was developed including all experiments carried out in stagnant water (i.e. including laboratory experiments). Hence, the larger similarity in the constituents of the different mesocosms (and closer resemblance to natural conditions) the higher is the predictability of toxic effects based on the various physical, chemical and toxicological properties of an experiment (see Table 2). Therefore, a PLS model for macroinvertebrates with an acceptable predictability needs to be based on mesocosm experiments containing sediments and preferentially macrophytes in the test system. The interpretation of the PLS model is therefore based on the PLS model for mesocosm data *with both macrophytes and sediment present in the test systems,* but excluding 2 experiments (76tll and 125flm) i.e. a total of 9 experiments.

5.4.1 Interpretation of the PLS model for macroinvertebrates

An overview of the model selected in the previous section is shown in Table 5.

Table 5. Predicted variation of the significant axis of the PLS model selected for macroinvertebrates. Q^2: Variation in the Y matrix predicted from the variation in the X matrix by the current axis. Q^2(cum): Cumulative variation in the Y matrix predicted from the variation in the X matrix.

PLS axis number	Q^2	Q^2(cum)
1	0.527	
2	0.207	0.625

As shown in Table 5 the first PLS axis predicts 52.7 % of the variation in the Y matrix (i.e. the toxic response) from the variation in the X matrix (system characteristics and toxicological and physico-chemical characteristics of the pesticide). The second PLS axis predicts 20.7% of the variation in the Y matrix from the variation in the X matrix. The fact that Q^2(cum) is lower than the sum of Q^2 for the two axis (i.e. 0.527 + 0.207) indicates some overlap between the predictions of the first and second PLS axis.

Figure 8. Weights (loadings) of variables contributing to the first PLS axis for macroinvertebrates. Day number through OECD represent variables in the X-matrix while the responses (LOEC) of the different macroinvertebrate groups are shown at right. Weights with opposite signs can be interpreted as being negatively correlated (e.g. INTERVAL between pesticide doses and toxic response of either macroinvertebrate group), while weights with identical signs can be interpreted as being positively correlated (e.g. mesocosm Depth, Number of dosings and toxic response of macroinvertebrates).

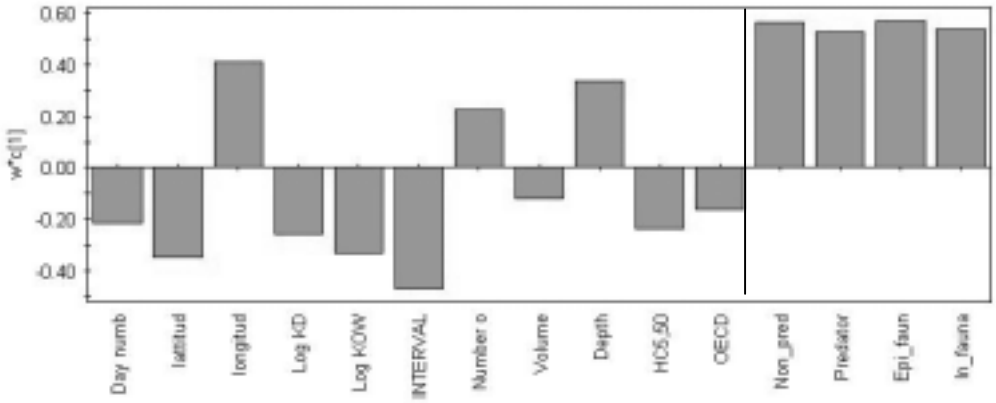
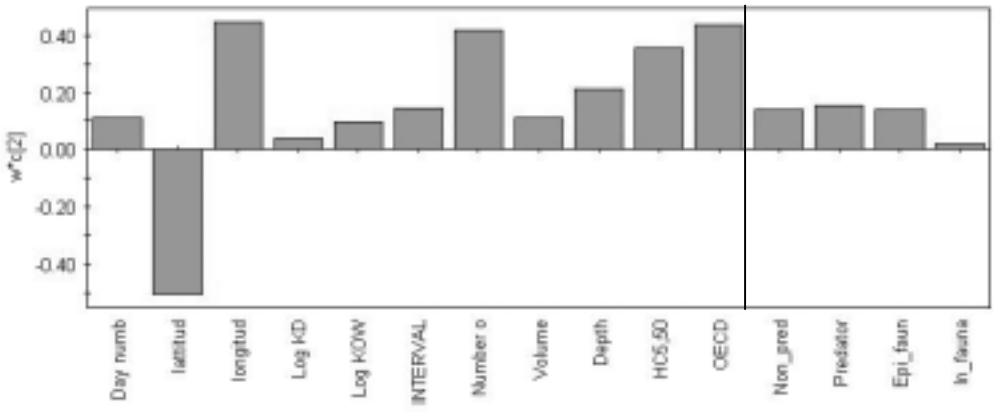


Figure 9. Weights (loadings) of variables contributing to the second PLS axis for macroinvertebrates.



As described in Section 4.2 the PLS axis was interpreted on the basis of the weights (loadings) of the variables to each PLS axis. The loading plots are shown in Figs 8 & 9.

- The first PLS axis showed positive loadings of almost equal magnitude in all groups of macroinvertebrates, which means that the four groups are equally sensitive to pesticides and the conditions in the mesocosms.
- For the second PLS axis a much lower positive loading is obtained for the infauna (i.e. borrowing animals) than for the other functional groups. Thus the two PLS axis seems to be indicative of different toxic responses of the macroinvertebrates according to their habitat.
- For the X variable longitude positive loadings are seen for both PLS axis, whereas for latitude, negative loadings are seen for both PLS axis. Thus all macroinvertebrate groups in the mesocosms seem to be most sensitive when the experiments are conducted at high latitudes and low longitudes in mesocosms. Therefore, toxic effects at lower concentrations are expected with increasing distance from Equator. A likely explanation is probably related to a slower turn-over of populations at high latitudes, i.e. fewer generations each year at lower temperatures. Therefore, recovery of populations affected by pesticide exposure takes longer time at northern latitudes. The positive loading for longitude suggests that organisms in experiments conducted in USA are less sensitive than the macroinvertebrates in experiments conducted in Europe. A possible explanation could be that most mesocosm experiments in USA, but not in Europe, are carried out with fish present in enclosures, which may override or mask the effect of pesticides.
- For the first axis the ”Interval” between pesticide dosings correlated negatively with LOEC of the macroinvertebrates. Thus by increasing the interval between dosings a lower LOEC will result. This may be due to the relativly long generation time of most macroinvertebrates. Hence, recovery will be hampered if pesticides are dosed at intervals close to the generation time. Interestingly, the related variable ”Number of pesticide Dosings” correlated positively with LOEC’s (especially on the second PLS axis), meaning that a low but persistent pesticide concentration will have a lower effect on the macroinvertebrates than a high but temporary pesticide concentration.
- High positive loading for the variable Depth on both axis could be related the fate of pesticides in mesococms. At decreasing mesocosm depth a

larger fraction of the pesticides will end up in the sediment compartment and thus increase the exposure to the sediment living macroinvertebrates.

- For the X variables Log K_D, Log K_{OW} and Interval (between dosings) significant negative loadings are obtained for the first PLS axis. The loadings of these variables to the second axis were considered as insignificant. Thus the toxic response of the macroinvertebrates expressed by the first PLS axis are most pronounced for hydrophobic, adsorbable (high log K_D) substances added to the mesocosms over a long time period (Interval). In effect, the toxic response of the macroinvertebrates expressed by the first PLS axis might be considered as a long term response probably involving sorption of the pesticides to particles, sedimentation of the particles and a subsequent exposure of the organisms to pesticides adsorbed to sediment particles.
- High positive loadings to the second PLS axis are obtained for the X variables hazard concentrations ($HC_{5,50}$ and LC50/10 (i.e. OECD procedure)), while their significance on the 1. axis are considered insignificant. As stated above the loadings of the infauna to the second axis is insignificant (see Fig. 9). Hence, the toxic response of the macroinvertebrates expressed by the second axis can be considered as a short-term response attributable to a direct exposure through the water phase, which consequently do not affect the macroinvertebrates living within the sediment. As the hazard concentrations are calculated from standardised short term (48-96 h) toxicity tests the loadings (correlations) are expected.
- The variables day number (i.e. season) and volume are considered as insignificant (low loadings and of opposite sign).

A summary of the effect of experimental mesocosm and pesticide characteristics is shown in Table 6.

Table 6. Summary of influences of mesocosm and pesticide characteristics and toxicology (extrapolated effect concentrations) on toxic response on macroinvertebrates. ↑↑ = major decrease in toxicity; ↑ = minor decrease in toxicity (i.e. higher LOEC); ↓↓ = major increase in toxicity; ↓ = minor increase in toxicity (i.e. lower LOEC); - = no effect. See Table 2 for an explanation of system variables.

Macroinvertebr. group	Season	Latitude	Longitude	Log K_d	Log K_{ow}	Interval	# of doses	Depth	$HC_{5,50}$	LOEC/10
Non-pred.	-	↓↓	↑↑	↓	↓	↓	↑↑	↑↑	↓	↓
Predatory	-	↓↓	↑↑	↓	↓	↓	↑↑	↑↑	↓	↓
Epi-fauna	-	↓↓	↑↑	↓	↓	↓	↑↑	↑↑	↓	↓
In-fauna	-	↓	↑	↓	↓	↓	↑	↑	-	-

The arrows in Table 6 indicate if numeric increases in system variables (see Table 2) will decrease (↑) or increase (↓) the toxic response in the different groups of macroinvertebrates. Double arrows denote that a system variable have the same significant influence in both PLS axes.

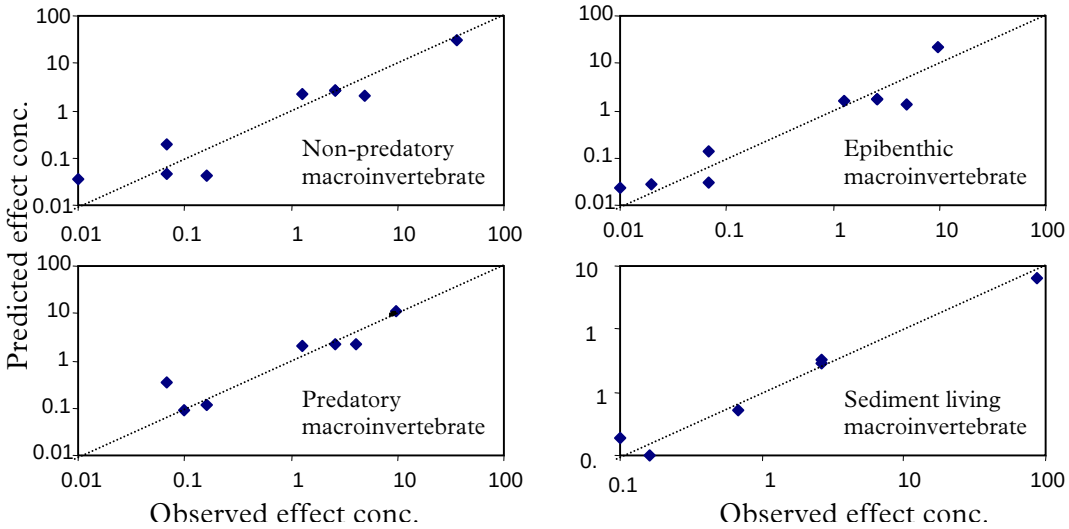
5.4.2 Predicting effect concentrations for the macroinvertebrates with the aid of the PLS models

The observed and predicted effect concentrations with associated 95 % confidence intervals for all the mesocosm experiments analysed by the PLS model appear in Table 7 and Figure 10. The asymmetric confidence interval is due to the logarithmic transformation of data before the PLS analysis. When the lower limit of the confidence intervals was below 0 (seemingly a bug occurring in the Simca program during log- and antilog transforming procedure) the lower limit of the confidence interval was set to 0.

Table 7. Comparison of observed and predicted LOEC (µg L^{-1}) with associated 95 % confidence interval for the mesocosm experiment calculated with the PLS model for macroinvertebrates. Macroinvertebrates have been grouped according to mode of feeding (non-predatory/predatory) and habitat (epifauna = macroinvertebrates living on sediment surface; infauna = macroinvertebrates living within the sediment).

Exp.	Pesticide	Non-pred		Confidence interval		Predatory		Confidence interval	
		Observ	Pred.	Low	Upp	Observ	Pred.	Low	Upp
83tll	cyfluthr	2.625	2.581	0.386	5.947	4.116	2.302	0.266	5.490
84tll	cyfluthr	2.625	2.577	0.572	5.516	2.625	2.252	0.428	4.977
42flm	chlorpyr	0.010	0.035	0.001	0.093	0.100	0.091	0.000	0.249
57flm	esfenval	0.160	0.041	0.004	0.100	0.160	0.116	0.007	0.294
123fl	lamb_cyh	0.068	0.200	0.071	0.374	0.068	0.343	0.113	0.661
57tll	Diazinon	36.800	30.70	0.000	97.13	9.600	11.35	0.000	37.61
110tll	carbofur	5.000	1.982	0.000	6.083	--	1.516	0.000	4.865
47flm	esfenval	1.279	2.206	0.091	5.684	1.279	2.119	0.010	5.675
60flm	lamb_cyh	0.068	0.045	0.007	0.102	--	0.113	0.014	0.267
Exp.	Pesticide	Epifauna		Confidence interval		Infauna		Confidence interval	
		Observ	Pred.	Low	Upp	Observ	Pred.	Low	Upp
83tll	cyfluthr	2.625	1.789	0.436	3.747	2.625	2.910	1.390	4.843
84tll	cyfluthr	2.625	1.782	0.553	3.495	2.625	3.274	1.723	5.197
42flm	chlorpyr	0.010	0.024	0.003	0.056	0.100	0.194	0.076	0.352
57flm	esfenval	0.020	0.028	0.005	0.061	0.160	0.101	0.044	0.174
123fl	lamb_cyh	0.068	0.136	0.059	0.237	0.680	0.504	0.310	0.735
57tll	Diazinon	9.600	21.13	0.000	58.89	88.00	64.90	17.32	132.8
110tll	carbofur	5.000	1.342	0.000	3.639	--	8.725	2.504	17.50
47flm	esfenval	1.279	1.532	0.221	3.550	--	2.148	0.866	3.844
60flm	lamb_cyh	0.068	0.030	0.008	0.063	--	0.179	0.087	0.296

Figure 10. Plots between observed (µg L^{-1}) and predicted LOEC by PLS models for macroinvertebrates allocated to different modes of feeding and their habitat. X=Y (stippled line) shown.



As depicted in Fig. 10 the LOEC predicted by the PLS models were in excellent agreement with the observed LOEC, irrespective of the chosen grouping of macroinvertebrates.

5.5 PLS models for zooplankton

An overview of raw data from the database used in the PLS models developed for zooplankton is shown in Table 8. The analyses are based on data from 31 different experiments with a total of 14 different pesticides.

The PLS models examined for zooplankton are summarised in Table 9.

The PLS model with the highest predictability (0.736) for zooplankton was obtained when the pesticides were added as single addition and the analysis was restricted to *insecticides* only (see Table 9). However, an almost as high predictability was obtained when the analysis included both *insecticides* and herbicides (0.655). For the remaining PLS models examined lower and more inconsistent predictabilities (Q^2(cum)) were obtained. The interpretation of the PLS models was therefore restricted to the PLS models for mesocosm experiments with a single addition of *insecticides* (i.e. a total of 11 experiments).

Table 8. Overview of raw data from the database for the PLS models developed for zooplankton. See Table 2 for abbreviations used. Sediment 1 refers to sediment present in mesocosm, 0 to no sediment; Macrophytes: 1 = present, 0 = no macrophytes, N= no information given on presence of marcophytes; Field/lab: 1 = Field study, 0 = Laboratory study. For all zooplankton groups the lowest effect concentrations observed for each taxonomic group in each mesocosm experiment were used as Y variables, expressing the toxic response of the organisms in the mesocosms (values shown in bold). L = Laboratory study at controlled temperature and light availability (hence latitude and longitude not relevant); F = flow-through study; - = no data. See Annex B for literature references.

Exp.	Pesticide	Day number	Latitude	Longitude	Log K_D	Log K_{OW}	Dosing interval *d*	Number of additions	Volume m^3	Depth *m*	Sediment	Macrophytes	Field/Lab	$HC_{5,50}$	EC50/10 (OECD)	Mesocosm LOEC µg l^{-1}		
																Cladoce	Copepo	Rotifer
38ank	Lindane	130	L	L	-	-	7	3	0.30	0.6	1	0	0	2.928	1.80	**48.0**	**12**	**12**
43flm	methoxyc	142	43,6	79,3	4.9	-	0	1	100	4	1	0	1	0.092	0.078	**3.0**	**3**	**3**
45flm	methoxyc	174	43,6	79,3	4.9	-	0	1	100	4	1	0	1	0.092	0.078	**20.0**	**20**	**20**
46flm	methoxyc	174	43,6	79,3	4.9	-	35	2	100	4	1	0	1	0.092	0.078	**20.0**	**20**	**20**
51flm	chlorpyr	54	L	L	3.78	4.6	0	1	0.70	0.7	1	0	0	0.044	0.0077	**35.0**	**35**	**35**
55flm	Linuron	110	L	L	2.6	3	3	10	0.60	0.5	1	1	0	19.67	5.0	**155.0**	**155**	**51.67**
57flm	esfenval	155	46,45	92,07	3.72	6.22	28	2	25	0.5	1	1	1	0.178	0.024	**0.02**	**0.02**	**0.16**
58flm	difluben	185	34,3	91,33	4	3.89	0	1	400	1	1	N	1	0.147	0.184	**30.0**	**30**	**30.0**
63flm	deltamet	181	48,3	2,5	6	4.6	0	1	16.0	0.4	1	1	1	0.013	0.005	**13.0**	**13**	**13.0**
26tll	difluben	148	31,15	89,5	4	3.89	150	2	700	1.5	1	0	1	0.147	0.184	**20.0**	**20**	**20.0**
27tll	difluben	148	31,15	89,5	4	3.89	15	3	700	1.5	1	0	1	0.147	0.184	**30.0**	**30**	**30.0**
38tll	gluf_amm	83	46,5	84,07	-	0.1	291	2	16.0	0.9	1	N	1	415295	56000	**200.0**	**2000**	**200.0**
57tll	Diazinon	134	38,58	95,14	3	3.3	7	4	11.2	1.3	1	1	1	0.028	0.0027	**9.60**	**17.2**	**9.60**
84tll	cyfluthr	174	33	100	5	6	14	11	1.90	1	1	N	1	0.070	0.014	**4.116**	**13.13**	**3.20**
85tll	permethr	135	43,6	79,3	5	6.1	0	1	120	4.8	1	0	1	0.386	0.0325	**0.50**	**0.50**	**0.50**
106tll	Lindane	158	48	12	-	-	14	3	1.00	0.8	1	N	1	2.928	1.80	**196.60**	**11.06**	**196.6**
109tll	esfenval	125	46,45	92,07	3.72	6.22	30	2	33.0	1.1	1	1	1	0.178	0.024	**0.40**	**0.40**	**0.40**
112tll	permethr	92	36,02	140,0	5	6.1	18	2	2.70	3.5	1	0	1	0.386	0.032	**3.0**	**3**	**20.0**
117tll	Atrazine	153	32,43	97,17	2	2.5	0	1	5.50	2	0	0	1	19.87	2.60	**200**	**20**	**20.0**

Exp.	Pesticide	Day number	Latitude	Longitude	Log K_D	Log K_{OW}	Dosing interval *d*	Number of additions	Volume *m³*	Depth *m*	Sediment	Macrophytes	Field/ Lab	$HC_{5,50}$	EC50/10 (OECD)	Mesocosm LOEC µg l^{-1}		
																Cladoce	Copepo	Rotifer
119tll	Atrazine	133	32,43	97,17	2	2.5	0	1	5.50	2	0	0	1	19.87	2.60	**250**	**250**	**250**
120tll	bifenth	133	32,43	97,17	5.38	6	0	1	5.50	2	0	0	1	0.038	0.007	**0.020**	**0.02**	**0.02**
44flm	methoxyc	129	43,6	79,3	4.9	-	0	1	100	4	1	0	1	0.092	0.078	**5.0**	**5**	**.**
47flm	esfenval	139	31,5	89,5	3.72	6.22	17	5	700	1.5	1	1	1	0.178	0.024	**1.28**	**1.28**	**.**
50flm	chlorpyr	54	L	L	3.78	4.6	0	1	0.70	0.7	1	1	0	0.044	0.0077	**5.0**	**35**	**.**
59flm	esfenval	106	32	87	3.72	6.22	10	8	1100	1	1	1	1	0.178	0.024	**.**	**0.24**	**3.60**
64flm	Lindane	181	48,3	2,5	-	-	0	1	10.0	0.4	1	1	1	2.928	1.80	**.**	**321**	**321.0**
123flm	lamb_cyh	144	51	0	5.26	7	14	4	25.0	1	1	1	1	0.080	0.0117	**0.68**	**0.68**	**.**
28tll	chlorpyr	129	46,45	92,07	3.78	4.6	0	1	25.0	0.5	1	1	1	0.044	0.0077	**0.50**	**0.50**	**.**
83tll	cyfluthr	174	33	100	5	6	14	11	634.7	1.3	1	N	1	0.070	0.014	**.**	**2.625**	**3.20**
111tll	permethr	106	36,02	140,04	5	6.1	14	2	2.70	3.5	1	0	1	0.386	0.032	**1.50**	**1.50**	**.**
118tll	bifenth	153	32,43	97,17	5.38	6	0	1	5.50	2	0	0	1	0.038	0.007	**0.02**	**0.02**	**.**

Table 9. Predictability (Q^2(cum)) of the examined PLS models for zooplankton. The procedure for removal of outliers is explained in Section 5.1.

Data included	Outliers	Q2(cum)
All experiments for stagnant water	experiment 38tll	0.346
All experiments with sediment	experiment 38tll	0.239
All experiments with macrophytes	none	0.596
All experiments with insecticides	none	0.206
All experiments with a single addition of pesticides	experiment 120tll, 64flm and 118tll	0.233
All experiments with sediment and a single addition of pesticides	experiment 64flm	0.424
All mesocosms experiments	experiment 38tll	0.454
All mesocosms experiments with sediment	experiment 57flm, 38tll, 106tll and 64flm	0.450
All mesocosms experiments with insecticides	experiment 106tll and 64flm	0.184
All mesocosms experiments with single addition	none	0.655
All mesocosms experiments with single addition restricted to insecticides	none	0.736

5.5.1 Interpretation of the PLS model for zooplankton.

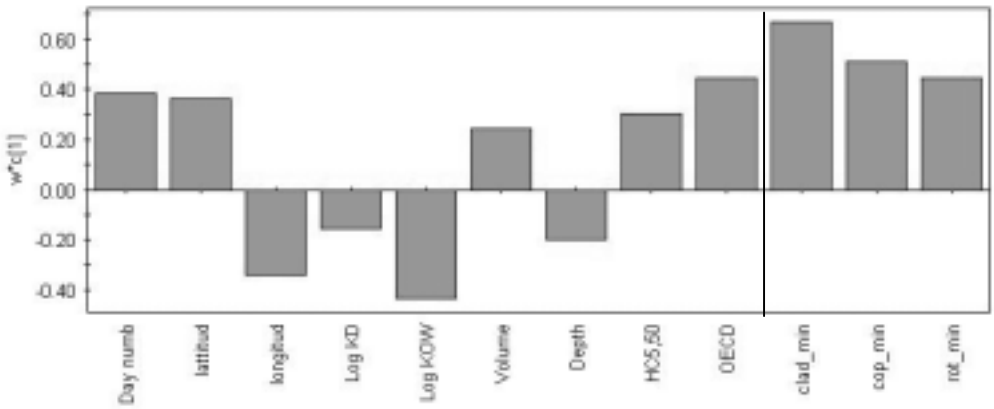
The prediction of the model selected in the previous section is shown Table 10.

Table 10. Predicted variation of the significant axis of the PLS model selected for zooplankton. Q^2: Variation in the Y matrix predicted from the variation in the X matrix by the current axis. Q^2(cum): Cumulative variation in the Y matrix predicted from the variation in the X matrix.

PLS axis number	Q^2	Q^2(cum)
1	0.736	0.736
2	-0.053	0.736

As shown in Table 10 the first PLS axis predicts 73.6 % of the variation in the Y matrix from the variation in the X matrix. The second PLS axis predicts 0 % of the variation in the Y matrix from the variation in the X matrix. Hence, the second axis does not contribute to the overall predictability of the model and an interpretation of the second axis was therefore not carried out.

Figure 11. Weights (loadings) of variables contributing to the first PLS axis for zooplankton. Day number through OECD represent variables in the X-matrix while the responses (LOEC) of the different zooplankton groups are shown at right.



From the loadings of the different variables (see Fig. 11) to the PLS axis it appears:

The axis primarily represents a ”traditional” toxicity axis with positive correlations between hazard concentrations ($HC_{5,50}$ and LC50/10 = OECD) and LOEC obtained in the mesocosms. In specific:

- Loadings were most positive for the cladocerans, least positive for the rotifers and intermediate for the copepods. Hence, cladocerans semingly are the most sensitive zooplankters to *insecticides* followed by copepods and rotifers.
- Positive loadings were obtained for the variables Day number and latitude, whereas a negative loading was obtained for longitude. Thus *insecticides* seem to be less toxic to the zooplankton (i.e. high LOEC) if experiments are conducted in cold climates (high latitude) and/or during in the summer (high Day #). The effect of latitude is in contradiction to the effect of climate on macroinvertebrates, but could be due to a higher activity of zooplankters and thus exposure to pesticide at higher temperatures. On the other hand, the negative correlation between Day# and LOEC, does not support such relationship. The negative loading for Longitude suggests that zooplankton in experiments conducted in USA are more sensitive than the zooplankton in experiments conducted in Europe. This is in contradiction to the response of macroinvertebrates. As for macroinvertebrates the deviation between European studies and studies carried out in USA could be related to the stocking of fish in enclosures in USA.
- For the variables expressing hazard concentrations ($HC_{5,50}$ and EC50/10 = OECD) positive loadings were obtained, whereas a negative loading was obtained for log K_{OW}. Hence, as expected hydrophobic substances characterised by high single species toxicity seems to be most toxic to the zooplankters in the mesocosmos.

A summary of the effect of experimental mesocosm and pesticide characteristics on response of zooplankton is shown in Table 11.

The arrows in Table 11 indicate if numeric increases in system variables (see Table 2) will decrease (↑ = high LOEC) or increase (↓ = low LOEC) the toxic response in the different groups of zooplankton.

Table 11. Summary of influences of mesocosm characteristics, pesticide characteristics and toxicology (extrapolated effect concentrations) on toxic response on zooplankton. ↑ = decrease in toxicity (i.e. higher LOEC); ↓ = increase in toxicity (i.e. lower LOEC);
- = no effect. See Table 2 for an explanation of system variables.

Zooplankt. group	Day#	Lati-tude	Longi-tude	Log K_d	Log K_{ow}	Volu-me	Depth	$HC_{5,50}$	LC50 /10
Cladocera	↑	↑	↓	-	↓	(↑)	-	↑	↑
Copepoda	↑	↑	↓	-	↓	(↑)	-	↑	↑
Rotifera	↑	↑	↓	-	↓	(↑)	-	↑	↑

5.5.2 Predicting the effect concentrations for the zooplankton with the aid of the PLS model

The observed and predicted effect concentrations with associated 95 % confidence interval for the mesocosmos experiment calculated with the PLS model for zooplankton are shown in Table 12 and Figure 12. When the lower limit of the confidence intervals was below 0 the lower limit of the confidence interval was set to 0 (see section 5.4.2).

Figure 12. Plots between observed LOEC (µg L^{-1}) and LOEC predicted by PLS model for zooplankton (Cladocera, Copepoda & Rotifera). X=Y (stippled line) shown.

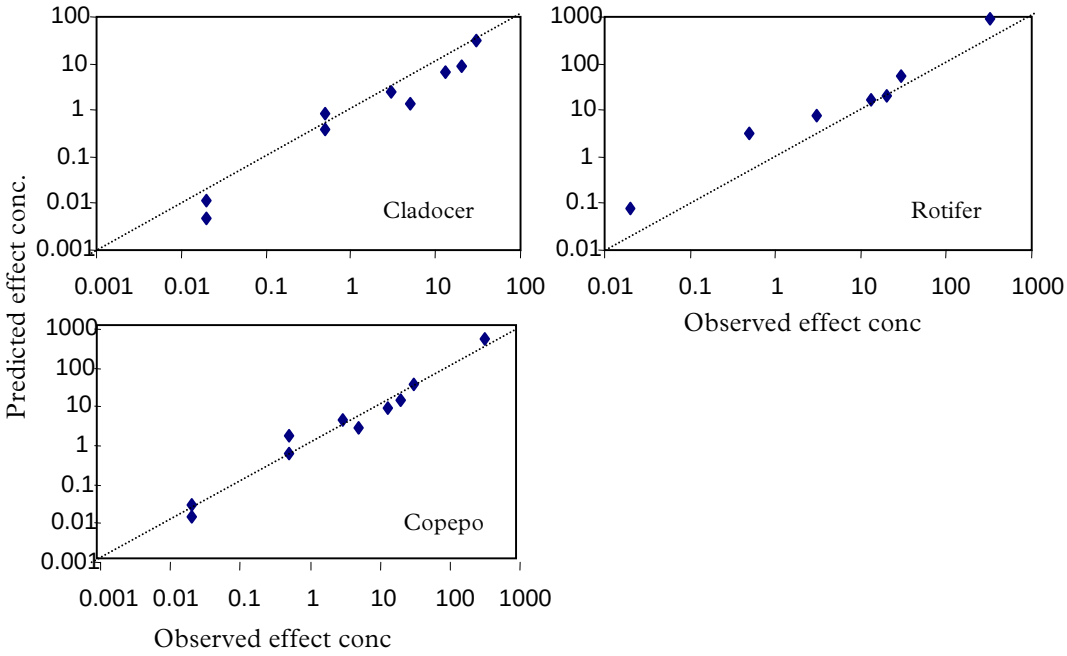


Table 12. Comparison of observed and predicted effect concentrations with associated 95 % confidence interval for the mesocosmos experiment calculated with the PLS model for zooplankton. -- = no observation. See Annex B for references.

Exp.	Pesticide	Cladocera		Confidence interval		Copepoda		Confidence interval		Rotifera		Confidence interval	
		Observ	Pred.	Low	Upp	Observ	Pred.	Low	Upp	Observ	Pred.	Low	Upp
43flm	methoxyc	3.0	2.46	0.35	5.76	3.0	4.80	1.99	8.52	3.0	7.59	0	22.2
45flm	methoxyc	20.0	8.50	0.64	21.41	20.0	14.52	5.30	27.1	20.0	19.29	0	61.57
58flm	difluben	30.0	30.5	5.34	69.07	30.0	35.93	15.8	62.3	30.0	53.44	0	150
63flm	deltamet	13.0	6.41	1.83	12.95	13.0	9.76	5.08	15.6	13.0	16.18	1.71	39.4
85tll	permethr	0.5	0.80	0.18	1.73	0.5	1.71	0.81	2.86	0.50	3.30	0.11	8.70
120tll	bifenth	0.02	0.01	0	0.02	0.02	0.02	0.003	0.033	0.02	0.08	0	0.33
44flm	methoxyc	5.0	1.37	0.22	3.15	5.0	2.84	1.22	4.98	--	4.88	0	14.0
64flm	Lindan	--	1074	0	5088	321	566	0.0	1661	321	866	0	5989
28tll	chlorpyr	0.5	0.37	0.03	0.92	0.50	0.60	0.23	1.11	--	2.02	0	6.35
118tll	bifenth	0.02	0.01	0	0.03	0.02	0.03	0.01	0.06	--	0.14	0	0.53

Generally, within the observed interval the effect concentrations predicted by the PLS model were in excellent agreement with the observed effect concentrations for both cladocerans and copepods, while the PLS model tended to over-estimate effect concentrations for rotifers. Such deviation could be expected, however, as effects of *insecticides* on rotifers primarily was of indirect nature (i.e. increases in abundance due to reduced competition from crustecean zooplankters).

5.6 PLS models for microalgae

An overview of raw data from the database used in the PLS models developed for microalgae is shown in Table 13. In total only 9 mesocosm experiments with toxicity data for microalgae were available from the data base. Thus it was only possible to consider the scenarios including either all experiments or all mesocosm experiments carried out in the field. Of these two scenarios the highest predictability (0.721) was obtained for the scenario of field mesocosm experiments (Table 14).

Table 14. Predictability of the examined PLS models for microalgae

Data included	Outliers	Q^2(cum)
All experiments	none	0.671
Field Mesocosm experiments	none	0.721

5.6.1 Interpretation of the PLS model for microalgae

For the selected PLS model with micro algae only one significant PLS axis was present (Table 15).

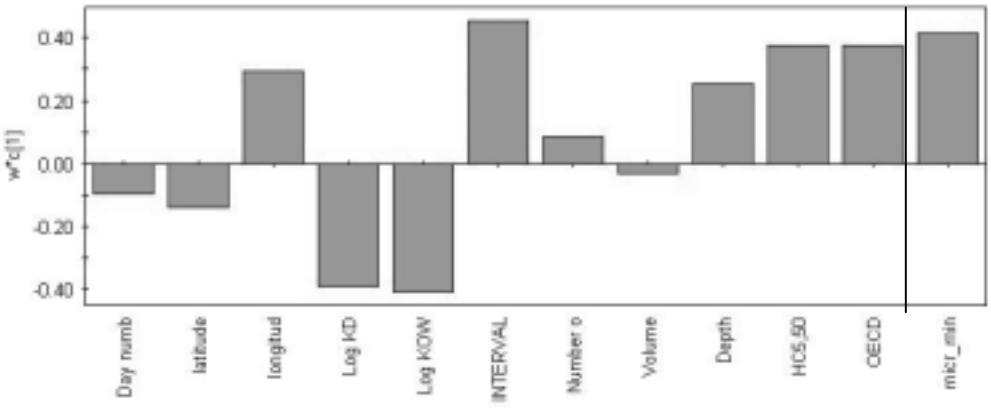
Table 15. Predicted variation of the significant axis of the PLS model selected for micro algae. Q^2: Variation in the Y matrix predicted from the variation in the X matrix by the current axis. Q^2(cum): Cumulative variation in the Y matrix predicted from the variation in the X matrix.

PLS axis number	Q^2	Q^2(cum)
1	0.721	0.721

From the plots of loadings and of variable importance the following interpretation of the PLS axis of the PLS model for micro algae is conducted:

- The highest (and positive) loading was found for the X variable Interval (between pesticide dosings). Thus, pesticides added over a short period are most toxic to the algae in the mesocosmos. This is probably related to the short generation time of microalgae: frequent dosings will prevent microalgae to recover, while one or less frequent dosings will allow the microalgae to recover, when the pesticide dissipates.
- Positive loadings were seen for the X variables expressing the extrapolated hazard concentrations ($HC_{5,50}$ and EC50/10 = OECD procedure), whereas negative loadings were seen for the variables log K_D and log K_{OW}. Hence, hydrophobic and adsorpable substances with high single species toxicity were most toxic to the micro algae in the mesoscosmos.

Figure 13. Weights (loadings) of variables contributing to the PLS axis for microalgae.



5.6.2 Predicting the effect concentrations for the micro algae with the aid of the PLS model

The observed and predicted effect concentrations with associated 95 % confidence interval for the mesocosmos experiment handled with the PLS model for micro algae appear is shown in Table 16 and Fig.14. When the lower limit of the confidence intervals was below 0 the lower limit of the confidence interval was set to 0 (see section 5.4.2).

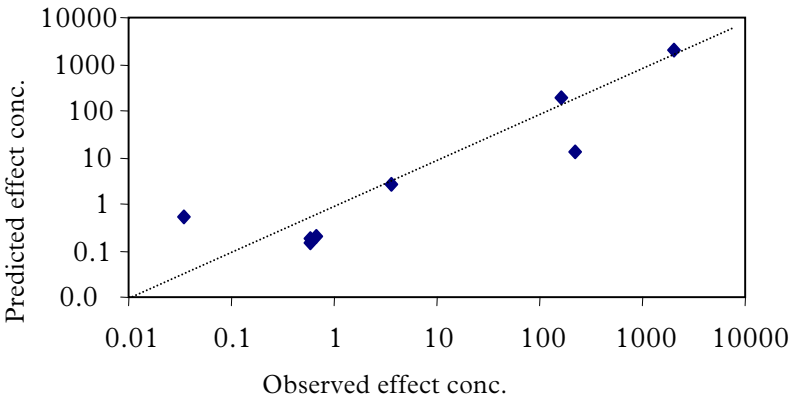
Table 16. Comparison of observed and predicted LOEC's (µg L^{-1}) with associated 95 % confidence interval for the mesocosmos experiment calculated with the PLS model for microalgae.

Exp.	Pesticide	Microalgae		Confidence interval	
		Observ	Pred.	Lower	Upper
16ank	Atrazin	225	13.134	0	37.23
59flm	esfenval	3.60	2.665	0	7.595
121flm	fenpropi	0.60	0.176	0	0.712
122flm	fenpropi	0.58	0.151	0	0.629
123flm	lamb_cyh	0.68	0.195	0	0.778
38tll	gluf_amm	2000	1954.7	0	11728
72tll	Atrazin	160	180.86	0	710.5
113tll	esfenval	0.035	0.5381	0	1.826

Table 13. Overview of raw data from the database for the PLS models developed for microalgae (phytoplankton & periphytes). See Table 2 for abbreviations used. For Sediment 1 refer to sediment present in mesocosm, 0 to no sediment; Macrophytes: 1 = present, 0 = no macrophytes; Field/lab: 1 = Field study, 0 = Laboratory study. For all microalgal groups tested the lowest effect concentrations observed in each mesocosms experiment were used as Y variables, expressing the toxic response of the organisms in the mesocosms (values shown in bold). L = Laboratory study at controlled temperature and light availability (hence latitude and longitude not relevant); - = no data. See Annex B for literature references.

Exp.	Pesticide	Day number	Latitude	Longitude	Log K_D	Log K_{OW}	Dosing interval *d*	Number of additions	Volume m^3	Depth *m*	Sediment	Macrophytes	Field/Lab	$HC_{5,50}$	EC50/10 (OECD)	Micr_min
16ank	Atrazin	84	48,15	11,34	2	2.5	26	3	1.00	0.8	1	-	1	19.87	2.6	**225.0**
55flm	Linuron	110	L	L	2.6	3	3	10	0.60	0.5	1	1	0	19.67	5	**516.7**
59flm	esfenval	106	32	87	3.72	6.22	10	8	1100.0	1	1	1	1	0.178	0.024	**3.60**
121fl	fenpropi	173	48,23	11,44	-	-	14	2	0.70	0.7	1	1	1	-	-	**0.60**
122fl	fenpropi	180	47	8,0	-	-	14	2	20.0	0.7	1	1	1	-	-	**0.58**
123fl	lamb_cyh	144	51	0	5.26	7	14	4	25.0	1	1	1	1	0.080	0.0117	**0.68**
38tll	gluf_amm	83	46,5	84,07	-	0.1	291	2	16.0	0.9	1	-	1	415295	56000	**2000**
72tll	Atrazin	151	43,6	79,3	2	2.5	223	2	120.0	4.8	1	0	1	19.87	2.60	**160.0**
113tll	esfenval	68	56,5	10	3.72	6.22	0	1	36.0	0.5	1	-	1	0.178	0.024	**0.035**

Figure 14. Plots between observed LOEC (µg L^{-1}) and LOEC predicted by PLS model for microalgae (phytoplankton + periphytes). X=Y (stippled line) and linear regression equation shown.



Within the observed interval the effect concentrations predicted by the PLS model were in excellent agreement with the observed effect concentrations for microalgae.

5.7 Summary of PLS models

The amounts of data available for the different communities were quite variable and a direct comparison of PLS models should therefore be conducted with caution.

Macroinvertebrates
To obtain a PLS model with a reasonable predictability of the toxic effects to various macroinvertebrate groups, mesocosms should contain sediment and preferably macrophytes in the test system. Overall, the model developed was able to predict 63 % of the observed effects among macroinvertebrates.

In summary, the PLS analysis showed that

5. All macroinvertebrate groups in the mesocosms seem to be most sensitive when the experiments are conducted at high latitudes. Therefore, toxic effects at lower concentrations are expected with increasing distance from Equator, which may be due to a slower turn-over of populations at high latitudes, i.e. fewer generations each year at lower temperatures. Therefore, recovery of populations affected by pesticide exposure takes longer time at northern latitudes.
6. Macroinvertebrates living within the sediment (i.e. infauna) were less sensitive the pesticides than macroinvertebrates living on the sediment surface.
7. At a given total dose the effect of pesticides decreases with number of pesticide additions. Therefore, a low but persistent pesticide concentration will have a lower effect on the macroinvertebrates than a high but temporary pesticide concentration.
8. The toxic effects of pesticides are most pronounced in shallow mesocosms. At decreasing mesocosm depth a larger fraction of the

pesticides will end up in the sediment compartment and thus increase the exposure to the sediment living macroinvertebrates. This interpretation is further reinforced by the inverse relation between Log K_D of pesticides and toxicity to invertebrates.

Zooplankton

The PLS model with the highest predictability for zooplankton was obtained when *the pesticides were applied as single addition* and the analysis was restricted to *insecticides* only.

The PLS analysis showed that

3. Hydrophobic *insecticides* with high single species toxicity were the most toxic to the zooplankters in the mesocosmos.
4. Cladocerans were the most sensitive group to *insecticides* followed by copepods and rotifers.
2. The effect of climate zone (latitude) and season was contradictory, as the highest sensitivity was obtained at low latitudes but outside the summer months.

Microalgae

The highest predictability of pesticide effects to microalgae was obtained when only field mesocosm experiments were included in the analysis.

The PLS analysis showed that

3. Hydrophobic and adsorpable pesticides with high single species toxicity were the most toxic to the micro algae in the mesoscosmos.
4. At a given total dose pesticides added over a short period were more toxic to the algae in mesocosmos than pesticides dosed at longer intervals. Frequent dosings will prevent microalgae to recover, while microalgae characterised by short generation times will be able to recover in between dosings applied at longer intervals.

6 Effect of pesticides in mesocosms

The previous PLS analysis was carried out at a rather high level of taxonomy and organism functionality to satisfy the requirement of data abundance within each group. In effect, detailed information on specific effects of pesticides and differences in sensitivity among different taxonomic groups have not been dealt with. In the following the specific effects (mortality, changes in abundance and sublethal effects) of individual *herbicides* and *insecticides* to different taxonomic groups within the major groups (microalgae, zooplankton and macroinvertebrates) are evaluated. In contradiction to the PLS analysis the evaluation has encompassed all mesocosm studies contained in the data base (see Annex B).

6.1 Phytoplankton and microalgae

The *insecticide* investigations contained in the data base have not demonstrated any directly significant effects such as reduced phytoplankton abundance at the prevalent *insecticide* concentrations. Therefore, the currently available data do not allow us to determine the maximum permissible insecticide-associated reduction that a phytoplankton population may suffer without becoming extinct. On the contrary it can be concluded that compared with zooplankton and benthic invertebrates, higher concentrations must prevail before a reduction in phytoplankton abundance occurs. This implies that for *insecticides* the various zooplankton and also invertebrates are affected before the phytoplankton community is directly affected.

A total of 193 records on effects of *herbicides* on algae (including phytoplankton, epibenthic microalgae and filamentous algae) were distributed between the following end-points LOEC: 83; NOEC: 101 and EC50: 9. We have not attempted to discriminate between phytoplankters and epibenthic algae as their environment (pelagic or benthic) especially in the shallow mesocosms will change rapidly according to mixing conditions. For filamentous algae the number of records was low which excludes a specific analysis. In line with zooplankton and macroinvertebrates structural parameters dominate the effect measures (abundance and biovolume by cell counts, biomass as fresh or dry weight for filamentous algae, chlorophyll a for microalgae). Primary production estimated by oxygen production or 14-C fixation was measured in two experiments (9 records).

In the data base direct effects of herbicides on algae were examined and quantified by relating the dosing of herbicide to changes in abundance relative to corresponding controls (without herbicide dosing). Mesocosm studies with herbicides ranged in duration between 14 and 373 days. Except for one study LOEC's were recorded during or shortly after termination of herbicide exposure. Hence, most of the data presented below are from this initial period. In the following the relative sensitivity of algae to 3 different herbicides is visualised in diagrams showing LOECs and numeric changes in abundance (Figs. 15-17).

Figure 15. Summary of effects of Alachlor on abundance of different microalgal species. Tests were carried out in recirculating flumes (175 l) in laboratory dosed at 5 concentrations (1-150 µg l^{-1}). Samples were taken 5 times during 3 weeks. Position of bars along the concentration axis refer to LOEC for the different groups/species. Numbers shown along bars denote decrease (-) or increase in abundance (in %) of corresponding controls. As a comparison the hazard concentration ($HC_{5,50\%}$) calculated from distribution based extrapolation of single species toxicity data for Alachlor was 0.73 µg l^{-1} (see Table 1).

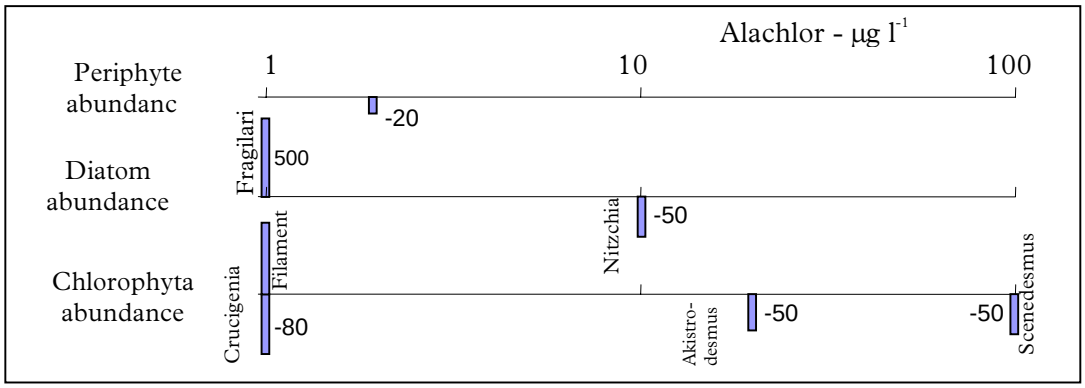


Figure 16. Summary of effects of Atrazine on different microalgae. A: recirculating microcosms in laboratory. B: primary production in phytoplankton in large mesocosms (470 m^3) dosed once (regular samplings: 39-374 days). C: 1 m^3 mesocosms dosed 3 times during 52 days. D: 120 m^3 mesocosms dosed twice during 36 days. Biomass was reduced even 280 days after the last application. E: 120 m^3 mesocosms dosed twice during 223 days. Periphytes recovered within 7 weeks; phytoplankton still reduced after 7 weeks. F: recirculating microcosms in laboratory. Numbers shown along bars denote decrease in abundance, concentration or primary production (in %) compared to corresponding controls. As a comparison the hazard concentration ($HC_{5,50\%}$) calculated from distribution based extrapolation of single species toxicity data for Atrazine was 19.9 µg l^{-1} (see Table 1).

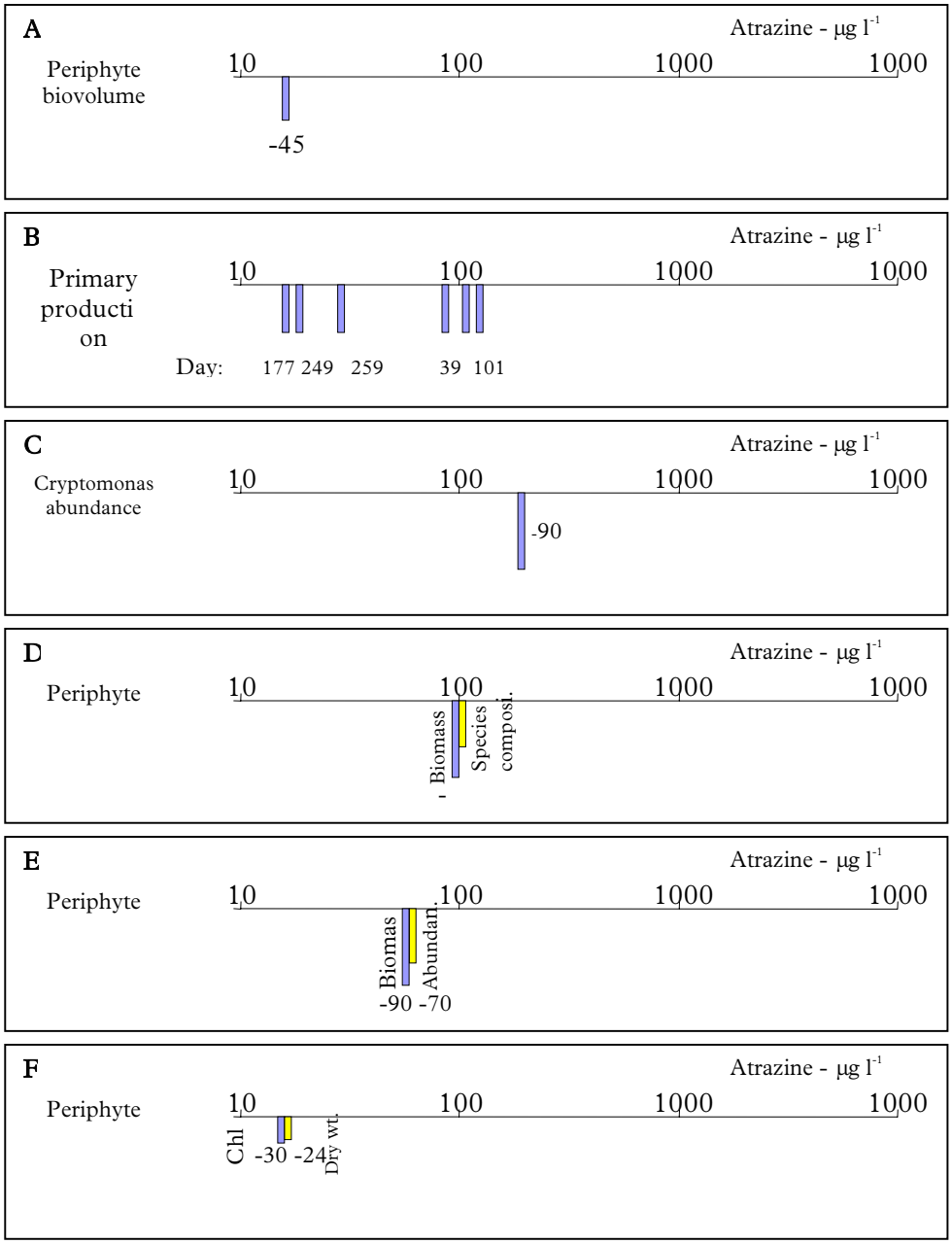
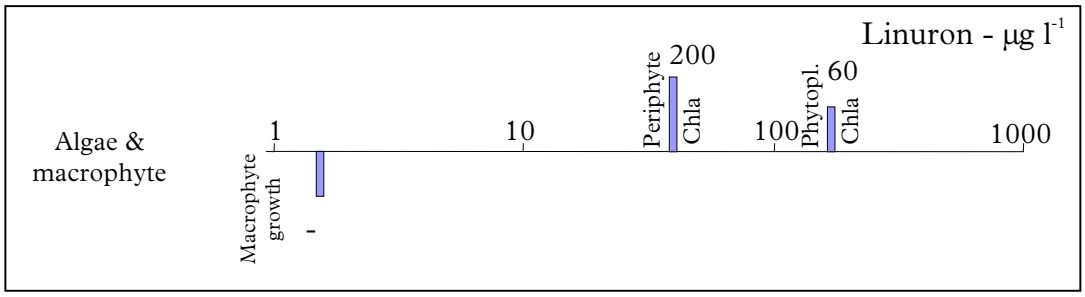


Figure 17. Summary of effects of Linuron on microalgae (abundance) and macrophytes (*Elodea nuttalii*, growth, EC50). Tests were carried out in laboratory mesocosms (600 l) dosed almost continuously through 28 days at 5 concentrations (0.5 – 150 µg l^{-1}). Numbers shown along bars denote decrease (-) or increase (%) in abundance or growth of corresponding controls. As a comparison the hazard concentration ($HC_{5,50\%}$) calculated from distribution based extrapolation of single species toxicity data for Linuron was 19.7 µg l^{-1} (see Table 1).



On the basis of these comparisons it is evident that:

1. The effect of herbicides on the different algal groups (and macrophytes) is very variable, with both reductions and increases occurring within one systematic group (Fig. 14). For Alachlor increases were observed only at the lowest test concentration (1 µg l^{-1}).
2. Atrazine as the mostly studied herbicide consistently led to reductions in algal biomass (Fig. 16). Generally, the effects were rather persistent in accordance with the slow dissipation of Atrazine, e.g. in one study primary production was impaired more than one year after application (Fig. 16 B).

In a study with a mixture of Atrazine, Diuron and Alachlor, some of the most sensitive species were *Cyanophytae* filaments and *Monoraphidium* sp. demonstrating inhibited growth. *Cryptomonas* sp., *Chlorophyceae coccales*, *Diatoma* sp. (single cell) and *Scenedesmus* sp. were less adversely affected, while the growth of *Chlamydomonas* sp. and *Stephanodiscus* sp. was stimulated. Several species were virtually unaffected by herbicides, e.g. pennate diatoms, *Cyanophytae coccales* and *Anabaena* sp.

Whether the phytoplankton can recover after a herbicide-related reduction is difficult to conclude from the mesocosm studies contained in the data base. In one study with Atrazine dosed at 100 µg l^{-1}, primary production was not fully recovered even one year after the application, while in a comparable study (80 µg l^{-1}) periphyton biomass and species composition recovered within 49 days. For Alachlor, almost full recovery was attained within 3 weeks for most algal groups except at the highest concentration tested (1000 µg l^{-1}). However, based on the dynamics of the phytoplankton communities observed in lakes, phytoplankton seem capable of recovering even after a pronounced reduction. It is a well-known phenomenon that some phytoplankton species may disappear from lakes for several years, only to reappear when growing conditions improve.

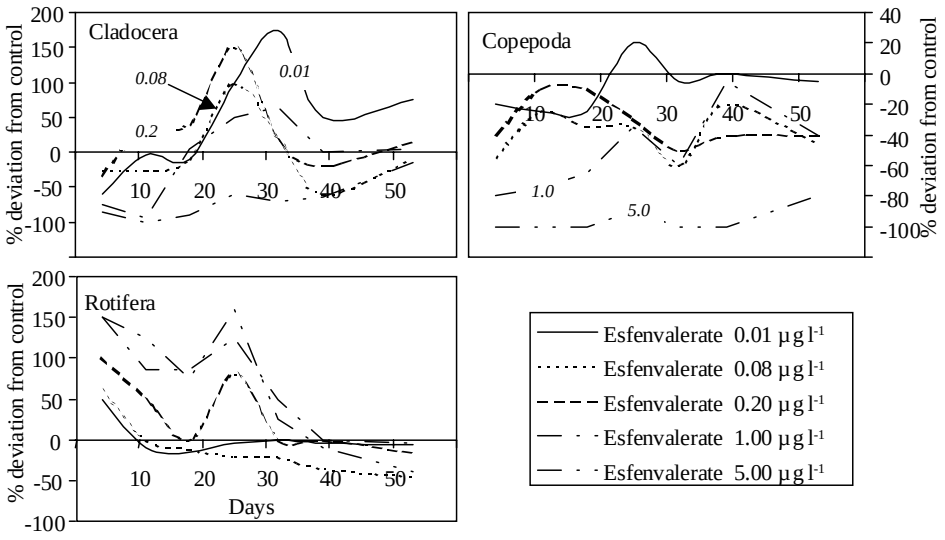
6.2 Zooplankton

Eighteen studies in the data base have examined the effects of *herbicides*. None of these studies have demonstrated direct effects on zooplankton.

The data base contains a total of 43 individual mesocosm experiments with zooplankton effect concentrations distributed among 18 different *insecticides*. A total of 1564 records on effects of *insecticides* on zooplankton were distributed between the following end-points LOEC: 706; NOEC: 817 and EC50: 14. The majority of records concern Copepods (674 records), Cladocerans (543 records) and Rotifers (279 records), while unspecified zooplankton records amounts to 32. Abundance of individuals is by far the most used effect parameter (1549 records), while species diversity and biomass are scarce at 13 and 2 records, respectively.

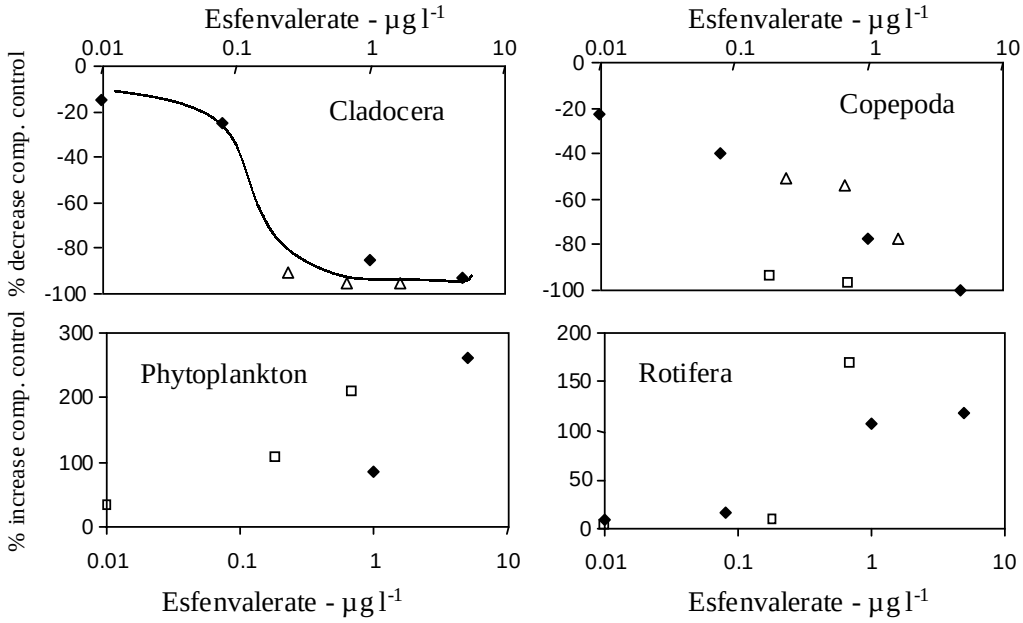
In the data base direct effects of insecticides on zooplankton were examined and quantified by relating the dosing of insecticides to changes in abundance relative to corresponding controls (without insecticide dosing). To include results from both single and multiple pesticide application experiments the average decrease in abundance within the period 3-14 days after the first application of insecticide was used. By this approach studies with single and multiple applications could be compared and bias due to different recovery was eliminated. In cases where sufficient data were available EC50 was calculated after probit transformation.

Figure 18. Temporal variation in abundance (% of control) of zooplankton following a single dose of esfenvalerate in 5 different concentrations (µg l^{-1})to mesocosms (after Lozano et al. 1992).



In Fig. 18 is shown an example of the temporal variation in abundance of Cladocera, Copepoda and Rotifera after a single dose of esfenvalerate. Both the initial impact and the subsequent recovery were dependent on the dose. By combining 2-3 different mesocosm studies distinct dose-response curves can be established (see Fig. 19). Noticeable features are dose dependent decreases in Cladocera and Copepoda and increases in phytoplankton and Rotifera.

Figure 19. Dose-response relations of plankters in mesocosms exposed to esfenvalerate. Mesocosms were shallow (0.5-1.1 m depth), had sediment and macrophytes and ranged between 25 – 1100 m^3 in volume (from Fairchild et al. 1992;Lozano et al. 1992, Webber et al. 1992).



Overall, during the first 1-2 weeks after insectide application Cladocerans were more sensitive to insecticides than Copepoda, even though large variation were evident in the data (see Figure 20). Within the copepod population nauplii were more sensitive (≈ 10%) than adult copepods (see Figure 21). Besides, the variation in the plot was limited reflecting that the two groups probably represent the same species within each experiment.

Figure 20. Scatter plot of decreases in abundance of Cladocera and Copepoda 3-14 days after insecticide application (Diflubenzuron; Methoxychlor, Hexazinon, Chlorpyrifos, Esfenvalerat, Deltamethrin, Permethrin, Bifenthrin). Regression line and X=Y shown (stippled). Decrease in Cladocera was significantly larger (i.e. Cladocera being more sensitive) than corresponding decrease in Copepoda (Kolgomorov-Smirnov test).

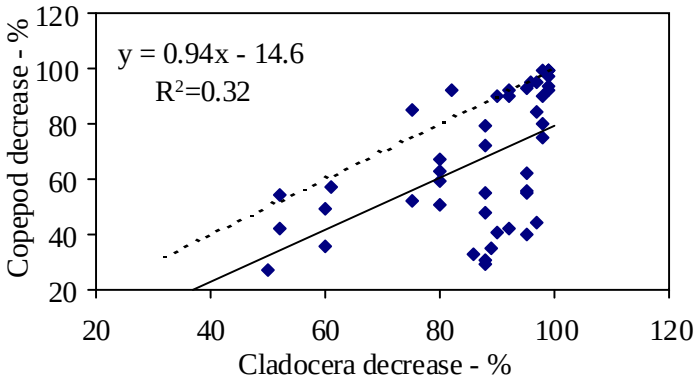
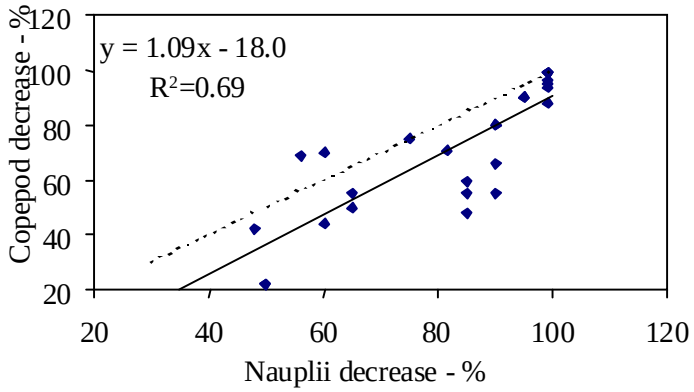
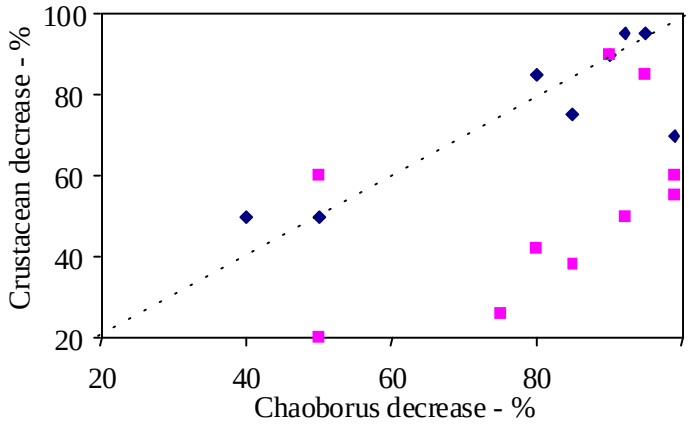


Figure 21. Scatter plot of decreases in populations of Nauplii and adult Copepoda after insecticide application (Fenvalerat, Methoxychlor, Chlorpyrifos, Esfenvalerat, Deltamethrin, Permethrin, Bifenthrin, Trahalomethrin). Regression line and X=Y shown. Only reductions lower than 100% were included. Decrease in Nauplii was significantly larger (i.e. Nauplii being more sensitive) than decrease in adult Copepoda (Kolgomorov-Smirnov test).



The larval stage of the dipteran Chaoborus is an important pelagic predator in lakes and ponds. In 6 mesocosm studies the abundance of Chaborus was sufficient to calculate the impact of insecticides and compare its sensitivity to Cladocera and Copepoda (Figure 22). In these studies representing different classes of insecticides Chaoborus consistently was more sensitive than Copepoda, but had a sensitivity similar to Cladocera's.

Figure 22. Scatter plot of decreases in abundance of Chaoborus and crustacean zooplankton after insecticide application (Chlorpyrifos, Permethrin, Lindan, Methoxychlor). X=Y shown. Only reductions lower than 100% were included. Decrease in Chaoborus was significantly larger (i.e. Chaoborus was more sensitive) than decrease in adult Copepoda (■) but identical to impact on Cladocera (◆) (Kolgomorov-Smirnov test).

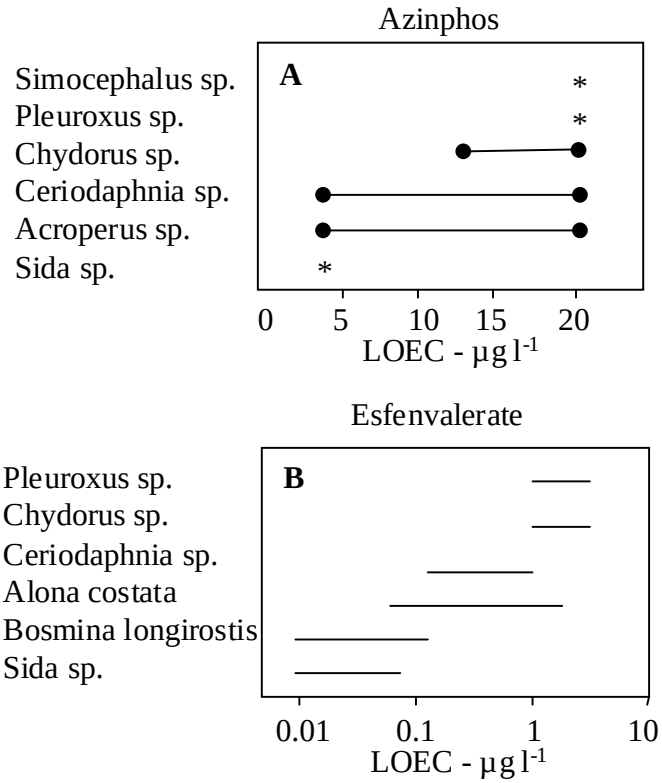


Hitherto, effects have been evaluated at the Order level (e.g. Cladocera) as dictated by the level of taxonomy reported in the majority of mesocosm studies. This invariably will lead to variation in the aggregated data in case of

interspecies differences in sensitivity (see Figure 20). Two studies allow extracting quantitative information on variability in sensitivity within Cladocera.

In mesocosms exposed to Azinphos-methyl LOEC ranged between 4 and 20 µg l^{-1} while for esfenvalerate the observed range in LOEC was markedly wider at 0.01-5 µg l^{-1} (Figure 23). In both studies *Sida* was the most sensitive genus and *Pleuroxus* the least sensitive. The difference may be related to size and habitat of species. For comparison the calculated Hazard Concentration of esfenvalerate to Cladocera is 0.18 µg l^{-1} and 0.02 µg l^{-1} for $HC_{5,50}$ and $OECD_{10}$, respectively (see Table 1). The range in LOEC for different species within Copepoda varied between 0.08 – 5 µg esfenvalerate l^{-1}.

FIGURE 23. LOWEST OBSERVED EFFECT CONCENTRATION FOR DIFFERENT CLADOCERAN SPECIES IN MESOCOSMS EXPOSED TO AZINPHOS-METHYL (A) AND ESFENVALERATE (B); (*) ALL OBSERVATIONS WERE IDENTICAL, (• — •) RANGE OF LOEC RECORDED DURING EXPOSURE.



In summary, mesocosm studies have demonstrated that zooplankters are very sensitive to insecticide exposure. At the group level:

1. Cladocerans and Chaoborus are the most sensitive followed by copepod nauplii and adult Copepoda.
2. At a given concentration the Cladoceran population on average will show larger reductions (20 %) than the copepod population (based on regression analysis).
3. Copepod nauplii on average will show 10 % larger reductions than the adult population and observed reductions in one group are very good predictors of the reductions of the other group.

The variation in sensitivity within each zooplankton group as demonstrated in mesocosm studies is considerable. For esfenvalerate LOEC varied 2.5 orders of magnitude for the different species among cladocerans. This variation is probably related to the size of the different species, their habitat and/or feeding mode.

6.2.1 Statistical power of impact of insecticides on zooplankton in mesocosm experiments

Overall, the statistical power in the mesocosm studies was rather low. The average reduction in abundance of zooplankters (i.e. excluding indirect effects) exposed to insecticides at recorded LOEC's was 75.4 % (± 21.3 %; SD). The low power is due to low number of replicates, low number of and/or large range in test concentrations. The use of few test concentrations spanning 2-3 orders of magnitude invariably will lead to crude estimates of LOEC.

In Table 17 is shown the distribution of reductions in abundance of zooplankton at the various combinations of replicate number and number of test concentrations applied in the different studies. The different combinations are based on observations ranging from 6 to 124 in number and from 1 to 4 different studies carried out at different locations and using different mesocosms (volume, ± macrophytes etc.). Hence, conclusions drawn should not be too firm. Still, the data suggest that in order to obtain a sufficient resolution and sensitivity the experimental design should be a hybrid approach encompassing more than 4 test concentrations and at least two replicates at each concentration. As the size of experimental design usually is constrained by economy with a maximum number of units of 15-16 (see Table 17) based on the results shown in Table 17 they should be distributed between 5 (8) test concentrations each with 2 (3) replicates. Still, to achieve a sufficient sensitivity the range in concentrations applied should not be unduly large, i.e. less than 2.5-3 orders of magnitude.

Table 17. Average reduction (%)±SD in zooplankton abundance at LOEC in mesocosm studies of different experimental design. Number of observations in brackets. – # observations below 5.

Number of replicates	Number of insecticide levels						
	2	3	4	5	6	7	8
1.5*	-	-	-	-	-	-	71±15 (15)
2	78±22 (24)	-	-	53±10 (73)	-	-	64±24 (19)
3	94±9 (124)	82±12 (9)	79±15 (10)	56±14 (29)	-	-	-
4	81±21 (18)	78±23 (6)	-	-	-	-	-

* 2 replicates in control and one replicate per test concentration.

6.2.2 Recovery of zooplankton populations

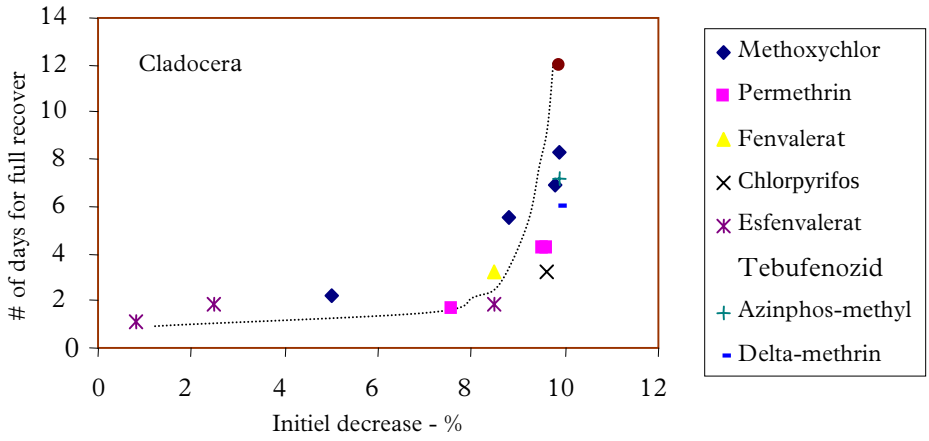
Recovery of zooplankton populations following insecticide exposure relies on reproduction from surviving individuals, hatching of resting stages (eggs) or immigrations from outside of the system. For the dipteran Chaoborus recovery may also take place by egg laying from imagos. Whether the

zooplankton community may endure a 100% reduction depends on whether the resting stages of the various zooplankton groups are tolerant to pesticides, which remains to be elucidated.

To be able to examine recovery of zooplankters, mesocosms need to include sediment and in addition to be in operation for several weeks-months after pesticide dosing has stopped. However, in more than 50 % of the mesocosm studies where zooplankton were followed the post exposure period was too short and/or the doses of insecticides too high to observe complete recovery of zooplankton.

Based on the recovery studies, an attempt can be made at defining the lowest level to which zooplankton populations may be reduced as a consequence of pesticides without being at risk of extinction. For Cladocera the time elapsed for full recovery after the insecticide dosage varied between 10 and 120 days. In Figure 23 is shown a plot of the initial (and maximum) reduction in population size (relative to corresponding control) and the time elapsed after dosage had stopped until full recovery of the population. In mesocosm experiments where cladocerans had been reduced severely (i.e. > 95 %) it took more than 12-15 weeks for full recovery. Such lengthy recovery is probably the result of slow dissipation of the insecticide in the mesocosm and thus continued toxic effects after dosage was stopped.

FIGURE 24. SCATTERPLOT BETWEEN INITIAL REDUCTION IN ABUNDANCE OF CLADOCERA AND TIME ELAPSED FOR FULL RECOVERY OF THE POPULATION. THE RELATION CAN BE DESCRIBED BY: $R = 8.5\ e^{0.019x}$, r^2=0.6, WHERE 8.5 (Y-AXIS INTERCEPT) INDICATE THE GENERATION TIME FOR NON-AFFECTED POPULATIONS. ONLY REDUCTIONS BELOW 100 % WERE INCLUDED.

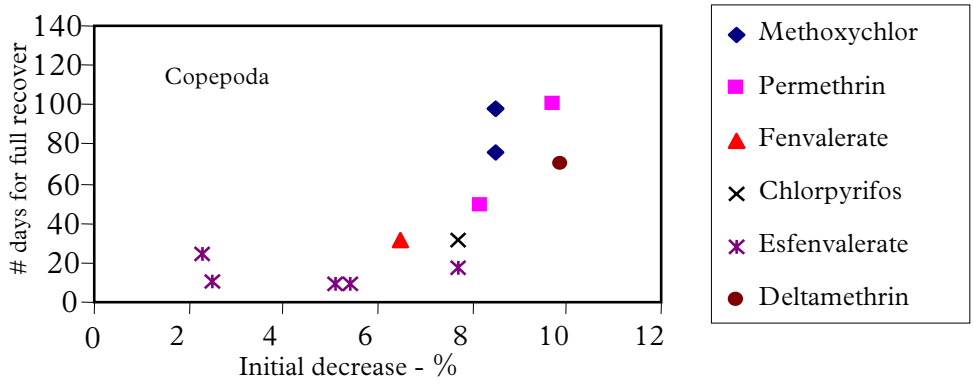


The relation between the initial reduction in population size and time until full recovery was met could be described by an exponential function (see Figure 24). At reductions below 80 % of the initial population size recovery was fast, i.e. less than 20 days. However, recovery time increased markedly if the initial population was reduced by more then 85 %. Still, even at population reductions close to 100 % full recovery of Cladocerans was observed in the mesocosms where the length of observation period was sufficient long.

For copepods an almost identical relation between initial decrease and recovery was obtained (see Figure 25). Fast recovery within Cladocera (usually analysed at Order level) as observed in numerous studies is likely to be governed by parthenogenetic reproduction. However, to maintain

populations of cladoceran species sexual reproduction is essential at intervals. Therefore, recovery studies terminated successfully within 3-4 months may not be sufficient to describe the recovery on the long term.

Figure 25. Scatterplot between initial reduction in abundance of Copepoda and time elapsed before full recovery of the population. Curve fitted by eye.



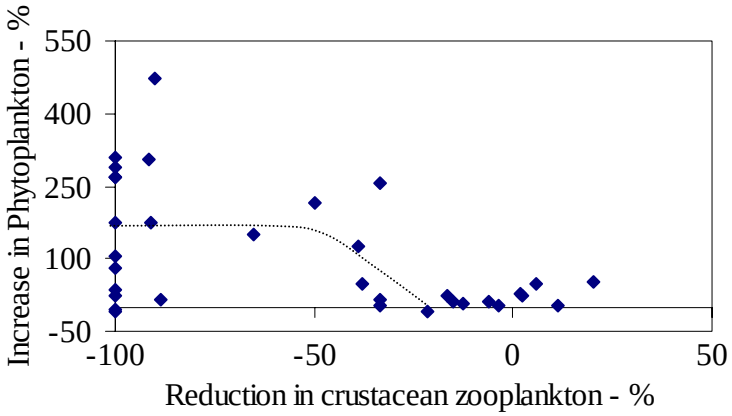
Without being at risk of extinction, a significant reduction of cladoceran and copepod numbers may, however, result in reduced species diversity and thus a decline of environmental quality. It may also be that a less diverse community/ecosystem is more sensitive to sudden outside influences such as increased nutrient input or additional pesticide inputs during the recovery phase. Unfortunately, the data contained in the data base do not allow examination of such relations.

6.3 Indirect effects of insecticides on plankton.

The most prominent indirect effect of insecticides on the plankton community includes increases in phytoplankton and rotifers. Following a decrease in population size of crustacean zooplankton, phytoplankton biomass generally will increase due to relaxation of grazing control. In addition, planktonic rotifers that are less sensitive to insecticides will increase in abundance due to increased food availability and reduced competition from crustacean zooplankton (see Figs. 18&19).

In Figure 26 is shown that the phytoplankton biomass increases, when the crustacean zooplankton becomes affected by insecticides. As expected being an indirect effect the scatter is substantial, however, the relation is highly significant. It seems that low impacts on the crustacean zooplankton will not result in increased growth of phytoplankton. If however, zooplankton becomes reduced by more than 50 % dramatic increases in phytoplankton (>100 %) must be expected (Fig. 26).

Figure 26. Decrease in crustacean zooplankton (Copepoda & Cladocera) and corresponding change (increase) in phytoplankton biomass (Chla) in mesocosm experiments with insecticides (diflubenzuron, endosulfan, deltamethrin, esfenvalerate).



Planktonic rotifers constitute direct competitors to cladocerans and copepods. Reductions caused by insecticides in these groups generally will lead to increases within Rotifera. Due to high reproductive potential increases in abundance up to 3000 % have been observed. In Figure 27 is shown a scatter-plot of changes in crustacean zooplankton and corresponding observations in rotifer abundance in mesocosm experiments with insecticides. Note that the increase in rotifer abundance has been scaled to 100 % within each experiment. On average the decrease in crustacean zooplankton only explains about 20 % of the observed variation in rotifer abundance. Still, the inverse relation is highly significant. Despite increases in rotifer abundance the pelagic grazing control in insecticide affected systems become impaired and phytoplankton biomass will increase (Fig. 26).

Figure 27. Decrease in crustacean zooplankton (Copepoda & Cladocera) and corresponding change (increase) in Rotifer abundance in mesocosm experiments with insecticides (methoxychlor, esfenvalerate, fenvalerate, cyfluthrin). Within each experiment the increase in Rotifera has been normalised to 100 %.

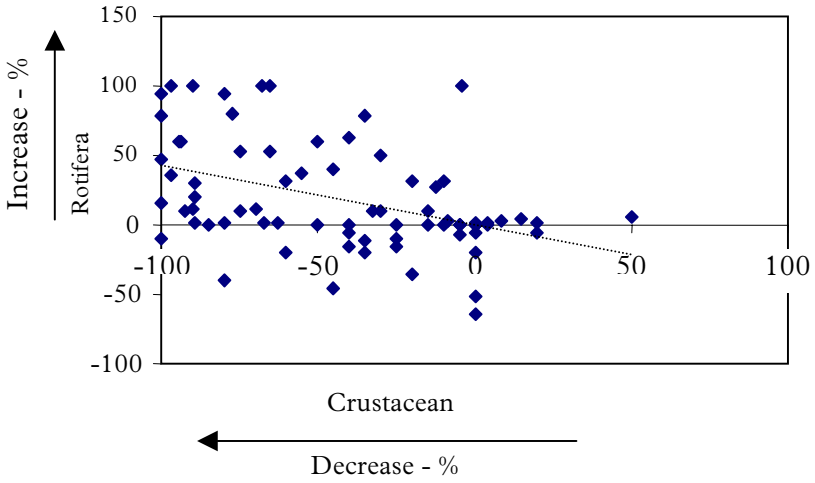


Table 18 shows an overview of recorded effects on plankton communities with 12 different *insecticides* in 19 different mesocosm studies. While direct effects on Cladocera and Copepoda are very consistent, indirect effects on

Rotifera are more variable. In addition, it is striking that changes in phytoplankton biomass were observed in only 5 out of 19 mesocosm studies. Presence of non-eatable phytoplankters may be responsible

Table 18 Overview of direct og indirect effects of insecticides in mesocosm experiments. ↓ = significant and consistent decrease in abundance (direct effect), → = no effects, ↑ = significant and consistent increase in abundance (indirect effect), ↑↓ = both decrease and increase observed , - no records.

Insecticide	Cladocera	Copepoda	Rotifera	Phytoplankton
Methoxychlor	↓	↓	↑	-
Diflurobenzuron	↓	-	-	↑
Lindan	-	↓	-	-
Fenvalerat	↓	↓	↑	→
Endosulfan	↓	-	-	↑
Deltamethrin	↓	-	-	↑
Cyfluthrin	↓	↓	↑↓	-
Permethrin	↓	↓	↑	-
Chlorpyrifos	↓	↓	→	-
Azinphos-methyl	↓	→	→	-
Tebufenozid	↓	↓	↑	-
Esfenvalerat	↓	↓	↑↓	↑-

In conclusion, indirect effects of insecticides on the plankton community have been recorded in more than 50 % of mesocosm studies. In those studies the indirect effects were at least as sensitive as direct effects, e.g. a 75 % reduction in crustacean zooplankton on average will be followed by a 500 % increase in rotifer abundance and a 200 % increase in phytoplankton biomass (see Figure 26). However, indirect effects are very variable in both magnitude and direction and thus less robust compared to direct effects.

6.4 Effect of Insecticides on Macroinvertebrates

Macroinvertebrates generally are insensitive to *herbicides*. Hence, in only 3 out of 7 mesocosm experiments involving macroinvertebrates in the data base were effects on the macroinvertebrate community detected. They included reduced emergence of Chironomids due to food limitation (reduction of epibenthic algae due to Atrazine), increased drift in streams (Triclopyr-ester & Hexazinone). However, effect concentrations were above calculated hazard concentrations for these herbicides (see Table 1).

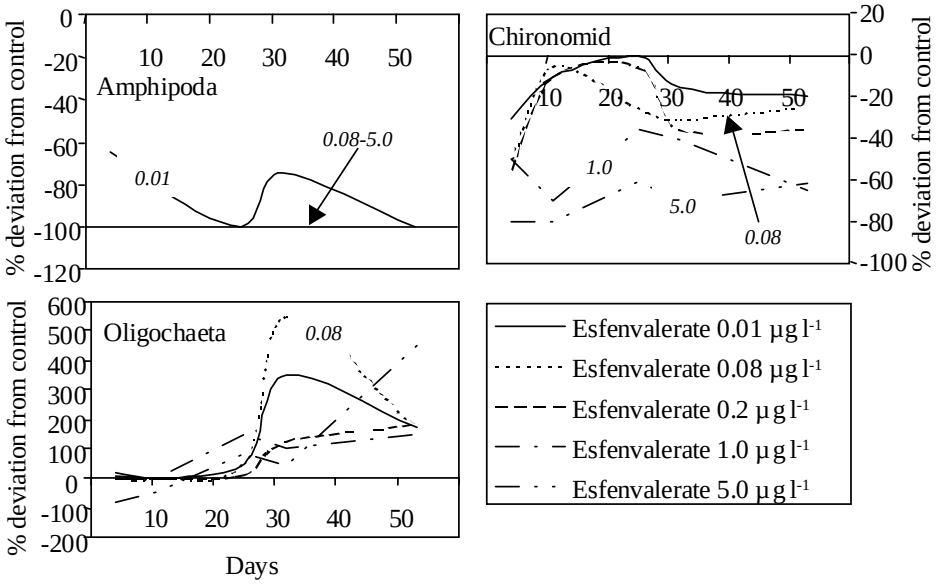
The data base contains a total of 41 individual mesocosm experiments with macroinvertebrates distributed among 19 different *insecticides*. A total of 935 records on effects of *insecticides* on macroinvertebrates were distributed between the following end-points LOEC: 424, NOEC: 491, EC50: 17 and NEC: 3. Dipterans were used in 29% of the experiments followed by mayflies, Ephemeroptera, which was used in 21% of the experiments. All other macroinvertebrate orders were followed in less than 10% of the experiments. In total, insects constituted 73% of all macroinvertebrates sampled and non-insects 27%.

The majority of recorded effect concentrations concern Dipteran (316 records) with the majority belonging to the family Chironomidae, Ephemeroptera (131 records), Amphipoda (93 records), Isopoda (75 records), Tricoptera (67 records), Hemioptera (58 records), Gastropoda (53 records), Coleptera (50 records), Oligochaeta (32 records), Odonata (29 records), Plecoptera (10 records) and Lepidoptera (4 records). Abundance of individuals was by far the most used effect parameter (872 records), followed by drift (26 records), mortality (17 records), emergence (13 records) and survival (4 records).

In the data base direct effects of *insecticides* on macroinvertebrates were examined and quantified by relating the dosing of insecticides to changes in abundance relative to corresponding controls (without insecticide dosing). To be able to compare studies with different application schemes the average decrease in abundance within the period 28-56 days after the first application of insecticide was used. By this approach studies with single and multiple applications could be compared.

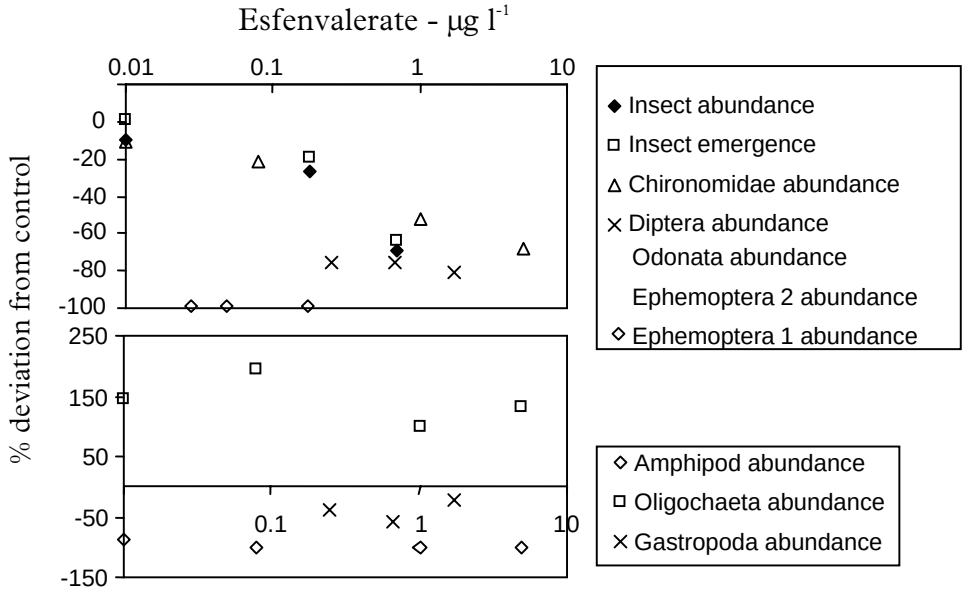
In Fig. 28 is shown an example of the temporal variation in abundance of Amphipoda, Chironomidae and Oligochaeta after a single dose of esfenvalerate. Both the initial impact and the subsequent recovery (Chironomidae and Oligochaeta only) were dependent on the dose.

Figure 28. Temporal variation in abundance (% of control) of macroinvertebrates following a single dose of esfenvalerate in 5 different concentrations to mesocosms (after Lozano et al. 1992).



The sensitivity of different macroinvertebrate groups and effect parameters to *insecticide* exposure in mesocosms were evaluated by comparing corresponding LOECs and numeric reductions. In Figure 29 is shown an example of reductions in abundance of various macroinvertebrate groups exposed to esfenvalerate along with effect on the emergence of insects. These studies demonstrate the general pattern among macroinvertebrates: amphipods and mayflies being rather sensitive to insecticides, while gastropods, Odonata and oligochaetes are rather insensitive.

Figure 29. Dose-response relations of macroinvertebrates in mesocosms exposed to esfenvalerate. Mesocosms were shallow (0.5-1.1 m depth), had sediment and macrophytes and ranged between 25 – 1100 m^3 in volume (from Fairchild et al. 1992; Lozano et al. 1992, Webber et al. 1992).



In the following the relative sensitivity of macroinvertebrate groups to individual insecticides is visualised in diagrams showing LOECs and numeric reductions of macroinvertebrate abundance or alternative endpoints such as increase in drift in artificial streams. Only experiments with more than one group or two or more endpoints followed within one group are presented and discussed. Because of differences in mesocosm volume, season and latitude that all influence the measured toxicity of insecticides (see chapter 5) comparisons can only be evaluated within single mesocosm experiments.

On the basis of these comparisons it is evident that:

1. The sublethal effect drift in stream macroinvertebrates generally appears to be a more sensitive endpoint than changes in abundance (Figure 30AB). In stream ecosystems drift is a natural behaviour of crustaceans and insect larvae for dispersal and colonisation of substrate. When exposed to insecticides (and several other toxic substances) arthropod macroinvertebrates may leave the substrate and drift to avoid the toxicant. Hence, in the short term, drift and population size are reciprocal measures: increased drift invariably will lead to reduced abundance. The seemingly higher sensitivity of drift compared to abundance presumably is related to differences in sample size and stronger statistics in drift data.
2. The endpoint emergence of adult insects seems to be as sensitive as changes in abundance of larvae (e.g. Figure 30). Insecticides may increase the mortality of larvae and reduce growth rate. In effect, emergence will decrease or be delayed. Rate of emergence usually is assessed using float traps that integrate samples from a fairly large bottom area and therefore show less spatial variability than benthos samples. On the other hand, the timing of emergence in affected populations of insect larvae often will differ from the emergence in non-affected populations (i.e. controls) which may complicate sampling and interpretation.
3. The insect order Tricoptera consistently was the most sensitive macroinvertebrate group to insecticides (Figs. 32-34), followed by Plecoptera/Hemiptera/Ephemeroptera/Cole-optera/Amfipoda/Isopoda (no particular order). Chironomidae as a very diverse group (individual size, mode of feeding etc.) showed a rather large variation in sensitivity (e.g. Fig. 31).
4. Odonata, Gastropoda and Oligochaeta consistently were the groups with the lowest sensitivity to insecticides.

Figure 30. Summary of effects of Lindane on drift, insect emergence and abundance of different macroinvertebrate groups. Experiment A&B (artificial streams) received Lindane continuously for 4 weeks, while in experiment C (1000 l stagnant mesocosm) Lindane was dosed only once. Numbers shown along bars denote the increase in drift (in percentage) or decrease in emergence or abundance of corresponding controls. As a comparison the hazard concentration ($HC_{5,50\%}$) calculated from distribution based extrapolation of single species toxicity data for Lindane was 2.9 µg l^{-1} (see Table 1).

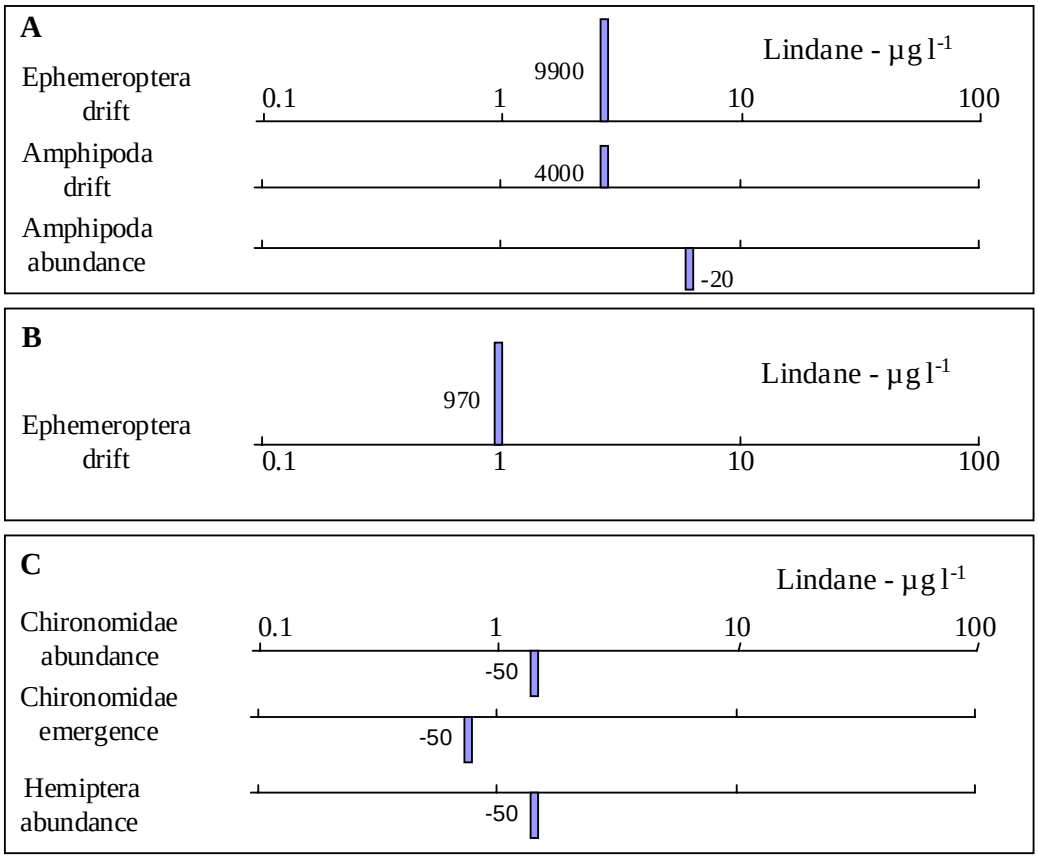


Figure 31. Summary of effects of Chlorpyrifos on macroinvertebrate groups in laboratory mesocosms (A & B), experimental ditches (C) and in artificial streams (D). In all 4 experiments reduction in abundance was used as end-point. Numbers shown along bars denote the reduction in percentage of corresponding controls. Experiment A-C received Chlorpyrifos as a single dose, while in experiment D Chlorpyrifos was dosed continuously for 21 days. The different colours denote different species within one group. As a comparison the hazard concentration ($HC_{5,50\%}$) calculated from distribution based extrapolation of single species toxicity data for Chlorpyrifos was 0.04 µg l^{-1} (see Table 1).

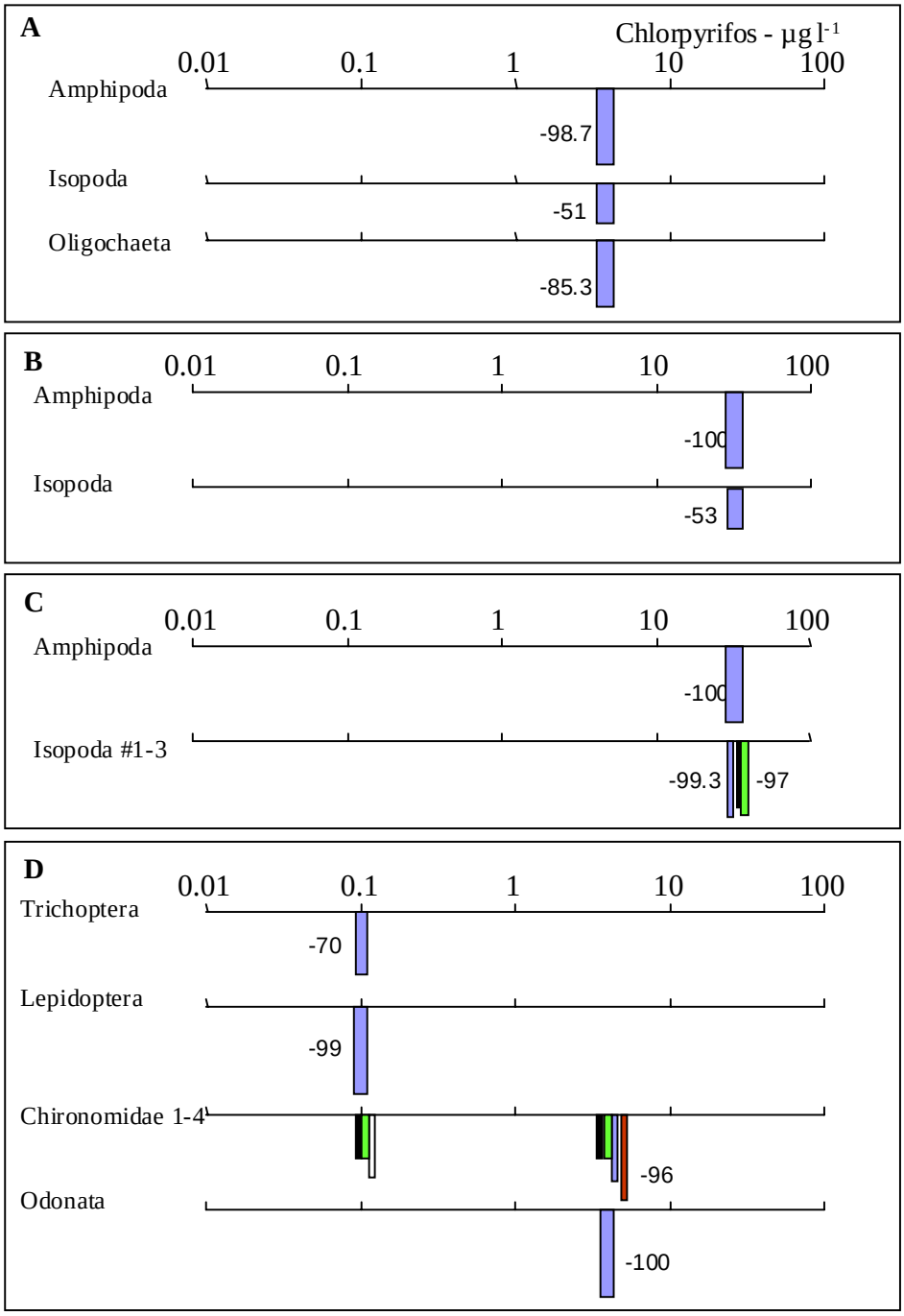


Figure 32. Summary of effects of Lambda-cyhalothrin on abundance of different macroinvertebrates in mesocosms. In experiment A and C (25 m^3) Lambda-cyhalothrin was dosed 4 times during 42 days, while B (450 m^3) was dosed every 14 days through 147 days. Numbers shown along bars denote the reduction in percentage of corresponding controls. The different colours denote different species within one group. As a comparison the hazard concentration ($HC_{5,50\%}$) calculated from distribution based extrapolation of single species toxicity data for Lambda-cyhalotrin was 80 ng l^{-1} (see Table 1).

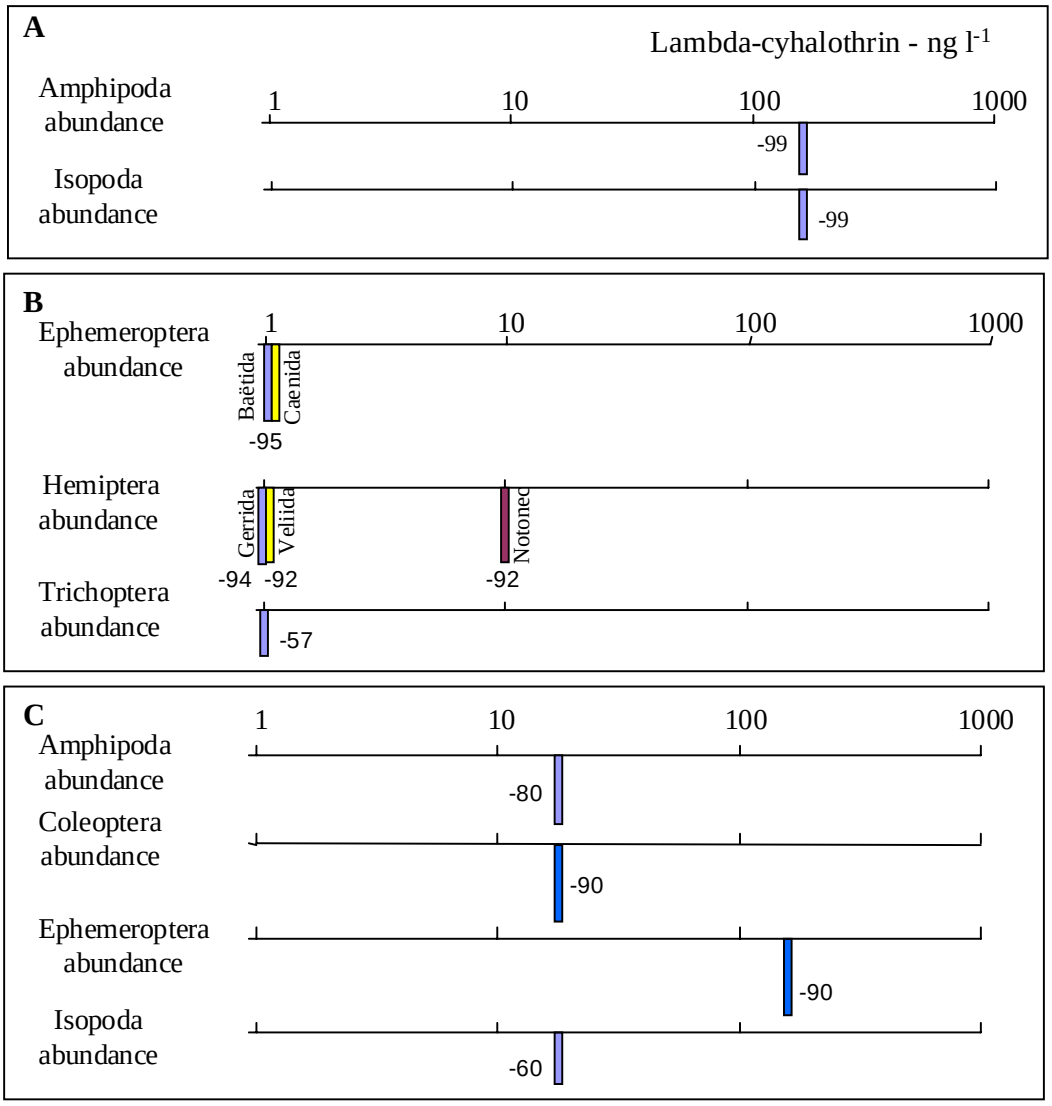


Figure 33. Summary of effects of Diazinone on abundance of different macroinvertebrates in mesocosms. Numbers shown along bars denote the reduction in percentage of corresponding controls. As a comparison the hazard concentration ($HC_{5,50\%}$) calculated from distribution based extrapolation of single species toxicity data for Diazinon was 0.03 µg l^{-1} (see Table 1).

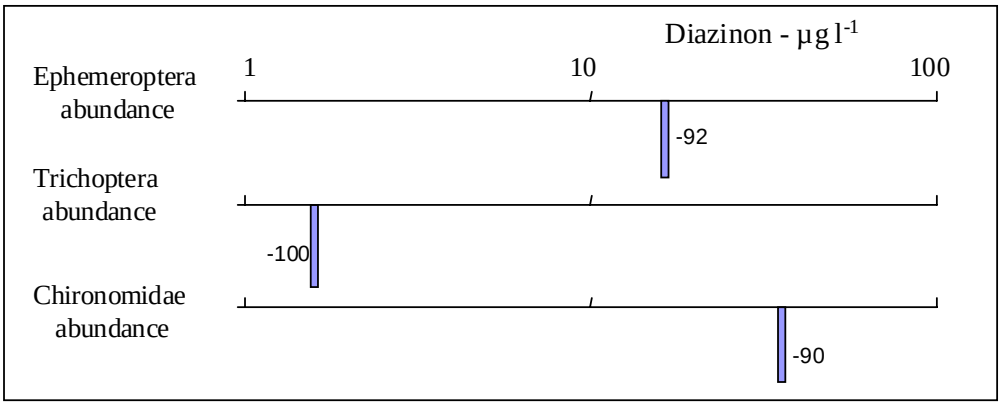


Figure 34. Summary of effects of Fenvalerate on abundance of different macroinvertebrates in mesocosms. In experiment A (small recirculating flume) Fenvalerate was dosed once times and abundance was monitored after 30 days, while B was followed through 84 days. Numbers shown along bars denote the reduction in percentage of corresponding controls. The different colours denote different species within one group. As a comparison the hazard concentration ($HC_{5,50\%}$) calculated from distribution based extrapolation of single species toxicity data for Fenvalerate was 50 ng l^{-1} (see Table 1).

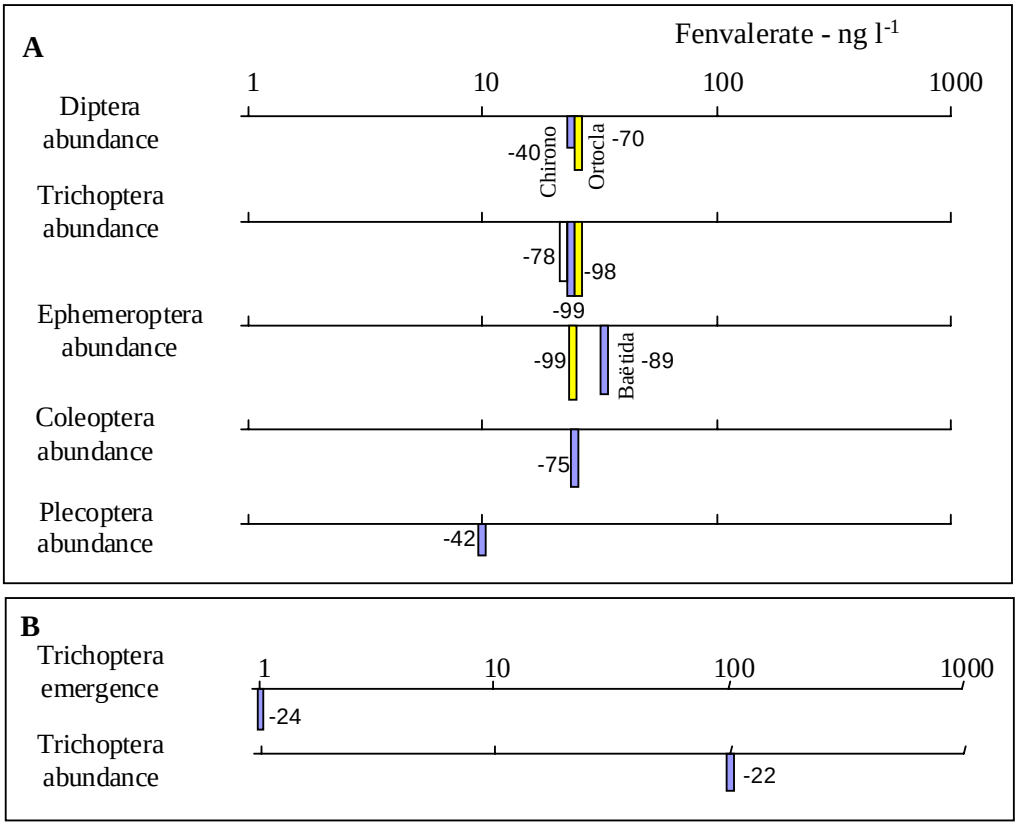
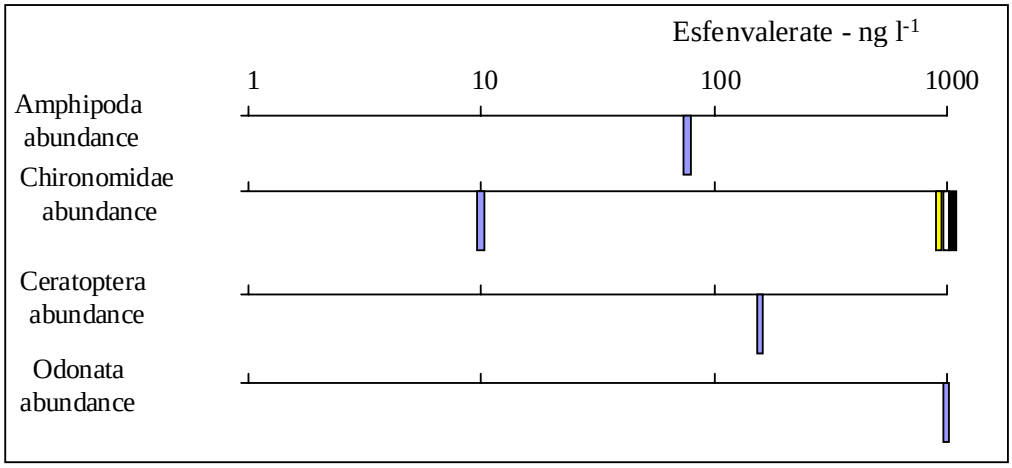


Figure 35. Summary of effects of Esfenvalerate on abundance of different macroinvertebrates in 1100 m^3 mesocosms. Esfenvalerate was dosed every week through 10 weeks. Only LOECs, but no numeric reductions were given in the report. The different colours denote different species within one group. As a comparison the hazard concentration ($HC_{5,50\%}$) calculated from distribution based extrapolation of single species toxicity data for Esfenvalerate was 180 ng l^{-1} (see Table 1).



6.4.1 Statistical power of impact of insecticides on macroinvertebrates in mesocosm experiments

The statistical power for effects on macroinvertebrates was comparable to the impact on zooplankton with an average reduction in abundance (i.e. excluding indirect effects) at recorded LOEC's of 76.5 % (± 20.3 %; SD). In Table 19 is shown the distribution of reductions in abundance of macroinvertebrates at the various combinations of replicate number and number of test concentrations applied in the different studies. The different combinations are based on observations ranging from 7 to 103 in number and from 1 to 4 different studies carried out at different locations and using different mesocosms (volume, ± macrophytes etc.), which may set limits to conclusions drawn.

Overall, the general pattern resembles the data for zooplankton suggesting that a sufficient sensitivity may be obtained by a hybrid approach with more than 4 test concentrations and at least two but preferably 3 replicates at each concentration.

Table 19. Average reduction (%)±SD in macroinvertebrate abundance at LOEC in mesocosm studies of different experimental design. Number of observations in brackets. – # observations below 5.

Number of replicates	Number of insecticide levels						
	2	3	4	5	6	7	8
2	81±18 (103)	-	-	51±5.5 (32)	-	-	84±18 (19)
3	-	75±14 (16)	74±18 (47)	-	-	-	-
4	93±12 (39)	80±18 (92)	-	24±5.3 (7)	-	-	-

6.4.2 Recovery of macroinvertebrates after insecticide exposure.

Recovery is essential when evaluating effects of pesticides. In macroinvertebrates recovery may take place by invasion from non-affected populations outside the affected area (e.g. by drift in streams, reproduction) and reproduction by surviving individuals within the affected area. In order to evaluate recovery, mesocosm studies need to be carried out in the field (to allow flying insects to lay eggs) and should at the minimum extend a full life cycle of the organisms studied after insecticide dosage. And obviously, repeated sampling of macroinvertebrates will be necessary to follow changes in populations. In the data base not all of the mesocosm experiments included a time series. Furthermore, the majority of experiments in the data base were terminated within 150 days although a few experiments ran for a whole year.

Taking the general life-cycle length for macroinvertebrates into consideration (ranging from less than a month to several years), the experimental time frames in most mesocosm studies appear to be too short. This might partly explain why there are only very few examples of recovery in mesocosms contained in the data base, none of them being a full recovery (Fig. 36). There was no sign of recovery in 81 % of the observations. Signs of recovery were found in 13% of the observations and a moderate recovery in 6% only.

Chironomids (belonging to the order Diptera) and Isopoda were the most important taxonomic groups in the "slight recovery group" (Fig. 37 left) whereas Chironomids and Ephemeropterans dominate the "moderate recovery group" (Fig. 37 right). Both Chironomids and Ephemeropterans in general are considered as good colonisers with short life cycles and this probably explains why they show the most rapid recovery.

Figure 36. Recovery of macroinvertebrate populations in mesocosm studies contained in data base. An observation includes changes found over time in a macroinvertebrate taxon. No recovery is defined as a less than 5% change (increase) after the initial decrease; slight recovery less than 25% change and moderate between 25 and 75% change.

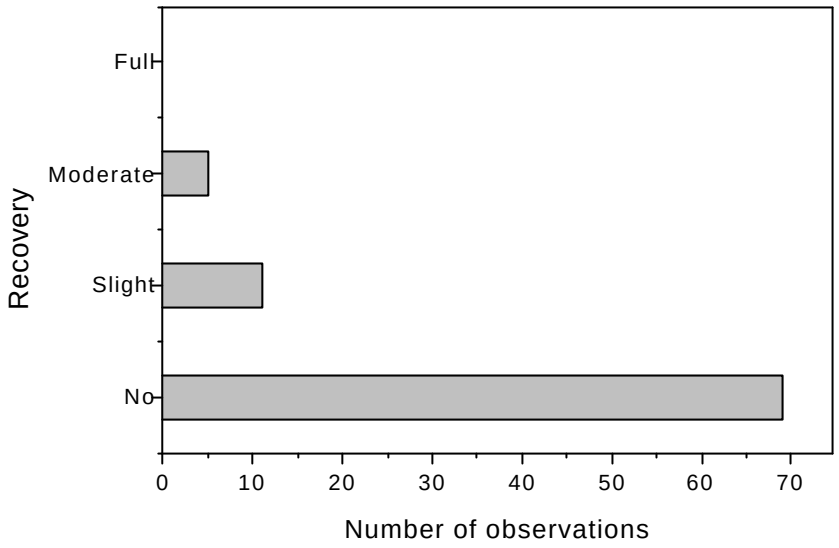
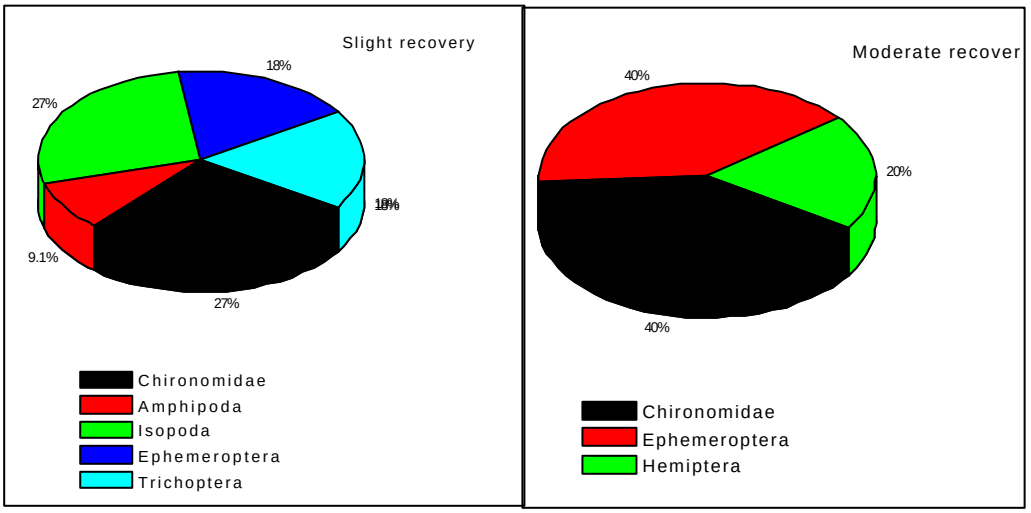


Figure 37. Percentage composition of macro-invertebrate orders that showed a slight recovery (left) or moderate recovery (right).



Overall, it is surprising that there is so little evidence of recovery within macroinvertebrates. This finding might reflect that most studies have been too short. One reason for this might be that studies in general involve other taxonomic groups such as zooplankters with shorter life spans and that the duration of the experiments are set to reflect their life span and not the macroinvertebrates. More specific studies targeted towards macroinvertebrates might be needed or the duration of the experiments should be increased when mimicking whole ecosystems. Generally, one should expect that organisms with limited ability for colonising, i.e. non-insect groups such as Isopods, Amphipods and Gastropods or insects with long generation times such as Odonata would be the slowest to recover following

insecticide exposure. However, both Gastropoda and Odonata are among the least sensitive to *insecticides* and the recovery will only be an issue after excessive insecticide exposure. For Amphipods, however, the limited ability to recover can be very critical as these organisms also are among the most sensitive to *insecticdes*.

7 Comparison of extrapolated hazard concentration and observed effects in mesocosms

As shown in this study only a limited number of "high quality" mesocosm experiments (see Chapter 4 for selection criteria) examining the effects of pesticides in freshwater systems have been reported. As a consequence, an alternative approach using the results from numerous standardised single species tests has been developed. Hazard concentrations for ecosystems may be calculated from distribution-based extrapolation of single species toxicity data (EC50, LC50) using (slightly) different statistical methods (e.g. Wagner & Løkke 1991, Miljøstyrelsen 1994, Emans 1994). The widely used calculation of hazard concentration, $HC_{5,50\%}$ aims to protect 95% of the organisms in an ecosystem with a 50% probability. Others consider that a 90% protection of ecosystem species is adequate to avoid adverse effects on the natural ecosystem, i.e. $HC_{10,50\%}$ (Hall et al., 1998). A alternative approach adopted by OECD multiplies the lowest effect concentration observed among all standardised tests by 0.1 (application factor of 10) (see Chapter 4 for more details).

The major limitation of these approaches is the availability of single species test results, as they are biased towards dominance of cladocerans, planktonic algae and fish. Thus, data on effect concentrations of insect larvae are scarce or not available for several pesticides (see Table 3 and Annex 1). For macrophytes, no standardised test results were available for the pesticides included in the data base! As standardised tests usually are short-term (48-96 hours) they may fail to reveal long-term effects caused by pesticides accumulated in organisms. And relying solely on standardised single species tests' extrapolation methods will never be able to account for behavioural effects and interactions between populations and trophic groups (i.e. indirect effects).

In Table 20 we have summarised a comparison of extrapolated hazard concentrations and the lowest observed effect concentrations in the mesocosm experiments contained in the data base. An extended version of the comparison including 66 mesocosm experiments is shown in Annex 3. Within single mesocosm experiments LOECs for different organisms can vary 2-3 fold (see Chapter 6 & 7), hence LOEC for one group can be NOEC for several others groups. Still, we have selected the lowest observed effect concentrations within an Order, genus or species and tested effects for significance (i.e. persistence) (see chapter 5).

We have used the ratio $HC_{5,50}$/LOEC or OECD/LOEC as a measure of the success of the extrapolated hazard concentration ($HC_{5,50}$ or OECD) to "protect the species" in an aquatic ecosystem. Table 20 only includes experiments, where the ratio $HC_{5,50}$/LOEC is above 1. With exception of experiment 57flm (that included 25 different taxons) the experiments in Table 20 included less than 20 taxons (range 1-13). Hence, in 13 out of 66 experiments the widely used approach failed to protect 95 % organisms in the

ecosystem (see Annex 3). Even using the more conservative OECD approach the hazard concentration failed to protect 95% of the organisms in 5 experiments. In about half of the experiments contained in Table 20 (i.e. 8 experiments) NOEC was not be established for the most sensitive parameter, because sufficient low concentrations were not tested. Hence the ratio $HC_{5,50}$/LOEC calculated for these experiments represents a minimum.

It is noticeable, that the vast majority of examples of "failures" of extrapolated hazard concentrations to protect sensitive species are found in experiments, where LOECs were recorded for macroinvertebrates and insects, while LOECs for phytoplankton and zooplankton except for two occasions (see Table 20) result in ratios of $HC_{5,50}$/LOEC well below 1 (see Annex 3). Therefore, extrapolated hazard concentrations generally will protect the plankton environment in ecosystems, which hardly is surprising as the extrapolated values primarily rely on standardised tests with cladocerans and phytoplankton. On the other hand, extrapolated hazard concentrations are much less successful in protecting the macroinvertebrate community.

The importance of including macroinvertebrates in mesocosm experiments is further demonstrated by an ANOVA, where the "failure" of extrapolated hazard concentrations ($HC_{5,50}$) to protect the aquatic ecosystem was explained by number of organism groups monitored, inclusion of macroinvertebrates and the number of insecticide doses (Table 21).

Intuitively, one would expect that the chance of "failure" would increase with increasing number of organism groups monitored and when the insecticide was dosed several times. In the analysis neither variable was important. However, if macroinvertebrates were monitored in mesocosms the risk that extrapolated hazard concentrations would fail to protect the whole ecosystem was substantial (significance level – $p = 0.017$) (see Table 21). Average ratio $HC_{5,50}$/LOEC in experiments with macroinvertebrates was 9.0 (1.10 if the high value of 80 in exp. 76tll was omitted) but much lower at 0.26 in experiments without macroinvertebrates.

Table 20. Comparison of extrapolated hazard concentrations and the lowest observed effect concentrations in the mesocosm experiments. NOEC: Yes = lowest test concentration were lower than the lowest effect concentration observed in mesocosm; No = effect was observed at the lowest test concentration applied. $HC_{5,50}$/LOEC = ratio between extrapolated hazard concentration (see Table 1) and lowest observed effect concentration. OECD/LOEC = ratio between hazard concentration ($OECD_{10}$ approach) and lowest observed effect concentration. $HC_{5,50}$/low = ratio between hazard concentration and the lowest test concentration applied. OECD/low = ratio between extrapolated hazard concentration ($OECD_{10}$ approach) and the lowest test concentration applied. Observed effects at lowest concentration: ↓ decrease (mostly in abundance); ↑ increase.

Exp #	Pest	Trivial name	NOEC	$HC_{50,5}$ /LOEC	OECD/ LOEC	$HC_{50,5}$ /low	OECD /low	Observed significant effect at lowest concentration*
76tll	Ins	Lambda-cyhalothrin	Yes	80	10	800	100	↓Ephemeroptera
96mli	Ins	Fenvalerate	No	50	10	50	10	↓ Tricopoptera emergence
57flm	Ins	Esfenvalerate	No	18	2	18	2	↓ Chironomidae
44tll	Herb	2,4 D**	Yes	12	2.4	120	24	↓ macrophyte biomass
113tll	Ins	Esfenvalerate	No	5.14	0.571	5.14	0.571	↓macroinvertebr↑ phytoplankton
60flm	Ins	Lambda-cyhalothrin	No	4.71	0.588	4.71	0.588	↓ Amphipoda
123fl	Ins	Lambda-cyhalothrin	No	4.71	0.588	4.71	0.588	↓ most macroinvertebrat groups
102tll	Ins	Lindan	Yes	3.66	2.25	2.93	1.8	↓ Chironomid emergence
104tll	Ins	Lindan	Yes	3.26	2.00	2.93	1.8	↓ Chaoborus mortality
77mli	Ins	Lindan	Yes	2.93	1.80	11.72	7.2	↑ drift in Ephemeroptera
86mli	Ins	Fenvalerate	Yes	1.67	0.333	5	1	↓macroinvertebr abundance
117tll	Herb	Atrazin	No	1.33	0.173	1.33	0.173	↓ Phytoplankton ↑ rotifer
82mli	Herb	Atrazin	No	1.22	0.160	1.22	0.160	↓ Periphyte biovolume
118tll	Ins	Bifenthrin	No	1.03	0.256	1.03	0.256	↓ Zooplankton

* Only significant effects included (see chapter 4).
** 2,4 D are toxic to higher plants only, while extrapolated hazard concentrations were based on single species test with algae and zooplankton, only.

The failure of extrapolated hazard concentrations in protecting 95% of organisms and especially macroinvertebrates, against *insecticides* in the aquatic environment probably occurs because

- macroinvertebrates are the most sensitive organisms to insecticide exposure, probably related to the high K_d of most insecticides. Hence,

under natural conditions the exposure of sediment-dwellers will be higher than plankters.
- macroinvertebrates are underrepresented in the single-species tests used for extrapolation of hazard concentrations
- the duration of standardised single species tests is too short to reveal the potential effects on macroinvertebrates as the maximum effects on macroinvertebrates are recorded 2-8 weeks after exposure start in mesocosm experiments.

Table 21. Result of 1-way ANOVA for effect of number of organism groups (phytoplankton, periphytes, macrophytes, zooplankton, macroinvertebrates, fish) monitored, inclusion of macroinvertebrates (yes, no) and number of insecticide doses (1-10) during the experiment on the ratio $HC_{5,50}$/LOEC ($HC_{5,50}$/LOEC >1 → value = failure; $HC_{5,50}$/LOEC <1 → value = success). The analysis was restricted to experiments with insecticids, where NOEC was recorded for the most sensitive organism (n=23). Mean squared effect, mean squared error, F statistics and level of significance shown.

Independent variable	Mean sqr. effect	Mean sqr. error	F(df1,2) 1.16	p-level
# of groups monitored	0.250	1.110	0.225	0.641
Effect on macroinvertebrates	1.361	0.193	7.063	0.017
# of insecticide doses	14.69	5.276	2.785	0.115

In conclusion, long-term (abundance and emergence) and short-term effects (drift in streams) of insecticides on macroinvertebrates are among the most sensitive effect parameters recorded in mesocosms. Such effects cannot be explained in sufficient detail by extrapolations based on calculations of Hazard Concentrations from standardised single species test. Therefore, the data bases used for extrapolation ought to be extended with tests on macroinvertebrates and preferentially the duration of these test should be increased. Alternatively, mesocosm experiments should be carried out. To arrive at environmentally realistic effect concentrations and protect the whole ecosystem, mesocosms need to include a benthic compartment encompassing a diverse fauna including important and sensitive taxonomic groups such as Tricoptera, Ephmeroptera and Amphipoda.

8 Conclusions and recommendations

The regulation of pesticide use and protection of non-target species primarily relies on evaluations based on single species tests. If a pesticide is evaluated to constitute a hazard to aquatic life, further and extended analysis must be carried out to show that the pesticide does not constitute a risk to the aquatic environment (EU directive 91/414). In line with several other countries Denmark relies on extended risk evaluations based on tests carried out under near-natural conditions at an ecosystem level by using experimental mesocosms of various size and design. Several guidelines describe protocols of how to carry out mesocosm experiments and what endpoints should be measured. Still, a general (uniform) procedure of how to interpret the results from mesocosm experiments and apply these results in a regulatory procedure has not been accepted at an international level.

In this study we have carried out a critical analysis of published results of mesocosm experiments, extracting and quantifying the influence of the experimental set-up on the sensitivity of organisms and the statistical power of observed effects, when and where the experiments were carried out, which taxonomic and functional groups were the most sensitive, and to what extent available single species test results can be used to protect the environment using various extrapolation procedures.

For a number of taxonomic and functional groups we have developed regression models using a PLS technique relating effects of pesticides to system characteristics and physico-chemical characteristics of the pesticides. The predictability of the models was rather high at 0.63-0.73. As the PLS models are based on all appropriate data in the database it is possible to develop a evaluation procedure taking all the available information into account, rather than on a restricted use of a single or a few mesocosm experiments for each pesticide. Thus with the aid of the PLS models it is possible to evaluate all mesocosm experiments with pesticides on a common basis.

The following presents an extract of the results of the analysis and thus constitutes a checklist for managers evaluating mesocosms in connection with the approval procedure.

CHECKLIST TO BE APPLIED WHEN EVALUATING RESULTS FROM MESOCOSM STUDIES. THE LEFT COLUMN CONTAINS GENERAL INFORMATION AND DEFINITIONS. THE RIGHT COLUMN CONTAINS THE IMPORTANT RESULTS FROM THE ANALYSIS WITH REFERENCES TO THE APPROPRIATE SECTIONS IN THE REPORT (IN BRACKETS).

Experimental design	
Hypothesis test (i.e. Anova design) is used to study whether the response of a mesocosm unit differs from that of a control unit. Hypothesis tests are used for comparing averages and are characterised by having *multiple replicates* in control and treatment groups. The greater number of replicates, the greater is the power of the test for resolving differences. Point estimate tests (i.e. Regression design) are used to evaluate *regression relationships* and, ideally estimate an exposure concentration which will not cause an adverse effect (NOEC or threshold concentration) or predict the intensity of an effect at a given exposure level. Regression design requires multiple treatments at various concentrations related to a response. The greater the number of treatment concentrations, the greater is the confidence in the fitted concentration-response line. As Point estimate tests assume a monotonic response of an effect parameter along a concentration gradient only direct effects can be evaluated. Even then indirect effects can mask the relationship. Hybrid tests incorporate features of both hypothesis and point estimate tests by employing both multiple replicates and multiple doses. Fewer replicates will reduce the power to resolve significant effects and fewer dose levels will reduce the confidence in estimating the fit and the NOEC.	The majority of mesocosm experiments in the data base belong to the "Anova Design" or "Hybrid design" (**6.2.1 & 6.4.1**). We have evaluated the statistical power of the various designs by comparing the average reduction in abundance of zooplankton and macroinvertebrates at the lowest observed effect (significant) concentration (LOEC). Overall, the statistical power in the mesocosm studies was rather low. The average *reduction* in abundance of zooplankters exposed to insecticides at recorded LOECs was 75.4 % (± 21.3 %; SD) (**see 6.2.1**). For macroinvertebrates the significant reduction was almost identical at 76.5 % (± 20.3 %; SD) (**see 6.4.1**). The low power is due to low number of replicates, low number of and/or large range in test concentrations. Overall, the data suggest that in order to obtain a sufficient resolution and sensitivity the experimental design should be a hybrid design encompassing more than 4 test concentrations and at least two replicates at each concentration. As the size of experimental design usually is constrained by economic considerations with a maximum number of units of 15-18 they should be distributed between 5-6 test concentrations each with 2-3 replicates. Therefore, in evaluating results from a mesocosm experiment one should take account of the experimental design, e.g. the results from a hybrid design with 5-6 test concentrations and 2-3 replicates each would produce the most reliable estimates of LOECs and NOECs.

Mesocosm design – size and depth	
Mesocosms intend to mimic nature and ideally they should allow different groups of organisms to survive, behave and interact with other groups as in natural systems. Logistics and economy ultimately set limits to the maximal size that can be applied. If fish are to be included, systems need to be large, which invariably will impose patchiness and may introduce biases in the sampling procedure. Therefore, mesocosms of intermediate size are usually preferred.	The size of the mesocosm studies contained in the database varies widely. Systems with volumes from 0.003 m^3 to 1,100 m^3 and average depths ranging 0.1–5 m are included in the database, with the small systems primarily representing flow-through experiments. The influence of volume and depth on the sensitivity to pesticide exposure of different functional and taxonomic groups was tested using PLS analysis (Chapter 4). The **volume** of mesocosm units had no influence on the toxicity of pesticides to either microalgae, zooplankton or macroinvertebrates (**5.4.1, 5.5.1, 5.6.1**), while the depth of the mesocosm significantly influenced the toxicity of insecticides to macroinvertebrates (**5.4.1**) with increasing effects (i.e. lower LOEC) at decreasing average depth of mesocosm.
Location and season of mesocosm tests	
Length of growth season, solar insolation and temperature vary on a continuum of scales determined by geographical location and time of year. As each of these "external" variables affects populations of aquatic organisms (*length of growth season*: number of generations; *insolation*: algal growth; *temperature*: growth and metabolism) and the fate of pesticides (*insolation & temperature*: degradation) both the geographical location where mesocosm studies are carried out and time of year when carried out are expected to influence the expression of pesticide effects.	In the mesocosm studies contained in the data base neither temperature nor solar insolation are explicitly given for each sampling occasion. Therefore, we have used a sinusoidal function of the day no. to integrate these variables (e.g. day no. 183 attain the value 1 and day no. 1 and 365 attain the value 0). All macroinvertebrate groups were most sensitive when the experiments were conducted at high latitudes. Toxic effects are expected to occur at lower insecticide concentrations with increasing distance from Equator probably due to a slower turn-over of populations at high latitudes, i.e. fewer generations each year at lower temperatures (**5.4.1**). Consequently, recovery of macroinvertebrate populations after pesticide exposure takes longer time at northern latitudes. For zooplankton effects of season and latitude of mesocosm was contradictory and no conclusion could be drawn.

Dosage of pesticides in mesocosms – single or multiple dosage	
Pesticides enter the aquatic environment during field application as spray drift, in association with surface run-off during heavy rainfall and through subsurface run-off (e.g. drainage). The importance of the different routes of entry is rather specific to site, crop, method of application and physico-chemical characteristics of the pesticide. For these reasons mesocosm tests usually are tailored to answer specific questions and accordingly single dosage or multi-dosage of dissolved pesticides, or pesticides dosed in slurries have been applied. Such different application schemes make it difficult to compare the outcome of the various studies, as the application mode invariably will affect the concentration and fate of pesticides in the mesocosms, e.g. multiple dosing every week at a low concentration may result in a higher temporal-averaged concentration than a single dose containing an identical amount of pesticide.	At a given total dose effects of pesticides on macroinvertebrates will increase with interval between individual doses but decrease with number of doses. Therefore, a low but persistent pesticide concentration will have a lower effect on the macroinvertebrates than a high but temporary pesticide concentration (**5.4.1**). This may be due to the relativly long generation time of most macroinvertebrates. Hence, recovery will be hampered if pesticides are dosed at intervals close to the generation time. For zooplankton the PLS models tested had the highest predictability (Q^2 = 0.736) when the only studies with a single addition of *insecticide* were included in the analysis (**see 5.5**). Inclusion of studies with multiple application of insecticides led to much lower goodness of fit and accordingly they were excluded in the analysis. Therefore, we cannot explicitly evaluate the influence of application mode on plankton.
Influence of sediment and macrophytes in mesocosms	
Presence of sediment in a meso-cosm should be a prerequisite for studying effects on macroinverte-brates. However, most zooplankters also rely on sediment for storage of resting eggs that constitute a "bank" for recolonisation. Macrophytes are a natural component of shallow freshwater systems. They have an important structural role, providing habitat, shelter and food for a number of organisms, influencing the physical environment and, therefore, affect the biogeochemical fluxes near the sediments. Macrophytes may prevent sediment from erosion and resuspension, while promoting sediment deposition. In addition, macrophytes directly may influence the availability of pesticides by adsorption and uptake.	For macroinvertebrates the PLS analysis showed that the highest predictability (Q^2(cum)) was obtained for a PLS model based on mesocosm experiments when both sediment and macrophytes were present in the test system (**5.4**). However, an almost similar high predictability was obtained for the PLS models for mesocosm experiments with sediment but without macrophytes in the test system. On the contrary, a much lower predictability was obtained when the PLS model was applied to all data for stagnant water including laboratory experiments without sediment. Therefore, effects of pesticides on macroinvertebrates must be studied in mesocosms including sediment and preferentially also macrophytes in the test system. Omission of sediment in test systems may lead to erroneous results out of line with the majority of

	high-quality studies. For zooplankton no consistent modifying effect of either sediment or macrophytes was found for the toxicity of pesticides (**5.5**).
Most sensitive groups – plankton - benthos	
Aquatic organisms differ in their sensitivity to pesticides according to their taxonomy, generation time, functional role in the ecosystem and their habitat. Generally, non-target arthropods in aquatic habitats (crustaceans and insect larvae) are very sensitive to insecticides aimed to control insects in crops, while molluscs are considered less sensitive probably due to their ability to reduce exposure by shell closure.	**Zooplankton**: The PLS analysis showed that cladocerans are the most sensitive zooplankters to insecticides followed by copepods and rotifers (**5.5.1**). This was confirmed and detailed by regression analysis revealing that Cladocerans and Chaoborus are the most sensitive zooplankters followed by copepod nauplii and adult Copepoda (**6.2**). The variation in sensitivity within each zooplankton group as demonstrated in mesocosm studies is considerable. Results from 3-4 detailed studies showed that LOEC varied 2 – 2.5 orders of magnitude within Cladocera (**6.2**). Hence, studies analysing Cladocera at the level of Order invariably will neglect effects on the species composition. **Macroinvertebrates**: The PLS analysis showed no difference in sensitivity between predatory and non-predatory macroinvertebrates (**5.4.1**). Detailed evaluation focussing on the sensitivity to *insecticides* of different taxonomic groups revealed that the insect order ***Tricoptera*** consistently was the most sensitive macroinverte-brate group, followed by Plecoptera /Hemiptera/Ephemeroptera/ Coleoptera/Amphipoda/Isopoda (**6.4**). Chironomidae as a very diverse group showed a rather large variation in sensitivity within a study (1-2 orders of magnitude). Hence, studies analysing effects on macroinvertebrates at the level of Order probably will neglect effects on the species composition. Odonata and Gastropoda consistently were the groups with the lowest sensitivity to *insecticides*. When comparing effects on zooplankton and macroinvertebrates the most sensitive organisms within

	macroinvertebrates generally will show lower LOEC than the most sensitive organisms within zooplankton (**Chapter 7**). Therefore, mesocosm studies must include and focus on macroinverte-brates, as effects cannot be extrapolated from available single species tests (because macroinverte-brates are underrepresented in the single-species tests used for extrapolation of hazard concentrations). The macroinvertebrate community must include important and sensitive taxonomic groups such as Tricoptera, Ephmeroptera and Amphipoda.
Most sensitive effect parameter	
Traditionally, mortality (and growth rate in algae) is the most widely used effect parameter in the regulatory procedure of pesticides because of ease of detection and obvious ecological significance. However, prior to death in an individual and reduction of a population sublethal effects will occur, which theoretically make sublethal effects excellent early warnings and sensitive effect parameters.	Abundance is by far the dominant effect parameter while functional effect parameters such as production and growth have seldom been measured and are therefore represented only at a limited scale (**Chapter 4**), which makes it difficult to compare the sensitivities. The sublethal effect drift in stream macroinvertebrates generally appears to be a more sensitive endpoint than changes in abundance (**6.4**). The endpoint emergence of adult insects generally is as sensitive as changes in abundance of larvae, however, sampling and interpretation can be difficult (**6.4**).
Duration of mesocosm experiments - recovery	
Recovery of zooplankton populations following insecticide exposure relies on reproduction from surviving individuals, hatching of resting stages (eggs) or immigration. To be able to examine recovery of zooplankters, mesocosms therefore need to include sediment and, in addition, to be in operation for several weeks-months after pesticide dosing has stopped. In macroinvertebrates recovery may take place by invasion from non-affected populations (e.g. by drift in	**Zooplankton**: Less than 50 % of the mesocosm studies where zooplankton was followed, the post exposure period was too short and/or the doses of insecticides too high to observe complete recovery of zooplankton. For Cladocera the time elapsed for full recovery after the insecticide dosage varied between 10 and 120 days (**6.2.2**). In mesocosm experiments where Cladocerans had been *reduced severely (i.e. > 95 %) it took more than 12-15 weeks* for full recovery. At reductions below 80 % of the initial population size recovery was fast, less than 20 days. For copepods an almost

streams, reproduction in insects) and reproduction by surviving individuals. In order to evaluate recovery, mesocosm studies need to be carried out in the field (to allow flying insects to lay eggs) and should at the minimum extend a full life cycle of the organisms studied after insecticide dosage.	identical relation between initial decrease and recovery was obtained. It should be noted that most recovery studies analysed the organisms at a "crude" taxonomic level (e.g. Cladocera). Therefore, recovery may take place by increase in "robust" species at the expense of sensitive species resulting in reduced species diversity and thus a decline of environmental quality. Macroinvertebrates: The majority of experiments in the database were terminated within 150 days. Taking the general life cycle length for macroinvertebrates into consideration (ranging from less than a month to several years), the experimental time frames in most mesocosm studies appear to be too short. Based on the few lengthy studies, Chironomids and Isopoda were the most important taxonomic groups in the "slight recovery group" whereas Chironomids and Ephemeropterans dominated the "moderate recovery group" (**6.4.2**). Both groups are considered as good colonisers with short life cycles and this probably explains why they show the most rapid recovery.

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10 Annex

10.1 Annex A

Single species toxicity data used for calculation of Hazard Concentrations. Effect parameter: BMS = biomass; PGR = population growth; IMM = immobilisation; MOR = mortality; Exp Typ (exposure type): S = static, F = flow through; NR = not recorded. Ref#: numbers denote reference number in Aquire data base; MST = data provided by the Danish EPA, Pesticide Manual.

Species	Endpoint	Effect	Dura (days)	Exp Typ	Conc (ug l^{-1})	Ref #
Endosulfan; cas# 115297						
Alonella sp	LC50	MOR	2	S	0.2	786
Anabaena doliolum	EC50*	GRO	10	S	2150	3418
Brachionus calyciflorus	LC50	MOR	1	S	5150	5702
Brachionus calyciflorus	LC50	MOR	1	S	5150	3967
Brachionus calyciflorus	LC50	MOR	1	S	5150	5096
Brachionus calyciflorus	LC50	MOR	1	S	5150	9597
Ceriodaphnia dubia	EC50	IMM	2	S	491	13678
Chlorella vulgaris	EC50*	GRO	10	S	41500	3418
Daphnia carinata	EC50	IMM	2	S	180	5194
Daphnia longispina	LC50	MOR	2	S	0.3	11147
Daphnia magna	EC50	IMM	2	S	307	10526
Daphnia magna	EC50	IMM	2	S	393	615
Daphnia magna	LC50	MOR	2	S	166	632
Daphnia magna	LC50	MOR	2	S	342.69	9479
Daphnia magna	LC50	MOR	2	S	220	9597
Daphnia magna	LC50*	MOR	2	S	97	890
Diaptomus sp	LC50	MOR	2	S	0.6	786
Eucyclops sp	LC50	MOR	2	S	0.1	786
Gammarus fasciatus	LC50	MOR	1	S	10	887
Gammarus fasciatus	LC50	MOR	4	S	6	887
Gammarus lacustris	LC50	MOR	1	S	9.2	885
Gammarus lacustris	LC50	MOR	2	S	6.4	885
Gammarus lacustris	LC50	MOR	4	S	5.8	885
Gammarus lacustris	LC50	MOR	4	S	5.8	666
Lepomis macrochirus	LC50	MOR	4	S	1.2	666
Lepomis macrochirus	LC50*	MOR	4	S	3.85	8096
Moinodaphnia macleayi	EC50	IMM	2	S	215	13678
Oncorhynchus mykiss	LC50	MOR	4	S	1.15	9479
Oncorhynchus mykiss	LC50	MOR	4	S	1.6	10526
Oncorhynchus mykiss	LC50	MOR	4	S	1.31	9479
Oncorhynchus mykiss	LC50	MOR	4	S	1.93	2085
Oncorhynchus mykiss	LC50	MOR	4	S	1.01	9479
Oncorhynchus mykiss	LC50	MOR	4	S	1.4	666
Oncorhynchus mykiss	LC50*	MOR	4	S	0.55	890
Pimephales promelas	LC50	MOR	4	S	1.96	9479

Pimephales promelas	LC50	MOR	4	S	1.13	10526
Pimephales promelas	LC50	MOR	4	S	2.16	9479
Pimephales promelas	LC50	MOR	4	S	1.5	666
Pteronarcys californica	LC50	MOR	1	S	24	889
Pteronarcys californica	LC50	MOR	2	S	5.6	889
Pteronarcys californica	LC50	MOR	4	S	2.3	889
Pteronarcys californica	LC50	MOR	4	S	2.3	666
Spicodiaptomus chilospinu	LC50*	MOR	1	S	50	5264
Spicodiaptomus chilospinu	LC50*	MOR	2	S	40	5264
Fenitrothion; cas# 122145						
Anabaena sp	EC50	BMS	4	NR	2200	15085
Anabaena sp	EC50	PGR	4	NR	1100	15085
Ankistrodesmus falcatus	EC50	BMS	4	NR	2500	15085
Ankistrodesmus falcatus	EC50	PGR	4	NR	3400	15085
Chironomus plumosus	EC50	IMM	2	S	3.3	15574
Chlamydomonas reinhardti	EC50	PGR	4	NR	4800	15085
Chlamydomonas segnis	EC50	PGR	4	NR	6600	15085
Chlorella vulgaris	EC50	PGR	4	NR	24400	15085
Daphnia carinata	LC50	MOR	2	S	20	5194
Daphnia magna	EC50	IMM	2	S	50	984
Daphnia magna	EC50	IMM	2	S	17	15574
Daphnia magna	EC50	IMM	2	S	11	666
Daphnia magna	LC50	MOR	2	S	50	984
Daphnia magna	LC50	MOR	2	S	10.3	3695
Isonychia sp	LC50	MOR	2	F	49	12682
Lepomis macrochirus	LC50	MOR	4	S	2463.6	15574
Lepomis macrochirus	LC50	MOR	4	S	3966.6	666
Moina macrocopa	LC50	MOR	2	S	38.7	984
Navicula sp	EC50	PGR	4	NR	3500	15085
Oncorhynchus mykiss	LC50	MOR	4	S	1818.1	15574
Oncorhynchus mykiss	LC50	MOR	4	S	2600	5867
Oncorhynchus mykiss	LC50	MOR	4	S	1950	3695
Oncorhynchus mykiss	LC50	MOR	4	S	2400	666
Pimephales promelas	LC50	MOR	4	S	3700	15574
Pimephales promelas	LC50	MOR	4	S	4000	666
Pteronarcys californica	LC50	MOR	2	S	17	889
Scenedesmus acutus	EC50	BMS	4	NR	6600	15085
Selenastrum capricornutu	EC50	BMS	4	NR	5020	15085
Staurastrum sp	EC50	PGR	4	NR	800	15085
Dimethoate; cas# 60515						
Baetis rhodani	LC50	MOR	4	F	7	13409
Chlorella pyrenoidosa	EC50	GRO	3	S	470000	5180
Chlorella pyrenoidosa	EC50	GRO	4	S	480000	5180
Daphnia magna	EC50	IMM	2	S	1008.5	600
Daphnia magna	LC50	MOR	2	S	1292.8	600
Daphnia magna	EC50	IMM	2	S	2900	5180
Daphnia magna	LC50	MOR	2	S	6400	5180
Daphnia magna	LC50	MOR	2	S	580	5370
Daphnia magna	LC50	MOR	2	S	6400	5675
Daphnia magna	LC50	MOR	2	S	3220	18476
Gammarus lacustris	LC50	MOR	2	S	400	885
Heptagenia sulphurea	LC50	MOR	4	F	81	13409
Lepomis macrochirus	LC50	MOR	4	S	6000	666
Oncorhynchus mykiss	LC50	MOR	4	S	6200	666

Pteronarcys californica	LC50	MOR	2	S	140	889
Selenastrum	EC50	NR	3	NR	282000	PM
Carbaryl; cas# 63252						
Asellus brevicaudus	LC50	MOR	1	S	320	887
Asellus brevicaudus	LC50	MOR	4	S	280	666
Asellus brevicaudus	LC50	MOR	4	S	240	887
Brachythermis contaminat	LC50	MOR	1	S	0.0144	17128
Brachythermis contaminat	LC50	MOR	2	S	0.0106	17128
Brachythermis contaminat	LC50	MOR	3	S	0.0008	17128
Brachythermis contaminat	LC50	MOR	4	S	0.0006	17128
Ceriodaphnia dubia	LC50	MOR	2	S	11.6	3590
Chauliodes sp	LC50	MOR	1	S	650	5589
Chauliodes sp.	LC50	MOR	3	S	200	5589
Chironomus plumosus	EC50	IMM	2	S	10	15574
Chironomus riparius	EC50	IMM	1	S	110.33	3278
Chironomus riparius	EC50	IMM	1	S	218	18935
Chironomus riparius	EC50	IMM	1	S	110	7293
Chironomus riparius	EC50*	IMM	1	S	104.5	6830
Chironomus riparius	LC50	MOR	1	S	127	12261
Chironomus tentans	EC50	IMM	1	S	4.2666	6267
Chironomus tentans	EC50	IMM	1	S	18000	7796
Chironomus tentans	EC50	IMM	2	S	18000	7796
Chironomus tentans	EC50	IMM	3	S	12000	7796
Chironomus tentans	EC50	IMM	4	S	5900	7796
Cloeon sp.	LC50	MOR	2	S	480	5589
Cloeon sp.	LC50	MOR	3	S	390	5589
Claassenia sabulosa	LC50	MOR	2	S	6.8	889
Cypretta kawatai	EC50	IMM	2	S	5280	7796
Cypridopsis vidua	EC50	IMM	2	S	115	666
Daphnia carinata	EC50	IMM	2	S	35	5194
Daphnia magna	EC50	IMM	2	S	5.6	15574
Daphnia magna	LC50	MOR	2	S	16760	7558
Daphnia magna	LC50	MOR	2	S	10	984
Daphnia magna	LC50	MOR	2	S	9.5	5370
Daphnia magna	LC50	MOR	2	S	7.2	4888
Daphnia pulex	EC50	IMM	2	S	6.4	888
Daphnia pulex	EC50	IMM	2	S	6.4	666
Echinogammarus tibaldii	LC50	MOR	4	NR	6.5	18621
Gammarus lacustris	LC50	MOR	2	S	22	885
Gammarus pulex	LC50	MOR	2	S	29	5589
Lepomis macrochirus	LC50	MOR	4	S	5850	15574
Lepomis macrochirus	LC50	MOR	4	S	5900	942
Lepomis macrochirus	LC50	MOR	4	S	6760	666
Lepomis macrochirus	LC50	MOR	4	S	6850	936
Lepomis macrochirus	LC50*	MOR	4	S	6760	610
Macrobrachium dayanum	LC50	MOR	1	S	42.35	12422
Moina macrocopa	LC50	MOR	2	S	100	984
Oncorhynchus mykiss	LC50	MOR	4	S	2830	12182
Oncorhynchus mykiss	LC50	MOR	4	S	1537.5	15574
Oncorhynchus mykiss	LC50	MOR	4	S	1215	10656
Oncorhynchus mykiss	LC50	MOR	4	S	1950	666
Oncorhynchus mykiss	LC50	MOR	4	S	1470	964
Oncorhynchus mykiss	LC50*	MOR	4	S	4340	610
Oncorhynchus mykiss	LC50*	MOR	4	S	1350	522

Orthetrum albistylum sp.	LC50*	MOR	1	NR	550	7119
Orthetrum albistylum sp.	LC50*	MOR	2	NR	430	7119
Palaemonetes kadiakensis	LC50	MOR	1	NR	410	849
Palaemonetes kadiakensis	LC50	MOR	1	S	120	887
Palaemonetes kadiakensis	LC50	MOR	2	NR	240	849
Palaemonetes kadiakensis	LC50	MOR	3	NR	140	849
Palaemonetes kadiakensis	LC50	MOR	4	NR	120	849
Palaemonetes kadiakensis	LC50	MOR	4	S	5.6	666
Palaemonetes kadiakensis	LC50	MOR	4	S	5.6	887
Palaemonetes kadiakensis	LC50*	MOR	1	S	132.7	2665
Paratya compressa imp.	LC50	MOR	2	S	32	984
Pimephales promelas	LC50	MOR	4	S	14600	15574
Pimephales promelas	LC50	MOR	4	S	14600	666
Pimephales promelas	LC50	MOR	4	S	15940	936
Pimephales promelas	LC50	MOR	4	S	13000	936
Pimephales promelas	LC50*	MOR	4	S	14600	610
Pteronarcella badia	LC50	MOR	2	S	3.6	889
Pteronarcys californica	LC50	MOR	2	S	13	889
Simocephalus serrulatus	EC50	IMM	2	S	7.6	888
Simocephalus serrulatus	EC50	IMM	2	S	7.6	666
Simuliidae	EC50*	DET	1	F	106	2828
Spicodiaptomus chilospin.	LC50*	MOR	1	S	240	5264
Spicodiaptomus chilospin.	LC50*	MOR	2	S	130	5264
Methyxochlor; cas# 72435						
Aedes cantans	LC50	MOR	1	S	31.5	2914
Asellus aquaticus	LC50	MOR	4	S	1	6273
Asellus brevicaudus	LC50	MOR	4	S	34	666
Asellus brevicaudus	LC50	MOR	4	S	3.2	887
Ceriodaphnia dubia	LC50	MOR	2	S	14.1	3590
Pimephales promelas	LC50	MOR	4	F	65	12665
Pimephales promelas	LC50	MOR	4	R	1900	5230
Chironomus tentans	EC50	BEH	4	S	3.33	18128
Chironomus tentans	EC50*	IMM	4	F	2.78	5961
Chironomus tentans	LC50	MOR	4	F	1.62	5070
Chironomus tentans	LC50*	MOR	4	F	5.5	5961
Chlorella pyrenoidosa	LC50	BMS	14	S	1800	17259
Chlorococcum sp	LC50	BMS	14	S	10000	17259
Culex pipiens molestus	LC50	MOR	1	S	18.9	2914
Culex pipiens pipiens	LC50	MOR	1	S	8.9	2914
Culiseta annulata	LC50	MOR	1	S	38.3	2914
Daphnia magna	LC50	MOR	2	S	16	6273
Daphnia pulex	EC50	IMM	2	S	0.78	888
Daphnia pulex	EC50	IMM	2	S	0.78	666
Gammarus fasciatus	LC50	MOR	4	S	1.9	666
Gammarus fasciatus	LC50	MOR	4	S	1.8	887
Gammarus lacustris	LC50	MOR	4	S	0.8	885
Gammarus lacustris	LC50	MOR	4	S	0.8	666
Lepomis macrochirus	LC50	MOR	4	S	56.67	2085
Lepomis macrochirus	LC50	MOR	4	S	32	666
Lepomis macrochirus	LC50	MOR	4	S	47.33	936
Lepomis macrochirus	LC50*	MOR	4	S	62	878
Lumbriculus variegatus	LC50	MOR	1	S	1620	6273
Lumbriculus variegatus	LC50	MOR	2	S	1230	6273
Lumbriculus variegatus	LC50	MOR	4	S	440	6273

Oncorhynchus mykiss	LC50	MOR	4	S	42	2085
Oncorhynchus mykiss	LC50	MOR	4	S	62	666
Oncorhynchus mykiss	LC50*	MOR	4	S	62.6	522
Pimephales promelas	LC50	MOR	4	S	7.5	5070
Pimephales promelas	LC50	MOR	4	S	7.5	5811
Pimephales promelas	LC50	MOR	4	S	39	666
Pimephales promelas	LC50*	MOR	4	S	49.5	878
Pteronarcella badia	LC50	MOR	4	S	5	666
Pteronarcys californica	LC50	MOR	4	S	1.4	889
Pteronarcys californica	LC50	MOR	4	S	1.4	666
Pteronarcys californica	LC50	MOR	2	S	8	889
Pteronarcys californica	LC50	MOR	4	S	1.4	889
Pteronarcys californica	LC50	MOR	4	S	1.4	666
Scenedesmus acutus	LC50	BMS	14	S	13000	17259
Scenedesmus quadricauda	LC50	BMS	14	S	7000	17259
Simocephalus serrulatus	EC50	IMM	2	S	5	888
Simocephalus serrulatus	EC50	IMM	2	S	5	666
Stenacron interpunctatum	LC50	MOR	4	F	1.96	5070
Stenonema candidum	EC50*	IMM	4	F	1.965	5961
Stenonema sp	EC50*	IMM	4	F	1.49	5961
Stichococcus sp	LC50	BMS	14	S	30000	17259
Azinphos-met; cas# 86500						
Asellus brevicaudus	LC50	MOR	4	S	21	666
Asellus brevicaudus	LC50	MOR	4	S	21	887
Daphnia magna	EC50	IMM	2	R	1.6	6449
Gammarus fasciatus	LC50	MOR	4	S	0.15	666
Gammarus fasciatus	LC50	MOR	4	S	0.24	887
Gammarus lacustris	LC50	MOR	4	S	0.126	528
Gammarus lacustris	LC50	MOR	4	S	0.15	885
Gammarus lacustris	LC50*	MOR	4	S	0.126	2094
Hyalella azteca	LC50	MOR	4	S	0.29	352
Lepomis macrochirus	LC50	MOR	4	S	9	14914
Lepomis macrochirus	LC50	MOR	4	S	6.17	2085
Lepomis macrochirus	LC50	MOR	4	S	120	942
Lepomis macrochirus	LC50	MOR	4	S	22	666
Lepomis macrochirus	LC50	MOR	4	S	15.52	936
Lepomis macrochirus	LC50*	MOR	4	S	22	610
Lepomis macrochirus	LC50*	MOR	4	S	5.2	2893
Oncorhynchus mykiss	LC50	MOR	4	S	7.1	501
Oncorhynchus mykiss	LC50	MOR	4	S	6.2	2085
Oncorhynchus mykiss	LC50	MOR	4	S	4.3	666
Oncorhynchus mykiss	LC50*	MOR	4	S	14	610
Oncorhynchus mykiss	LC50*	MOR	4	S	3.2	522
Pimephales promelas	LC50	MOR	4	S	205.67	14914
Pimephales promelas	LC50	MOR	4	S	235	666
Pimephales promelas	LC50	MOR	4	S	353.83	936
Pimephales promelas	LC50*	MOR	4	S	235	610
Pimephales promelas	LC50*	MOR	4	S	93	2893
Pteronarcys californica	LC50	MOR	4	S	22	528
Pteronarcys californica	LC50	MOR	4	S	1.5	889
Pteronarcys californica	LC50	MOR	4	S	1.9	666
Pteronarcys californica	LC50*	MOR	4	S	22	2667

2,4-D; cas# 94757						
Brachionus calyciflorus	EC50	REP	2	S	128000	3963
Brachionus calyciflorus	LC50	MOR	2	S	117000	3963
Ceriodaphnia dubia	LC50	MOR	2	S	422000	18961
Ceriodaphnia dubia	LC50	MOR	2	S	236000	3590
Daphnia magna	EC50*	IMM	2	S	100000	886
Daphnia magna	LC50	MOR	2	S	25000	11504
Daphnia magna	LC50*	MOR	2	S	135000	2877
Daphnia magna	LC50*	MOR	2	S	148682	2877
Daphnia pulex	EC50	IMM	2	S	3200	888
Gammarus fasciatus	LC50	MOR	4	S	2400	666
Gammarus fasciatus	LC50*	MOR	2	S	3200	886
Lepomis macrochirus	LC50	MOR	4	S	7400	666
Lepomis macrochirus	LC50	MOR	4	S	263000	11504
Oncorhynchus mykiss	LC50	MOR	4	S	12460	666
Oncorhynchus mykiss	LC50	MOR	4	S	358000	11504
Pimephales promelas	LC50	MOR	4	S	4500	666
Pimephales promelas	LC50	MOR	4	S	263000	11504
Selenastrum capricornut.	EC50	PGR	4	S	41772	18093
Simocephalus serrulatus	EC50	IMM	2	S	4900	888
Stylonychia mytilus	LC50*	MOR	3	S	294500	2877
Mexacarbate; cas# 315184						
Chironomus riparius	EC50	IMM	1	S	23.4	7293
Chironomus riparius	EC50*	IMM	1	S	12.2	6830
Chironomus tentans	EC50	IMM	1	S	1.8	6267
Daphnia pulex	EC50	IMM	2	S	10	888
Daphnia pulex	EC50	IMM	2	S	10	666
Gammarus lacustris	LC50	MOR	2	S	76	885
Lepomis macrochirus	LC50	MOR	4	S	10413.	665
Lepomis macrochirus	LC50	MOR	4	S	22900	666
Lepomis macrochirus	LC50*	MOR	4	S	11200	610
Oncorhynchus mykiss	LC50	MOR	4	S	20000	501
Oncorhynchus mykiss	LC50	MOR	4	S	12000	666
Oncorhynchus mykiss	LC50*	MOR	4	S	10200	610
Pimephales promelas	LC50	MOR	4	S	23700	665
Pimephales promelas	LC50	MOR	4	S	17000	666
Pimephales promelas	LC50*	MOR	4	S	17000	610
Pteronarcys californica	LC50	MOR	2	S	16	889
Simocephalus serrulatus	EC50	IMM	2	S	13	888
Simocephalus serrulatus	EC50	IMM	2	S	13	666
Simulium venustum	LC50	MOR	2	F	124	12682
Linuron; cas# 330552						
Chlorella vulgaris	EC50	PGR	5	NR	50	11658
Daphnia magna	EC50	IMM	1	NR	590	11658
Daphnia magna	EC50	IMM	1	NR	310	11658
Daphnia sp	EC50	IMM	1	NR	360	11658
Diaptomus gracilis	EC50	IMM	1	NR	330	11658
Diazinon; cas# 333415						
Acroneuria ruralis	LC50	MOR	2	S	294	7581
Asellus communis	LC50	MOR	4	S	21	7581
Baetis intermedius	LC50	MOR	1	S	358	7581
Baetis intermedius	LC50	MOR	2	S	55	7581
Baetis intermedius	LC50	MOR	4	S	24	7581
Brachionus calyciflorus	LC50	MOR	2	S	31000	3963

Ceriodaphnia dubia	LC50	MOR	2	S	0.5	821
Ceriodaphnia dubia	LC50	MOR	2	S	0.402	16043
Ceriodaphnia dubia	LC50	MOR	2	S	0.435	18190
Chironomus tentans	LC50	MOR	2	S	0.1	7581
Chironomus tentans	LC50	MOR	7	S	0.027	7581
Chironomus tentans	LC50	MOR	3	S	0.07	7581
Chironomus tentans	LC50	MOR	4	S	0.03	7581
Chironomus tentans	LC50	MOR	4	S	10.7	352
Daphnia magna	EC50	IMM	2	S	1.22	866
Daphnia magna	EC50	IMM	2	S	1.22	5894
Daphnia magna	LC50	MOR	2	S	0.8	821
Daphnia magna	LC50	MOR	2	S	1	984
Daphnia magna	LC50	MOR	2	S	0.75	5370
Daphnia magna	LC50	MOR	2	S	0.96	13007
Daphnia magna	LC50*	MOR	2	S	2	551
Daphnia pulex	EC50	IMM	2	S	0.9	888
Daphnia pulex	EC50	IMM	2	S	0.8	666
Daphnia pulex	LC50	MOR	2	S	0.65	821
Gammarus lacustris	LC50	MOR	2	S	229	7581
Gammarus lacustris	LC50	MOR	2	S	500	885
Gammarus pseudolimna.	LC50	MOR	2	S	4	7581
Hyalella azteca	LC50	MOR	2	S	22	7581
Lepomis macrochirus	LC50	MOR	4	S	22	13001
Lepomis macrochirus	LC50	MOR	4	S	120	551
Lepomis macrochirus	LC50	MOR	4	S	136	13000
Lepomis macrochirus	LC50	MOR	4	S	245	5311
Lepomis macrochirus	LC50	MOR	4	S	350	866
Lepomis macrochirus	LC50	MOR	4	S	350	5894
Moina macrocopa	LC50	MOR	2	S	10	984
Oncorhynchus mykiss	LC50	MOR	4	S	90	13001
Oncorhynchus mykiss	LC50	MOR	4	S	1350	551
Oncorhynchus mykiss	LC50	MOR	4	S	400	13000
Oncorhynchus mykiss	LC50	MOR	4	S	3200	12999
Oncorhynchus mykiss	LC50	MOR	4	S	90	666
Paraleptophlebia pallipes	LC50	MOR	1	S	243	7581
Paraleptophlebia pallipes	LC50	MOR	2	S	134	7581
Paraleptophlebia pallipes	LC50	MOR	6	S	43	7581
Paraleptophlebia pallipes	LC50	MOR	7	S	32	7581
Paraleptophlebia pallipes	LC50	MOR	3	S	85	7581
Paraleptophlebia pallipes	LC50	MOR	4	S	44	7581
Pimephales promelas	LC50	MOR	4	S	10300	551
Pimephales promelas	LC50	MOR	4	S	3700	866
Pimephales promelas	LC50	MOR	4	S	5591.5	5894
Pimephales promelas	LC50	MOR	4	S	5200	15462
Pteronarcys californica	LC50	MOR	2	S	60	889
Selenastrum capricornutu	EC50	PSR	7	S	6400	13002
Lindane; cas# 608731						
Daphnia pulex	EC50	IMM	2	S	680	666
Gammarus lacustris	LC50	MOR	4	S	78	666
Lepomis macrochirus	LC50	MOR	4	S	67	666
Lepomis macrochirus	LC50*	MOR	4	S	790	878
Oncorhynchus mykiss	LC50	MOR	4	S	18	666
Pimephales promelas	LC50	MOR	4	S	7562.5	666
Pimephales promelas	LC50*	MOR	4	S	2300	878

Pimephales promelas	LC50*	MOR	4	S	7500	878
Pteronarcys californica	LC50	MOR	4	S	18	666
Glyphosate; cas# 1071836						
Chironomus plumosus	EC50	IMM	2	S	55000	666
Chironomus plumosus	EC50	IMM	2	S	55000	5752
Chlorella pyrenoidosa	EC50	PGR	4	S	394638	4338
Daphnia magna	EC50	IMM	2	S	61720	17455
Daphnia spinulata	EC50	IMM	2	S	66180	17455
Lepomis macrochirus	LC50	MOR	4	S	166666	5752
Lepomis macrochirus	LC50	MOR	4	S	135000	666
Myriophyllum spicatum	EC50	DVP	5	NR	1600	13730
Oncorhynchus mykiss	LC50	MOR	4	S	6053235	4070
Oncorhynchus mykiss	LC50	MOR	4	S	4290800	4070
Oncorhynchus mykiss	LC50	MOR	4	S	96000	924
Oncorhynchus mykiss	LC50	MOR	4	S	240000	5752
Oncorhynchus mykiss	LC50	MOR	4	S	76333	924
Oncorhynchus mykiss	LC50	MOR	4	S	173333	5752
Oncorhynchus mykiss	LC50	MOR	4	S	140000	5752
Oncorhynchus mykiss	LC50	MOR	4	S	130000	666
Pimephales promelas	LC50	MOR	4	S	97000	5752
Pimephales promelas	LC50	MOR	4	S	97000	666
Scenedesmus acutus	EC50	PGR	4	S	10200	18456
Scenedesmus quadricauda	EC50	PGR	4	S	7200	18456
Carbofuran; cas# 1563662						
Brachythermis contaminat	LC50	MOR	2	S	0.19	17128
Chironomus riparius	EC50	MOR	2	S	56	12280
Chlorella pyrenoidosa	EC50*	PGR	4	S	272640	6353
Chlorella pyrenoidosa	EC50*	PGR	4	S	204480	6353
Daphnia magna	EC50	MOR	2	S	48	12280
Daphnia magna	EC50	MOR	2	S	86.1	17129
Gammarus pulex	LC50	MOR	2	R	12.5	15357
Lepomis macrochirus	LC50	MOR	4	S	80	942
Lepomis macrochirus	LC50	MOR	4	S	240	666
Oncorhynchus mykiss	LC50	MOR	4	S	380	666
Pimephales promelas	LC50	MOR	4	S	872	666
Pimephales promelas	LC50	MOR	4	F	844	3217
Pimephales promelas	LC50	MOR	4	F	844	17263
Atrazine; cas# 15912249						
Ceriodaphnia dubia	LC50	MOR	2	S	30000	3590
Chironomus riparius	EC50	MOR	2	S	1000	12280
Chironomus tentans	LC50	MOR	2	S	720	631
Daphnia magna	EC50	IMM	2	S	39000	13154
Daphnia magna	LC50	MOR	2	S	6900	631
Gammarus fasciatus	LC50	MOR	2	S	5700	631
Lepomis macrochirus	LC50	MOR	4	S	16000	546
Lepomis macrochirus	LC50	MOR	4	S	50000	546
Oncorhynchus mykiss	LC50	MOR	4	S	4500	12999
Oncorhynchus mykiss	LC50	MOR	4	S	12900	546
Pimephales promelas	LC50	MOR	4	R	15000	631
Scenedesmus abundans	EC50	GRO	4	S	110	11677
Selenastrum capricornutu	EC50	PGR	4	S	128.2	18933
Selenastrum capricornutu	EC50	PGR	4	S	235	18093
Selenastrum capricornutu	LC50	PGR	4	S	26	17098
Tetrahymena pyriformis	EC50	PGR	2	S	96000	4008

Aminocarb; cas# 2032599						
Algae	EC50	PSE	1.17	S	560	10875
Asellus racovitzai	LC50	MOR	4	R	21800	11218
Chironomus plumosus	EC50	IMM	2	S	162.5	15574
Chironomus plumosus	EC50	IMM	2	S	270	666
Daphnia magna	EC50	IMM	2	S	19	15574
Daphnia magna	EC50	IMM	2	S	32	666
Lepomis macrochirus	LC50	MOR	4	S	5340	15574
Lepomis macrochirus	LC50	MOR	4	S	1600	666
Oncorhynchus mykiss	LC50	MOR	4	S	15515	15574
Oncorhynchus mykiss	LC50	MOR	4	S	18465	10668
Oncorhynchus mykiss	LC50	MOR	4	S	1000	5867
Oncorhynchus mykiss	LC50	MOR	4	S	32000	666
Oncorhynchus mykiss	LC50	MOR	4	S	373166	10311
Oncorhynchus mykiss	LC50	MOR	4	S	6815	666
Pimephales promelas	LC50	MOR	4	S	4290	15574
Pimephales promelas	LC50	MOR	4	S	4287.5	666
Pteronarcella badia	LC50	MOR	4	S	24.33	5618
Chlorpyifos; cas# 2921882						
Asellus aquaticus	EC50	IMM	2	R	3.5	8107
Brachionus calyciflorus	LC50	MOR	2	S	12000	3963
Ceriodaphnia dubia	LC50	MOR	2	S	0.08	18190
Claassenia sabulosa	LC50	MOR	2	S	1.8	889
Copepoda	LC50	MOR	2	S	2.13	12821
Daphnia longispina	EC50	IMM	2	S	0.55	8107
Daphnia magna	LC50	MOR	2	S	1	16353
Daphnia pulex	EC50	IMM	2	S	0.25	18477
Daphnia pulex	LC50	MOR	2	S	0.25	18477
Gammarus lacustris	LC50	MOR	2	S	0.4	885
Lepomis macrochirus	LC50	MOR	4	S	30	942
Lepomis macrochirus	LC50	MOR	4	S	2.4	666
Lepomis macrochirus	LC50	MOR	4	F	10	10775
Oncorhynchus mykiss	LC50	MOR	4	S	24.37	2085
Oncorhynchus mykiss	LC50	MOR	4	S	7.1	666
Pimephales promelas	EC50	ABN	4	S	54.9	12885
Pimephales promelas	LC50	MOR	4	S	150	15462
Pimephales promelas	LC50	MOR	4	S	122.2	12885
Pteronarcella badia	LC50	MOR	2	S	1.8	889
Pteronarcys californica	LC50	MOR	2	S	18	889
Simocephalus vetulus	EC50	IMM	2	S	0.6	8107
Alachlor; cas# 15972608						
Ceriodaphnia dubia	LC50	MOR	2	S	7900	3590
Ceriodaphnia dubia	LC50	MOR	2	S	14360	13689
Chlorella pyrenoidosa	EC50	PGR	4	S	111	4338
Daphnia pulex	EC50	MOR	2	NR	9700	11433
Echinogammarus tibaldii	LC50	MOR	4	NR	13000	18621
Gammarus italicus	LC50	MOR	4	NR	19700	18621
Lemna minor	EC50	MOR	2	NR	12.3	11433
Lemna minor	EC50	PGR	4	S	198	18093
Oncorhynchus mykiss	LC50	MOR	4	S	1900	666
Pimephales promelas	LC50	MOR	4	F	5000	12858
Pimephales promelas	LC50	MOR	4	F	5000	15031
Pimephales promelas	LC50*	MOR	4	F	5000	10635
Selenastrum capricornut.	EC50	PGR	4	S	6	18093

Diflubenzuron; cas# 35367385						
Chironomus plumosus	EC50	IMM	2	S	560	939
Chironomus plumosus	EC50	IMM	2	S	560	666
Daphnia magna	EC50	IMM	2	S	15	939
Daphnia magna	EC50	IMM	2	S	16	666
Daphnia magna	LC50	MOR	2	S	5.29	11595
Daphnia magna	LC50	MOR	2	S	4.55	11595
Gammarus pseudolimnaeu	LC50	MOR	4	S	30	5238
Gammarus pseudolimnaeu	LC50	MOR	4	S	30	939
Gammarus pseudolimnaeu	LC50	MOR	4	S	27.5	666
Hyalella azteca	LC50	MOR	5	F	1.84	11595
Lepomis macrochirus	LC50	MOR	4	S	660000	939
Oncorhynchus mykiss	LC50	MOR	4	S	240000	939
Oncorhynchus mykiss	LC50	MOR	4	S	170000	666
Pimephales promelas	LC50	MOR	4	S	430000	939
Pimephales promelas	LC50	MOR	4	S	100000	666
Hexazinone; cas# 51235042						
Cyclotella meneghiniana	EC50	PSE	1	S	32	18372
Daphnia	LC50	NR	2	NR	442000	PM
Lepomis macrochirus	LC50	MOR	4	S	100000	666
Lepomis macrochirus	LC50	MOR	4	S	395000	PM
Nitzschia sp	EC50	PSE	1	S	61	18372
Oncorhynchus mykiss	LC50	MOR	4	S	1031000	13181
Oncorhynchus mykiss	LC50	MOR	4	S	100000	666
Oncorhynchus mykiss	LC50	MOR	4	S	380000	PM
Scenedesmus quadricauda	EC50	PSE	1	S	14	18372
Selenastrum capricornut.	EC50	CLR	3	S	56	95
Selenastrum capricornut.	EC50	CLR	5	S	85	95
Selenastrum capricornut.	EC50	CLR	7	S	126	95
Selenastrum capricornut.	EC50	PSE	1	S	9	18372
Fenvalerate; cas# 51630581						
Ceriodaphnia lacustris	EC50	IMM	2	S	0.21	12564
Chironomus decorus	LC50	MOR	1	S	18	6268
Chironomus utahensis	LC50	MOR	1	S	4.2	6268
Daphnia galeata mendotae	EC50	IMM	2	S	0.225	12564
Daphnia magna	EC50	IMM	2	S	1.675	12564
Daphnia magna	EC50	IMM	2	S	1.59	9991
Daphnia magna	LC50	MOR	2	S	4.3	5679
Daphnia magna	LC50	MOR	2	S	2.75	16674
Daphnia magna	LC50	MOR	2	S	1.2	16674
Lepomis macrochirus	LC50	MOR	2	S	1.21	708
Oncorhynchus mykiss	LC50	MOR	4	F	2.1	10536
Oncorhynchus mykiss	LC50	MOR	4	F	0.172	12019
Pimephales promelas	LC50	MOR	4	S	14.09	15277
Procladius sp	LC50	MOR	1	S	7.2	6268
Skistodiaptomus oregonen	EC50	IMM	2	S	0.12	12564
Permethrin; cas# 52645531						
Alonella sp	LC50	MOR	2	S	4	786
Anabaena inaequalis	EC50	BMS	13	S	1600	15991
Anabaena inaequalis	EC50	GRO	13	S	5000	15991
Ceriodaphnia dubia	LC50	MOR	2	S	0.55	85
Chlorella kessleri	LC50	MOR	5	S	44500	11852
Cypria sp	LC50	MOR	2	S	5	786

Daphnia carinata	EC50	IMM	2	S	50	5194
Daphnia magna	LC50	MOR	2	S	13.45	11852
Daphnia magna	LC50	MOR	2	S	1.25	85
Daphnia magna	LC50	MOR	2	S	1.95	12004
Daphnia magna	LC50	MOR	2	S	1.95	17559
Daphnia pulex	LC50	MOR	2	S	7.77	101
Diaptomus sp	LC50	MOR	2	S	7	786
Eucyclops sp	LC50	MOR	2	S	5	786
Gammarus pseudolimnaeu	LC50	MOR	2	S	0.33	12852
Gammarus pseudolimnaeu	LC50	MOR	2	S	0.25	12268
Lepomis macrochirus	LC50	MOR	4	F	5.185	12004
Lepomis macrochirus	LC50	MOR	4	F	5.81	17559
Oncorhynchus mykiss	LC50	MOR	4	S	5.26	10656
Pimephales promelas	LC50	MOR	4	S	23.24	15277
Spicodiaptomus chilospinu	LC50*	MOR	2	S	5	5264
Tanytarsus dissimilis	LC50	MOR	2	S	2.5	12004
Tanytarsus dissimilis	LC50	MOR	2	S	2.5	17559
Deltamethrin; cas# 52918635						
Chironomus decorus	LC50	MOR	1	S	1.1	6268
Chironomus decorus	LC50	MOR	1	S	0.27	3671
Chironomus utahensis	LC50	MOR	1	S	0.29	6268
Cricotopus sp	LC50	MOR	1	S	0.13	3671
Daphnia magna	EC50	IMM	2	S	60	7357
Daphnia magna	EC50	IMM	2	S	0.64	9991
Daphnia magna	LC50	MOR	2	S	0.05	225
Dicrotendipes californicus	LC50	MOR	1	S	1.75	3671
Oncorhynchus mykiss	LC50	MOR	1	S	2.37	225
Procladius sp	LC50	MOR	1	S	0.067	6268
Selenastrum cap	EC50	NR	4	NR	9100	PM
Tanypus nubifer	LC50	MOR	1	S	0.11	3671
Triclopyr ester; cas# 55335063						
Daphnia	EC50	NR	4	S	133000	PM
Daphnia pulex	EC50	IMM	4	S	1200	12591
Lepomis macrochirus	LC50	MOR	4	S	100000	666
Oncorhynchus mykiss	LC50	MOR	1	S	4750	12605
Oncorhynchus mykiss	LC50	MOR	1	F	790	13652
Oncorhynchus mykiss	LC50	MOR	2	S	4450	12605
Oncorhynchus mykiss	LC50	MOR	0.25	F	1950	13652
Oncorhynchus mykiss	LC50	MOR	3	S	4350	12605
Oncorhynchus mykiss	LC50	MOR	4	S	2200	12591
Oncorhynchus mykiss	LC50	MOR	4	S	100000	666
Oncorhynchus mykiss	LC50	MOR	4	S	4300	12605
Selenastrum capricornutu	EC50	NR	5	NR	45000	PM
Propiconazole; cas# 60207901						
Baetis rhodani	LC50	MOR	4	F	900	13409
Chlamydomonas noctigam	EC50	PGR	3	NR	0.8	16010
Chlamydomonas reinhardti	EC50	PGR	3	NR	6500	16010
Cyclotella sp	EC50	PGR	6	NR	3300	16010
Daphnia magna	LC50	IMM	1	NR	3.16	16005
Daphnia pulex	LC50	IMM	1.5	NR	3.16	16005
Gammarus lacustris	LC50	MOR	4	F	1300	13409
Heptagenia sulphurea	LC50	MOR	4	F	1000	13409
Microcystis aeruginosa	EC50	PGR	6	NR	1000	16010
Oncorhynchus mykiss	LC50	MOR	4	NR	5300	PM

Selenastrum capricornutu	EC50	PGR	3	NR	5000	16010
Synechococcus leopoliensi	EC50	PGR	5	NR	4500	16010
Esfenvalerate; cas# 66230044						
Daphnia magna	LC50	MOR	2	S	0.27	3897
Daphnia magna	LC50	NR	2	NR	0.24	PM
Lepomis macrochirus	LC50	MOR	4	S	0.44	14914
Lepomis macrochirus	LC50	MOR	4	S	0.31	3897
Pimephales promelas	LC50	MOR	4	S	0.26	14914
Trahalomethrin; cas# 66841256						
Anguilla japonica	LC50	MOR	1	NR	43.3	8570
Anguilla japonica	LC50	MOR	2	NR	12.1	8570
Ceriodaphnia dubia	LC50	MOR	2	S	0.26	85
Culex quinquefasciatus	LC50	MOR	1	S	0.58	11492
Daphnia magna	EC50	NR	2	NR	0.432	MST
Daphnia magna	EC50	NR	2	NR	0.250	MST
Daphnia magna	EC50	NR	2	NR	2.200	MST
Daphnia magna	LC50	MOR	2	S	0.15	85
Lepomis macrochirus	LC50	NR	4	NR	2.800	MST
Lepomis macrochirus	LC50	NR	4	NR	3.312	MST
Lepomis macrochirus	LC50	NR	4	NR	4.300	MST
Lepomis macrochirus	LC50	NR	4	NR	1.350	MST
Lepomis macrochirus	LC50	NR	4	NR	1.764	MST
Lepomis macrochirus	LC50	NR	4	NR	2.030	MST
Oncorhynchus mykiss	LC50	NR	4	NR	1.600	MST
Oncorhynchus mykiss	LC50	NR	4	NR	1.598	MST
Oncorhynchus mykiss	LC50	NR	4	NR	1.080	MST
Oncorhynchus mykiss	LC50	NR	4	NR	1.880	MST
Oncorhynchus mykiss	LC50*	NR	4	NR	4.320	MST
Cyfluthrin; cas# 68359375						
Ceriodaphnia dubia	LC50	MOR	2	S	0.14	85
Culex quinquefasciatus	LC50	MOR	1	NR	0.7	14514
Culex quinquefasciatus	LC50	MOR	1	S	0.3	11492
Daphnia magna	LC50	MOR	2	S	0.17	85
Daphnia magna	LC50	NR	2	NR	0.25	MST
Daphnia magna	LC50	NR	3	NR	0.141	MST
Daphnia magna	LC50	NR	4	NR	0.17	MST
Daphnia magna	LC50	NR	5	NR	0.16	MST
Lepomis macrochirus	LC50	MOR	4	NR	1.5	MST
Lepomis macrochirus	LC50	NR	4	NR	0.209	MST
Lepomis macrochirus	LC50	NR	4	NR	0.87	MST
Lepomis macrochirus	LC50	NR	4	NR	0.998	MST
Lepomis macrochirus	LC50	NR	4	NR	1.5	MST
Oncorhynchus mykiss	LC50	MOR	2	S	0.57	4175
Oncorhynchus mykiss	LC50	NR	4	NR	0.3	MST
Oncorhynchus mykiss	LC50	NR	4	NR	0.3	MST
Oncorhynchus mykiss	LC50	NR	4	NR	0.68	MST
Oncorhynchus mykiss	LC50	NR	4	NR	0.68	MST
Oncorhynchus mykiss	LC50	NR	4	NR	2.9	MST
Oncorhynchus mykiss	LC50	NR	4	NR	2.9	MST
Scenedesmus	EC50	NR	4	NR	100000	MST
Selenastrum	EC50	NR	4	NR	1000000	MST
Glufosinate-am; cas# 77182822						
Daphnia	EC50	NR	2	R	560000	PM
Daphnia	EC50	NR	2	R	1000000	PM

Lepomis macrochirus	LC50*	MOR	4	S	1000000	PM
Oncorhynchus mykiss	LC50	MOR	4	S	710000	PM
Selenastum	EC50	NR	2	R	37000	PM
Bifenthrin; cas# 82657043						
Ceriodaphnia dubia	LC50	MOR	2	S	0.07	85
Daphnia magna	LC50	MOR	2	S	0.32	85
Daphnia magna	LC50	MOR	2	S	0.32	MST
Daphnia magna	LC50	MOR	2	S	0.111	MST
Daphnia magna	LC50	MOR	2	S	1.5	MST
Daphnia magna	LC50	MOR	2	S	1.6	MST
Daphnia magna	LC50	MOR	2	S	0.16	PM
Lepomis macrochirus	LC50	MOR	4	S	0.26	MST
Lepomis macrochirus	LC50	MOR	4	S	0.35	MST
Lepomis macrochirus	LC50	MOR	4	S	0.35	PM
Oncorhynchus mykiss	LC50	MOR	4	S	0.1	MST
Oncorhynchus mykiss	LC50	MOR	4	S	0.15	MST
Oncorhynchus mykiss	LC50	MOR	4	S	0.15	PM
Lambda-cyhalothrin; cas# 91465086						
Ceriodaphnia dubia	LC50	MOR	2	S	0.3	85
Daphnia	EC50	MOR	2	S	0.36	PM
Daphnia magna	EC50	MOR	2	S	16	MST
Daphnia magna	EC50	MOR	2	S	90	MST
Daphnia magna	EC50	MOR	2	S	90	MST
Daphnia magna	EC50	MOR	2	S	1040	MST
Daphnia magna	EC50	MOR	2	S	190	MST
Daphnia magna	EC50	MOR	2	S	350	MST
Daphnia magna	EC50	MOR	2	S	380	MST
Daphnia magna	EC50	MOR	2	S	1800	MST
Daphnia magna	EC50	MOR	2	S	440	MST
Daphnia magna	EC50	MOR	2	S	260	MST
Daphnia magna	EC50	MOR	2	S	660	MST
Daphnia magna	LC50	MOR	2	S	1.04	85
Gambusia affinis	LC50	MOR	1	S	0.181	184
Gambusia affinis	LC50	MOR	1	S	0.076	184
Lepomis macrochirus	LC50	MOR	4	NR	0.21	PM
Lepomis macrochirus	LC50	MOR	4	NR	0.21	MST
Lepomis macrochirus	LC50	MOR	4	NR	0.284	MST
Lepomis macrochirus	LC50	MOR	4	NR	1.3	MST
Lepomis macrochirus	LC50	MOR	4	NR	0.46	MST
Lepomis macrochirus	LC50	MOR	4	NR	1.3	MST
Oncorhynchus mykiss	LC50	MOR	4	NR	0.24	PM
Oncorhynchus mykiss	LC50	MOR	4	NR	0.34	MST
Oncorhynchus mykiss	LC50	MOR	4	NR	0.24	MST
Oncorhynchus mykiss	LC50	MOR	4	NR	0.399	MST
Oncorhynchus mykiss	LC50	MOR	4	NR	0.44	MST
Oncorhynchus mykiss	LC50	MOR	4	NR	0.44	MST
Oncorhynchus mykiss	LC50	MOR	4	NR	0.54	MST
Oncorhynchus mykiss	LC50	MOR	4	NR	0.928	MST
Oncorhynchus mykiss	LC50	MOR	4	NR	3	MST
Oncorhynchus mykiss	LC50	MOR	4	NR	1.058	MST
Selenastrum capricornutu	EC50	NR	4	S	580000	MST
Selenastrum capricornutu	EC50	NR	4	S	27000000	MST
Selenastrum capricornutu	EC50	NR	4	S	71000000	MST

Tebufenozide; cas# 112410238						
Aedes aegypti	LC50	MOR	2	S	920	18476
Daphnia	EC50	NR	2	S	3800	PM
Daphnia magna	LC50	MOR	2	S	17370	18476
Oncorhynchus mykiss	LC50*	NR	4	NR	5700	PM
Oncorhynchus mykiss	LC50*	MOR	4	S	5700	PM
Oncorhynchus mykiss	LC50*	MOR	4	NR	830	CanEPA
Scenedesmus	EC50	NR	4	NR	160	CanEPA
Selenastrum c.	EC50	NR	5	NR	640	PM

10.2 Annex B

Overview of experimental conditions in mesocosm experiments contained in database. End of application = last day of pesticide dosing; Interval = interval between pesticide dosings; Sediment (1 = present; 0 = without sediment); Macrophytes (1 = present; 0 = without macrophytes);Field/lab (1 = field study; 0 = Laboratory study).

Experiment	Pesticide	End of application	Interval	Volume	Avr. Depth	Sediment	Makrophytes	field/lab	Latitude	Longitude	Reference
		day	day	litre	m						
16ank	Atrazine	52	26	1000	0.8	1		1	48,15N	11,34E	Juttner et al. 1995
25tll	Endosulfan	21	0	3	0.1	1	1	0			Barry 1998
26tll	Diflubenzuron	150	150	700000	1.5	1	0	1	31,15N	89,50W	Boyle et al. 1996
27tll	Diflubenzuron	30	15	700000	1.5	1	0	1	31,15N	89,50W	Boyle et al. 1996
28tll	Chlorpyrifos	0	0	25000	0.5	1	1	1	46,45N	92,07W	Brazner & Kline 1990
33tll	Fenvalerate	0		125000	5	1	0	1	43,6N	79,3W	Day et al. 1997
38ank	Lindan	14	7	300	0.6	1	0	0			Fliedner & Klein 1996
38tll	Glufosinate-am.	291	0	16000	0.9	1		1	46,5N	84,07W	Faber et al. 1998
42flm	Chlorpyrifos	0	0	55000	0.5	1	1	1	51,58N	5,40E	Brink et al. 1996
43flm	Methoxychlor	0	0	100000	4	1	0	1	43,6N	79,3E	Stephenson et al. 1986
44flm	Methoxychlor	0	0	100000	4	1	0	1	43,6N	79,3E	Stephenson et al. 1986
44tll	2,4 D	0	0	600	0.6	1	1	1	52,07N	106,38W	Forsyth et al. 1997
45flm	Methoxychlor	0	0	100000	4	1	0	1	43,6N	79,3E	Solomon et al. 1987
46flm	Methoxychlor	35	0	100000	4	1	0	1	43,6N	79,3E	Solomon et al. 1987
47flm	Esfenvalerate	70	17	700000	1.5	1	1	1	31,5N	89,5W	Fairchild et al. 1992b
48flm	Hexazinone	0	0	120000	4.8	1	0	1	46,5N	84,03W	Thompson et al. 1993
49flm	Tebufenozide	0	0	50000	2.5	1	0	1	46,5N	84,03W	Kreutzweiser & Thomas 1995
50flm	Chlorpyrifos	0	0	700	0.7	1	1	0			Brock, T. et al. 1992
51flm	Chlorpyrifos	0	0	700	0.7	1	0	0			Brock, T. et al. 1992

Experiment	Pesticide	End of application	Interval	Volume	Avr. Depth	Sediment	Makrophytes	field/lab	Latitude	Longitude	Reference
		day	day	litre	m						
52flm	Chlorpyrifos	0	0	55000	0.5	1	1	1	51,58N	5,40E	Wijngaarden et al. 1996
53flm	Chlorpyrifos	0	0	700	0.7	1	1	0			Donk et al. 1995
54flm	Azinphos-methyl	0	0	25000	0.5	1	1	1	46,45N	92,07W	Sierszen & Lozano 1998
55flm	Linuron	28	3	600	0.5	1	1	0			Brink et al. 1997
56flm	Chlorpyrifos	49	1	600	0.5	1	0	0			Brink et al. 1995
57flm	Esfenvalerate	28	0	25000	0.5	1	1	1	46,45N	92,07W	Lozano et al. 1992
57tll	Diazinon	21	7	11200	1.3	1	1	1	38,58N	95,14W	Giddings et al. 1996
58flm	Diflubenzuron	0	0	400000	1	1		1	34,30N	91.33W	Ludwig 1993
59flm	Esfenvalerate	70	10	1100000	1	1	1	1	32N	87W	Webber 1992
60flm	Lambda-cyhalothrin	42	14	25000	1	1	1	1	51,3N	1,20W	Farmer et al. 1995
61flm	Atrazine	36	0	120000	4.8	1	0	1	46,5N	84,03W	Herman et al. 1986
61tll	Atrazine	56	0	120000	4.8	1	0	1	43,6N	79,3W	Hamilton et al. 1987
62flm	Atrazine	0	0	450000	2	1	1	1	38N	94W	Dewey 1986
63flm	Deltamethrin	0	0	16000	0.4	1	1	1	48,3N	2,5E	Tidou et al. 1992
64flm	Lindane	0	0	10000	0.4	1	1	1	48,3N	2,5E	Tidou et al. 1992
65flm	Atrazine	35	0	120000	4.8	1	0	1	43,6N	79,3W	Hamilton et al. 1989
72tll	Atrazine	223	0	120000	4.8	1	0	1	43,6N	79,3W	Hamilton et al. 1987
73mli	Hexazinone	2	1			0	0	0			Kreutzweiser et al. 1992b
74mli	Triclopyr ester	2	1			0	0	0			Kreutzweiser et al. 1992b
75mli	Hexazinone	0	0			0	0	1			Kreutzweiser et al. 1995
75tll	Carbaryl	0	0	100	2	0	0	1	41,11N	81,2W	Havens 1995
76mli	Lindane	28	1	437.5	0.25	1	0	1			Mitchell et al. 1995
76tll	Lambda-cyhalothrin	147	14	450000	1	1	1	1	35,26N	77,59W	Hill et al. 1988
77mli	Lindane	28	1	437.5	0.25	1	0	1			Mitchell et al. 1995
78mli	Atrazine	12	1								Krieger et al. 1988
79mli	Atrazine	12	1								Krieger et al. 1988
82mli	Atrazine	0	0	114		0	0	0			Carder & Hoagland 1998

Experiment	Pesticide	End of application	Interval	Volume	Avr. Depth	Sediment	Makrophytes	field/lab	Latitude	Longitude	Reference
		day	day	litre	m						
83mli	Alachlor	0	0	114		0	0	0			Carder & Hoagland 1998
83tll	Cyfluthrin	133	14	634700	1.3	1		1	33N	100W	Johnson et al. 1994
84tll	Cyfluthrin	133	14	1900	1	1		1	33N	100W	Johnson et al. 1994
85tll	Permethrin	0	0	120000	4.8	1	0	1	43,6N	79,3W	Kaushik et al. 1985
86mli	Fenvalerate	0	0	55		1	0	0			Breneman et al. 1994
87mli	Alachlor	0	0	175	0.3	0	0	0			Spawn et al. 1997
88mli	Atrazine	14	0	35.25	0.4	1	0	1			Jurgensen & Hoagland 1990
90tll	Diflubenzuron	33	0	25000	0.5	1	1	1	47N	92W	Liber et al. 1996
91mli	Chlorpyrifos	21	1	7200	0.4	1	0	1			Ward et al. 1995
92mli	Chlorpyrifos	0	0	7200	0.4	1	0	1			Pusey et al. 1994
93mli	Propiconazol	35	1			1	0	0			Aanes & Baekken 1994
94mli	Methoxychlor	0.4	0			1	0	0			Scherer &McNicol 1986
95mli	Fenitrothion	0.4	0			1	0	0			Scherer &McNicol 1986
96mli	Fenvalerate	0.06	0			1	1	0			Liess & Schulz 1996
97mli	Permethrin	0	0	10		0	0	0			Poirier & Surgeoner 1987
97tll	Atrazine	0	0	470000	1	1		1	48N	12E	Larsen et al. 1986
98mli	fenitrothion	0	0	10		0	0	0			Poirier & Surgeoner 1987
99mli	aminocarb	0	0	10		0	0	0			Poirier & Surgeoner 1987
100mli	mexacarbate	0	0	10		0	0	0			Poirier & Surgeoner 1987
101mli	Triclopyr ester	1	0			1	0	0			Kreutzweiser &Capell 1992a
102mli	Permethrin	1	0			1	0	0			Kreutzweiser &Capell 1992a
102tll	Lindane	0	0	1000	0.8	1		1	48N	12E	Maund et al. 1992
103tll	Lindane	0	0	1000	0.8	1		1	48N	12E	Maund et al. 1992
104tll	Lindane	0	0	1000	0.8	1		1	48N	12E	Maund et al. 1992
105tll	Lindane	0	0	1000	0.8	1		1	48N	12E	Maund et al. 1992

Experiment	Pesticide	End of application	Interval	Volume	Avr. Depth	Sediment	Makrophytes	field/lab	Latitude	Longitude	Reference
		day	day	litre	m						
106tll	Lindane	29	14	1000	0.8	1		1	48N	12E	Peither et al. 1996
107tll	2,4 D	360	30	250000	1	1	0	1	22,56N	88,44E	Sarkar 1991
109tll	Esfenvalerate	30	0	33000	1.1	1	1	1	46,45N	92,07W	Tanner & Knuth 1996
110tll	Carbofuran	0	0	1200	0.6	1	1	1	53,33N	113,15W	Wayland 1991
111tll	Permethrin	14	0	2700	3.5	1	0	1	36,02N	140,04E	Yasuno et al. 1988
112tll	Permethrin	18	0	2700	3.5	1	0	1	36,02N	140,04E	Yasuno et al. 1988
113tll	Esfenvalerate	0	0	36000	0.5	1		1	56,5N	10E	Schroll et al. 1998
114tll	Atrazine	0	0	450000	2	1	1	1	38,58N	95,14W	Kettle et al. 1987
117tll	Atrazine	0	0	5500	2	0	0	1	32,43N	97,17W	Hoagland & Drenner 1991
118tll	Bifenthrin	0	0	5500	2	0	0	1	32,43N	97,17W	Hoagland & Drenner 1991
119tll	Atrazine	0	0	5500	2	0	0	1	32,43N	97,17W	Hoagland & Drenner 1991
120tll	Bifenthrin	0	0	5500	2	0	0	1	32,43N	97,17W	Hoagland & Drenner 1991
121flm	Fenpropidin	14	0	700	0.7	1	1	1	48,23N	11,44E	Huber 1995
122flm	Fenpropidin	14	0	20000	0.7	1	1	1	47N	8E	Neumann 1995
123flm	Lambda-cyhalothrin	42	14	25000	1	1	1	1	51N	0E	Hamer 1994
124flm	Azinphos-methyl	49	7	400000	2	1	1	1	37N	98W	Giddings et al. 1994
125flm	Trahalomethrin	63	13	635000	1.3	1	1	1	32N	97W	Mayasich et al. 1994

10.3 Annex C

Comparison of extrapolated hazard concentrations and the lowest observed effect concentrations in the mesocosm experiments. $HC_{5,50}$: extrapolated hazard concentration. $OECD_{10}$: Hazard concentration according to OECD approach. NOEC: Yes = lowest test concentration were lower than the lowest effect concentration observed; No = effect was observed at the lowest test concentration applied. $HC_{5,50}$/LOEC = ratio between extrapolated hazard concentration (see Table 1) and lowest observed effect concentration. OECD/LOEC = ratio between hazard concentration ($OECD_{10}$ approach) and lowest observed effect concentration. $HC_{5,50}$/low = ratio between hazard concentration and the lowest test concentration applied. OECD/low = ratio between extrapolated hazard concentration ($OECD_{10}$ approach) and the lowest test concentration applied. Observed effects at lowest concentration: ↓ decrease (mostly in abundance); ↑ increase.

Exp code	Pesticide	Trivial name	$HC_{50.5}$	$OECD_{10}$	NOEC	$HC_{50.5}$ /LOEC	OECD/ LOEC	$HC_{50.5}$/ low	OECD/ low	Lowest observed effect
76tll	Insecticide	Lambda-cyhalothrin	0.08	0.01	Yes	80	10	800	100	↓ Ephemeroptera
96mli	Insecticide	Fenvalerate	0.05	0.01	No	50	10	50	10	↓ Tricopoptera emergence
57flm	Insecticide	Esfenvalerate	0.18	0.02	No	18	2	18	2	↓ Chironomidae
44tll	Herbicide	2,4 D	1200	240	Yes	12	2.4	120	24	↓ macrophyte biomass
113tll	Insecticide	Esfenvalerate	0.18	0.02	No	5.14	0.571	5.14	0.571	↓ macroinvertebrates - ↑ phytoplankton
60flm	Insecticide	Lambda-cyhalothrin	0.08	0.01	No	4.71	0.588	4.71	0.588	↓ Amfipoda
123flm	Insecticide	Lambda-cyhalothrin	0.08	0.01	No	4.71	0.588	4.71	0.588	↓ macroinvertebrate groups
102tll	Insecticide	Lindan	2.93	1.8	Yes	3.66	2.25	2.93	1.8	↓ Chironomid emergence
104tll	Insecticide	Lindan	2.93	1.8	Yes	3.26	2.00	2.93	1.8	↓ Chaoborus mortality
77mli	Insecticide	Lindan	2.93	1.8	Yes	2.93	1.80	11.72	7.2	↑ drift in Ephemeroptera
86mli	Insecticide	Fenvalerate	0.05	0.01	Yes	1.67	0.333	5	1	↓ macroinvertebrate abundance
117tll	Herbicide	Atrazin	19.9	2.6	No	1.33	0.173	1.33	0.173	↓ Phytoplankton decrease & ↑rotifer (ind.eff)
82mli	Herbicide	Atrazin	19.9	2.6	No	1.22	0.160	1.22	0.160	↓ Periphyte biovolume
118tll	Insecticide	Bifenthrin	0.04	0.01	No	1.03	0.256	1.03	0.256	↓ Zooplankton
59flm	Insecticide	Esfenvalerate	0.18	0.02	Yes	1	0.111	18	2.000	↓ copepod nauplii
33tll	Insecticide	Fenvalerate	0.05	0.01	Yes	1	0.200	5	1.000	↓ crustacean zooplankton-↑ rotifers

Exp code	Pesticide	Trivial name	$HC_{50,5}$	$OECD_{10}$	NOEC	$HC_{50,5}$ /LOEC	OECD/ LOEC	$HC_{50,5}$/ low	OECD/ low	Lowest observed effect
62flm	Herbicide	Atrazin	19.9	2.6	No	0.99	0.130	0.99	0.130	↓ macrophyte coverage & ↓ insect emergence (ind.eff)
114tll	Herbicide	Atrazin	19.9	2.6	No	0.99	0.130	0.99	0.130	↓ macrophyte coverage
78&79mli	Herbicide	Atrazin	19.9	2.6	No	0.83	0.108	0.83	0.108	↓ periphyte chlorophyll
85tll	Insecticide	Permethrin	0.39	0.03	No	0.78	0.060	0.78	0.060	↓ Cladocera-increase in rotifers
76mli	Insecticide	Lindan	2.93	1.8	No	0.73	0.450	0.73	0.450	↑ drift in Ephemeroptera
87mli	Herbicide	Alachlor	0.73	0.6	No	0.73	0.600	0.73	0.600	↓ periphyte abundance
47flm	Insecticide	Esfenvalerate	0.18	0.02	No	0.72	0.080	0.72	0.080	↓ macroinvertebrate and zooplankton
49flm	Insecticide	Tebufenozide	87.3	16	Yes	0.67	0.123	1.25	0.229	↓ Cladocera
111tll	Insecticide	Permethrin	0.39	0.03	No	0.52	0.040	0.52	0.040	↓ Cladocera&Chaoborus
56flm	Insecticide	Chlorpyrifos	0.04	0.01	No	0.40	0.100	0.40	0.100	↓ Amfipods & ↑ fil. algae
91mli	Insecticide	Chlorpyrifos	0.04	0.01	No	0.40	0.100	0.40	0.100	↓ most insect larvae
55flm	Herbicide	Linuron	19.67	5.0	Yes	0.39	0.100	39.34	10.000	↓↑ zooplankton
102mli	Insecticide	Permethrin	0.39	0.03	No	0.39	0.030	0.390	0.030	↑ drift in Ephemeroptera
120tll	Insecticide	Bifenthrin	0.04	0.01	No	0.32	0.080	0.320	0.080	↓ zooplankton
16ank	Herbicide	Atrazin	19.9	2.6	Yes	0.29	0.038	3.980	0.520	↓ Copepod nauplii (ind.eff)
95mli	Insecticide	Fenitrothion	2.26	0.32	Yes	0.28	0.040	0.565	0.080	Δ Tricop. Behaviour
83tll	Insecticide	Cyfluthrin	0.07	0.01	No	0.28	0.040	0.280	0.040	↓ most insect larvae
84tll	Insecticide	Cyfluthrin	0.07	0.01	No	0.28	0.040	0.280	0.040	↓ most insect larvae
112tll	Insecticide	Permethrin	0.39	0.03	No	0.26	0.020	0.260	0.020	↓ Cladocera & Chaoborus
72tll	Herbicide	Atrazin	19.9	2.6	No	0.25	0.033	0.249	0.033	↓ periphyte abundance
38ank	Insecticide	Lindan	2.93	1.8	Yes	0.23	0.138	0.977	0.600	↓ copepod nauplii
61flm	Herbicide	Atrazin	19.9	2.6	No	0.2	0.026	0.199	0.026	↓ periphyte abundance & composition
109tll	Insecticide	Esfenvalerate	0.18	0.02	Yes	0.18	0.020	18.000	2.000	↓↑ zooplankton
75tll	Insecticide	Carbaryl	0.58	0.07	Yes	0.12	0.014	0.290	0.035	↓ Cladocera
28tll	Insecticide	Chlorpyrifos	0.04	0.01	No	0.08	0.020	0.080	0.020	↓ zooplankton
90tll	Insecticide	Diflubenzuron	0.15	0.18	Yes	0.06	0.072	0.214	0.257	↓ Diptera
119tll	Herbicide	Atrazin	19.9	2.6	No	0.05	0.007	0.052	0.007	↓ phyto- and zooplankton
74mli	Herbicide	Triclopyr ester	131.12	120	Yes	0.04	0.038	0.410	0.375	↑ drift in Tricoptera

Exp code	Pesticide	Trivial name	$HC_{50,5}$	$OECD_{10}$	NOEC	$HC_{50,5}$ /LOEC	OECD/ LOEC	$HC_{50,5}$/ low	OECD/ low	Lowest observed effect
124flm	Insecticide	Azinphos-methyl	0.05	0.02	Yes	0.02	0.009	2.000	0.800	↑ Cyclopoida
26tll	Insecticide	Diflubenzuron	0.15	0.18	No	0.02	0.018	0.015	0.018	↓ zooplankton; ↑ phytoplankton
27tll	Insecticide	Diflubenzuron	0.15	0.18	No	0.0	0.018	0.015	0.018	↓ zooplankton; ↑ phytoplankton
61tll	Herbicide	Atrazin	19.9	2.6	Yes	0.01	0.002	0.166	0.022	↓ periphyte abundance & composition
92mli	Insecticide	Chlorpyrifos	0.04	0.01	Yes	0.01	0.003	0.667	0.167	↓↑ macroinvertebrates
54flm	Insecticide	Azinphos-methyl	0.05	0.02	Yes	0.01	0.005	0.250	0.100	↓ Cladocera
64flm	Insecticide	Lindan	2.93	1.8	No	0.01	0.006	0.009	0.006	↓ zooplankton; ↑ phytoplankton
46flm	Insecticide	Methoxychlor	0.09	0.08	Yes	0.01	0.007	0.009	0.008	↓ crustacean zooplankton; ↑ rotifers
50flm	Insecticide	Chlorpyrifos	0.04	0.01	No	0.01	0.002	0.008	0.002	↓ Amfipod & Isopod
57tll	Insecticide	Diazinone	0.03	0.003	Yes	0.01	0.000	0.013	0.000	↓ Cladocera
125flm	Insecticide	Trahalomethrin	0.07	0.01	No	0.01	0.001	0.006	0.001	↓ Ephemeroptera
58flm	Insecticide	Diflubenzuron	0.15	0.18	No	0.01	0.006	0.005	0.006	↓ macroinvertebrate & zooplankton; ↑ phytoplankton
45flm	Insecticide	Methoxychlor	0.09	0.08	Yes	0.01	0.004	0.005	0.004	↓ crustacean zooplankton; ↑ rotifers
110tll	Insecticide	Carbofuran	0.05	0.02	Yes	0.00	0.001	0.010	0.004	↓ Diptera & Amfipoda
25tll	Insecticide	Endosulfan	0.02	0.01	Yes	0.00	0.001	0.020	0.010	↓ Ostracoda; ↑ phytoplankton
44flm	Insecticide	Methoxychlor	0.09	0.08	Yes	0.00	0.002	0.018	0.016	↓ crustacean zooplankton; ↑ rotifers
51flm	Insecticide	Chlorpyrifos	0.04	0.01	No	0.00	0.000	0.001	0.000	↓ Amfipod & Isopod
53flm	Insecticide	Chlorpyrifos	0.04	0.01	No	0.00	0.000	0.001	0.000	↓ most groups
101mli	Herbicide	Triclopyr ester	131.1	120	Yes	0.00	0.000	0.041	0.038	↑ Drift & mortality in macroinvertebrates
43flm	Insecticide	Methoxychlor	0.09	0.08	Yes	0.00	0.000	0.030	0.027	↓ zooplankton
63flm	Insecticide	Delta-methrin	0.01	0.003	No	0.00	0.000	0.000	0.000	↓ zooplankton; ↑ phytoplankton