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Threshold Levels for Effects of Insecticides in Freshwater Ecosystems: A Review

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Abstract. A literature review of freshwater (model) ecosystem studies with neurotoxic insecticides was performed to assess ecological threshold levels, to compare these levels with the first tier approach within European Union (EU) administration procedures, and to evaluate the ecological consequences of exceeding these thresholds. Studies published between 1980 and 2001 were reviewed. Most studies covered organophosphates and synthetic pyrethroids in lentic waters. The most sensitive taxa were representatives of crustaceans, insects and fish. Based on toxic units, threshold values were equivalent for compounds with a similar mode of action. This also accounted for the nature and magnitude of direct effects at higher concentrations. Although laboratory single species toxicity tests may not allow predictions on precise ecological effects, some generalisations on effects and recovery can be made with respect to acute standard laboratory EC_{50} data. The $NOEC_{ecosystem}$ usually is a factor of 10 or more higher than first tier acceptable concentrations, particularly in the case of single applications and acetylcholinesterase inhibitors. Acceptable concentrations, as set by the EU first tier approach, appear to be protective. Recovery of sensitive endpoints usually occurs within 2 months of the (last) application when peak concentrations remain lower than (0.1–1) $\times$ EC_{50} of the most sensitive standard test species. The consistency of response patterns found in model ecosystem studies can be useful when estimating the ecological risks of pesticides. The use of an effect classification system was also helpful in evaluating effects.

Keywords: Organophosphorus insecticides; carbamates; synthetic pyrethroids; freshwater ecosystems; risk evaluation

Introduction

From their introduction, the use of pesticides has increased tremendously since the time when they were successfully deployed in strategies to increase crop productivity. The quantity of pesticides sold world wide to the agricultural sector had reached over 1.3 million metric tons of active ingredients by 1995 (FAO, http://www.fao.org/statistical databases/mean of production/pesticide trade/). Of this amount, 295 thousand metric tons (about 23% of the 1995 total sales) was attributable to insecticides.

In many situations, aquatic ecosystems form highly integrated parts of agricultural areas because they provide water and drainage facilities. With the pesticide application techniques in use for crop protection, it is inevitable that fractions of applied insecticides will enter aquatic ecosystems. Entry routes of pesticides into adjacent bodies of water resulting from normal agricultural

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usage include spray drift, runoff, and leaching (e.g., Ganzelmeier et al., 1995; Capri and Trevisan, 1998; Van de Zande et al., 2000). Programmes and studies focusing on the detection of pesticides in an aquatic environment report traces of these toxicants in various bodies of water (Wan, 1989; Thoma and Nicolson, 1989; Frank et al., 1990; Teunissen-Ordelman and Schrap, 1996; Lahr and Banister, 1997; Liess and Schulz, 1999; Leonard et al., 2000). Hence, it is demonstrated that non-target species living in water catchments of agricultural areas are potentially at risk when they have similar toxicant receptors as the target organisms. Pesticide admission and regulatory authorities have therefore been set up to control and reduce the undesirable impacts of pesticide usage on the environment.

Essentially, risk assessment is done by comparing concentrations expected, or found, in the environment with concentrations of that pesticide considered acceptable by regulatory authorities. In many countries (e.g., EU countries and the US) a tiered approach for aquatic risk assessments is being applied. The concept of this approach is that when passing through the tiers, the estimates of exposure and effects become more accurate as uncertainty is reduced through the acquisition of more data. The lower tiers are more conservative while higher ones are more realistic (Campbell et al., 1999; Solomon, 2001).

In the first tier risk assessment, criteria are based on the toxicity data of a small set of standard test species generated in the laboratory which are then multiplied by a safety factor (EU, 1997; US-EPA, 1998). This method is sometimes considered to be very strict and has been the subject of debate (e.g., Maund et al., 1998; Giesy et al., 1999). Issues concerning the adequacy of the first tier risk assessment is one of the reasons calling for higher tier risk evaluation, as this type of evaluations consider the outcome of ecotoxicological studies under more realistic exposure conditions in combination with greater ecological realism.

Microcosm and mesocosm studies form an important part of the research that has been done to validate first tier water quality criteria for pesticides and/or to assess their 'regulatory acceptable concentration' in surface waters. These studies have been done with various active ingredients and under a wide range of conditions. Major differences in conditions between studies are location (e.g., climatological or biogeographical regions) and types of natural and experimental ecosystems used (e.g., plankton or macrophyte-dominated systems, experimental ponds or streams). The relatively large amount of data generated by these freshwater ecosystems provide the opportunity to detect whether there are predictable concentration-effect relationships and/or other generalities in effect patterns between studies.

The present review focuses on the ecological impact of neurotoxic insecticides. We considered two groups: acetylcholinesterase inhibitors (organophosphates and carbamates) and synthetic pyrethroids.

Data presented here are based mainly on experiments in freshwater model ecosystems since descriptive hydrobiological field research into the effects of insecticides is scarce. Following the terminology used by the European Workshop on Freshwater Field Tests (EWOFFT), these systems are also called microcosms (tanks/ponds with a water volume $<15\ m^3$ or experimental streams <15 m in length) or mesocosms (systems $>15\ m^3$ or >15 m, respectively) (Crossland et al., 1994). An advantage of experimental ecosystems is that they can be replicated, and several concentrations of a pollutant can be tested simultaneously. The pros and cons of working with model freshwater ecosystems are discussed by Brock et al. (1995a), ECETOC (1997), and Caquet et al. (2000).

Objectives of the present literature review are: (a) to list ecological threshold values (e.g., $NOEC_{eco}$ and $LOEC_{eco}$) for individual insecticides as established experimentally by means of freshwater model ecosystems or adequate field studies, (b) to compare $NOEC_{eco}$s with established first tier water quality criteria for insecticides in surface water and (c) to assess the ecological consequences of exceeding the first tier water quality criteria.

We consider $NOEC_{eco}$ to be the highest tested concentration at which no, or hardly any, effects on the structure and functioning of the studied (model) ecosystem are observed. The $LOEC_{eco}$ is the lowest tested concentration at which significant treatment-related effects occur.

Methods

Literature reviewed

The literature database available at our Institute served as a basis for the study. This database has been built up over the years and kept up-to-date by means of the literature bulletins 'Chemical Abstracts' and 'Current Contents'. The existing database was checked for possible gaps through a specific literature search, using the program 'Winspirs' (version 4.0). This program was used to search the databases of 'Agris Current' (from 1980 onwards), 'Biological Abstracts' (from December 1989 onwards), and 'CAB-Abstracts' (from 1980 onwards). Publications up to and including June 2001 were included in this search. Furthermore, we included recent studies done by our own research group, which are still in the process of being published (Van Wijngaarden et al., submitted 'a'; Roessink et al., in press; Van Wijngaarden et al., submitted 'b').

Criteria for the selection of suitable microcosm and mesocosm studies

The yielded ecotoxicological studies were screened on the following criteria:

1. Test systems used represent a realistic freshwater community (organisms of various trophic levels are present).
2. Description of the experimental set-up is adequate and unambiguous.
3. Exposure concentrations relevant to the study are reported or can be derived (at least nominal concentrations are known).
4. Investigated endpoints are sensitive to the substance in that direct effects on these endpoints are related to the working mechanisms of insecticides. Arthropods and fish are especially considered to be sensitive endpoints for insecticides (Hill et al., 1994a; Graney et al., 1994; this paper).
5. The effects are evaluated statistically and show an unambiguous concentration-effect relationship or, observed effects are in agreement with a concentration-effect relationship from additional studies.
6. To establish a $NOEC_{eco}$, at least the lowest test concentration within the study should not show a consistent effect attributable to the treatment. A concentration above the $NOEC_{eco}$ should show a significant treatment-related effect ($LOEC_{eco}$).
7. To enable a comparison of field concentrations with target concentrations for registration procedures, toxicity data of standard test organisms (at least for *Daphnia* or fish) should be known.
8. The results of the study were published in 1980 or later.

Subsequently, selected studies were classified according to the exposure regime (single application, multiple applications, or continuous exposure), type of test system (stagnant or running water), and working mechanism of the insecticides.

Comparison between insecticides

To enable comparison of studies using different insecticides, the reported field concentrations were normalised by dividing them by the 48 h-EC_{50} of the aquatic standard test species *Daphnia magna* or by the 96 h-LC_{50} of a standard test fish (the most sensitive species was used). The unit of the resulting variable is defined as TU_{mso} (=Toxic Unit based on the most sensitive standard test organism). In the case of EC_{50}s for *Daphnia magna*, the effect parameter could also be mortality.

Publications by Crommentuijn et al. (1997), Mayer and Ellersieck (1986), the AQUIRE database (http://www.epa.gov/ecotox/), and references in the papers about the evaluated microcosm and mesocosm studies have been used as a source of information for the toxicity data. If several EC_{50}s were available for the same standard test organism, the geometric mean of these values was calculated and referred to as 'gm-EC_{50}' (Table 1). When gm-EC_{50}s were available, they were used to calculate TU_{mso}. The toxicity data showed that *Daphnia magna* was usually the most sensitive standard test organism for the evaluated insecticides (Table 1). For some pyrethroids, *Daphnia* as well as fish are a representative sensitive standard test species.

Table 1. Toxicity data (μg/l) of the most sensitive standard test species used to calculate toxic units (TU_{mso}). First tier acceptable concentrations (NEC) are derived from the Uniform Principles criteria and based on the toxicity data in this table. *D. magna*: *Daphnia magna*. crust: crustacean. *P. promelas*: *Pimephalus promelas*. *O. mykiss*: *Oncorhynchus mykiss*. *L. macrochirus*: *Lepomis macrochirus*

Compound	Toxicity	Species	References	First tier NEC
Azinphos-methyl	gm-L(E)C_{50} = 2.0 (48 h)	*D. magna* (crust)	1, 2, 3	0.02
Bendiocarb	gm-L(E)C_{50} = 74 (48 h)	*D. magna* (crust)	4	0.74
Carbaryl	EC_{50} = 5.6 (48 h)	*D. magna* (crust)	2	0.056
Carbofuran	gm-L(E)C_{50} = 33.2 (48 h)	*D. magna* (crust)	5,6	0.33
Chlorpyrifos	gm-L(E)C_{50} = 1.3 (48 h)	*D. magna* (crust)	7,8	0.013
Cyfluthrin	gm-L(E)C_{50} = 0.15 (48 h)	*D. magna* (crust)	9	0.0015
Cypermethrin	gm-L(E)C_{50} = 0.68 (96 h)	*O. mykiss* (fish)	10, 11	0.0068
Deltamethrin	gm-L(E)C_{50} = 0.04 (48 h)	*D. magna* (crust)	12, 13	0.0004
Diazinon	gm-L(E)C_{50} = 1.0 (48 h)	*D. magna* (crust)	1, 14	0.01
Esfenvalerate	gm-L(E)C_{50} = 0.25 (96 h)	*P. promelas* (fish)	15	0.0025
Fenitrothion	gm-L(E)C_{50} = 11 (48 h)	*D. magna* (crust)	16, 17	0.11
Fenvalerate	gm-L(E)C_{50} = 0.82 (96 h)	*O. mykiss* (fish)	2	0.008
Lambda-cyhalothrin	LC_{50} = 0.21 (96 h)	*L. macrochirus* (fish)	2	0.0021
Parathion	gm-L(E)C_{50} = 1.1 (48 h)	*D. magna* (crust)	1	0.0011
Parathion-methyl	gm-L(E)C_{50} = 1.4 (48 h)	*D. magna* (crust)	1, 2, 19	0.014
Permethrin	gm-L(E)C_{50} = 0.65 (48 h)	*D. magna* (crust)	2, 9, 11	0.0065
Phorate	gm-L(E)C_{50} = 1.5 (48 h)	*D. magna* (crust)	18	0.015
Tralomethrin	LC_{50} = 0.15 (48 h)	*D. magna* (crust)	9	0.0015

1: Dortland (1980), 2: Mayer and Ellersieck (1986), 3: Giddings et al. (1994), 4: Visser and Linders (1990), 5: Trotter et al. (1991), 6: Jansma and Linders (1993), 7: Kersting and Van Wijngaarden (1992), 8: McCarthy (1977) in Barron and Woodburn (1995), 9: Mokry and Hoagland (1990), 10: Stephenson (1982), 11: Crommentuijn et al. (1997), 12: Xiu et al. (1989), 13: Day (1991), 14: AQUIRE database (http://www.epa.gov/ecotox/), 15: Stay and Jarvinen (1995), 16: Sanders et al. (1983), 17: LeBlanc (1984), 18: Fairchild et al. (1992a), 19: Oikari et al. (1992).

Criteria for effect classification

Reported endpoints were assigned to one of eight endpoint categories: (a) 'Microcrustaceans' (including Cladocera, Copepoda, Ostracoda), (b) 'Macrocrustaceans' (including Amphipoda, Isopoda, Anostraca), (c) 'Insects', (d) 'Fish', (e) 'Rotifers', (f) 'Other macroinvertebrates', (g) 'Algae & macrophytes', and (h) 'Community metabolism'. Within each category, the most sensitive endpoint was decisive for classification into an effect class (worst case approach). The categories 'a' to 'f' represent structural endpoints, while category 'h' represents functional responses. Structural endpoints concern densities (numbers) and biomass of populations. Functional endpoints in most cases concern oxygen balance, water chemistry, and decomposition of particulate matter. Effects reported on these endpoints were classified into five classes based on the following criteria:

Class 1: 'effect not demonstrated'

- No effects observed as a result of treatment (primarily, statistical significance plays an important role for this criterion) and/or,
- Observed differences between treatment and controls show no clear causal relationship. Causality in this context is judged through the use of guidelines similar to those developed for identifying causative agents of disease (Koch, 1942; Hill, 1965).

Class 2: 'slight effect'

- Effects only observed on individual samplings, especially shortly after treatment, and/or
- Short-term and/or quantitatively restricted response of sensitive endpoints.

Class 3: 'pronounced short-term effect'

- Clear response of sensitive endpoints, but full

recovery within eight weeks after (the last) application, and
- Effects observed on some subsequent sampling dates, and
- Effects reported on several sensitive species; temporary effects on less sensitive species and/or endpoints.

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

- Clear effects observed, but the study is too short to demonstrate complete recovery within 8 weeks after (the last) application of the insecticide for the endpoint concerned.

Class 5: 'pronounced long-term effect'

- Clear response on various subsequent sampling dates, and recovery time of sensitive endpoints is longer than 8 weeks after the last application, and
- Effects reported on many sensitive species and/or endpoints; elimination of sensitive species; effects on less sensitive species endpoints and/or other similar descriptions.

A recovery period of 8 weeks was applied in the classification to decide whether effects were short-term or longer-term. In relation to the lifecycles of macroinvertebrates, fish and macrophytes, it is common practice in microcosm and mesocosm studies to sample these groups of organisms on a biweekly or monthly basis. Consequently, the typical sampling intervals for macroinvertebrates may not establish actual times of recovery, but will be adequate for determining if effects are persisting beyond the short-term eight-week time frame. For short-cyclic organisms, such as phytoplankton and zooplankton, sampling frequencies are generally on a weekly basis. For this group of organisms there are enough observation points to establish the time of actual recovery within this time window.

Effects were reported in the literature in a variety of ways, and generally did not fit exactly into our effect criteria scheme. The process of assigning reported effects to one of the effect classes therefore normally consisted of evaluating both quantitative and qualitative information, and judging on a case-by-case basis into which combination of criteria this information fitted best. If in doubt, the information was evaluated by more than one expert to obtain a consensus answer.

Data analysis

The probability of effects occurring in microcosm and mesocosm studies was calculated by analysing the combined data set of the most sensitive endpoints of both the acetylcholinesterase inhibitors and pyrethroids using logistic regression. For this purpose, the effect classes were reclassified to a nominal variable: a 'no-effect class' (0) and an 'effect class' (1). The 'effect class' contained the former Classes 3, 4 and 5. 'No-effect class' analyses were performed using two definitions; one containing only the data of Effect Class 1, and the other containing the data of Effect Classes 1 and 2. The following logistic model was used for these calculations:

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

in which y is the response variable (effect/no effect), x is the concentration expressed in TU_{mso}; a is the concentration at which an effect has been reported for 50% of the studies, and b is the slope of the sigmoid curve at this concentration. Results of these analyses were expressed as Field Effect Concentrations (FEC) at 5, 50 and 95 percentages of probability. In other words, the model yielded fitted concentrations (expressed in TU_{mso}) for which it predicted that for 5, 50 and 95% of the studies, effects will occur. The calculations were performed using the GENSTAT statistical program (Payne and Lane, 1993).

Comparison of ecological threshold values with registration criteria

We compared the ecological threshold values ($NOEC_{eco}$s) obtained from microcosm and mesocosm studies with the acceptable concentrations established by the first tier registration criteria applied in the European Union. According to EU Uniform Principles (EU, 1997), in the first tier of the risk assessment, the peak concentration of a pesticide in surface water as calculated from ref-

erence tables for spray drift and/or fate models (Ganzelmeier et al., 1995; FOCUS, 2001), should not be higher than 0.01× the acute EC_{50} for the standard test species of fish or *Daphnia* and 0.1× the EC_{50} for standard test algae. In addition, the time weighted average exposure concentration should not be higher than 0.1× the chronic NOEC of *Daphnia* (21 days) and fish (28 days) with long-term exposure. A higher concentration, however, may be considered acceptable if it can be demonstrated by using higher tier tests that the real risk to aquatic organisms is less than predicted by the first tier criteria ('unless clauses').

We established first tier acceptable concentrations on the basis of acute toxicity data for the standard test organisms mentioned in OECD protocols (OECD, 1993). This is established by dividing the gm-EC_{50} of the most sensitive species by a factor of 100 (Table 1). We used acute toxicity data because: (a) adequate chronic toxicity data for the substances studied in microcosm and mesocosm experiments are in many cases not available in the open literature whereas acute toxicity data are; (b) in microcosm and mesocosm studies, only nominal or measured peak concentrations of the studied pesticide are usually reported; and (c) the compounds studied have relatively low environmental persistence making comparison of short-term exposures to acute toxicity data the most relevant.

Available information

Summaries were first made of the selected studies. Concise versions of these are given in Brock et al. (2000b).

Acetylcholinesterase inhibitors

Organophosphorous and carbamate insecticides inhibit the activity of the enzyme acetylcholinesterase. Inhibition of this enzyme results in the accumulation of acetylcholine at choline receptors and consequently in the disturbance of nerve impulses (Klaassen et al., 1986).

Microcosm and mesocosm experiments were only conducted on a small number of the 64 organophosphates listed by Tomlin (2000). After testing against the selection criteria, 26 studies remained. They yielded adequate information on ecological risks of seven active ingredients (Table 2). The selected studies were mainly conducted on chlorpyrifos (twelve studies), fenitrothion (five studies), and azinphos-methyl (four studies). Five microcosm and mesocosm studies provided adequate information on the active ingredients bendiocarb, carbaryl, and carbofuran [three out of the twenty acetylcholinesterase inhibiting carbamates listed (Tomlin, 2000)]. The study locations were quite diverse, and done under climatological conditions ranging from temperate to subtropical and tropical (Table 2).

Synthetic pyrethroids

Pyrethroids also affect the functioning of the nervous system. Their primary mode of action is by interference with ion channels in the nerve axon, resulting in hyperactivity of the nervous system with a subsequent lack of control of normal function (Clark and Brooks, 1998).

Eighteen microcosm and mesocosm studies of eight active ingredients – out of the 39 listed pyrethroids – (Tomlin, 2000), yielded adequate information after testing against our selection criteria. The studies were performed predominantly in North America and Europe under various climatological conditions (Table 3).

Application method and pesticide behaviour

Most studies were conducted using formulated materials (Tables 2 and 3). Exposure of aquatic organisms to insecticides, and observed effects during microcosm and mesocosm studies, are strongly related to the method of application and the environmental behaviour of these substances. Pollution of watercourses by insecticides may be the result of spray drift. Most studies focusing on acute risks simulated this entry route and applied the insecticide by spraying the water surface. In studies with a chronic exposure regime, insecticides are usually directly mixed into the water column.

In the studies with organophosphates and carbamates, active ingredients were almost always applied in dissolved form via the aqueous phase (spray drift or direct mixing in the water column).

Table 2. Experiments with acetylcholinesterase inhibitors included in this report

Active ingredient	Test form	Experiment	Location	Authors
Organophosphorous insecticides				
Azinphos-methyl	F	S-stag	USA (lab)	Stay and Jarvinen 1995
–	F	S-stag	USA (Minnesota)	Tanner and Knuth 1995
–	F	M-stag	USA (Kansas)	Giddings et al. 1994
–	F	S-stag	USA (Minnesota)	Knuth et al. 1992
Chlorpyrifos	F	S-stream	Australia	Pusey et al. 1994
–	F	L-stag	NL (lab)	Van den Brink et al. 1995
–	F	L-stream	Australia	Ward et al. 1995
–	F	S-stag	USA (Kansas)	Biever et al. 1994
–	F	M-stag	USA (Kansas)	Giddings et al. 1997
–	F	S-stag	NL	Van Wijngaarden et al. 1996, Van den Brink et al. 1996, Kersting and Van den Brink 1997
–	F	S-stag	NL (lab)	Brock et al. 1992a, b, 1993
–	F	S-stag	NL (lab)	Van Donk et al. 1995; Brock et al. 1995b; Cuppen et al. 1995
–	F	S-stag	USA (Minnesota)	Siefert et al. 1989, Brazner et al. 1989, Brazner and Kline 1990
–	-	S-stag	USA (lab)	Stay et al. 1989
–	F	S-stag	Canada	Hughes et al. 1980
–	F	S-stag	Canada (Manitoba)	Zrum et al. 2000
–	F	S-stag	NL (lab)	Van Wijngaarden et al. subm. b
Diazinon	A	M-stag	USA (Kansas)	Giddings et al. 1996
Fenitrothion	F	S-stag	Senegal	Lahr and Diallo 1993
–	F	M-stag	Canada	Fairchild and Eidt 1993
– (∗)	F	S-stream	UK	Morrison and Wells 1981
– (∗)	F	S-stream	Canada	Poirier and Surgeoner 1988
– (∗)	F	S-stream	Japan	Yasuno et al. 1981
Parathion-ethyl	A	L-stag	NL	Dortland 1980
Parathion-methyl	S	S-stag	UK	Crossland 1984, Crossland and Bennett 1984
–	S	S-stag	UK	Crossland 1988
Phorate	F	S-stag	USA (S. Dakota)	Dieter et al. 1996
Carbamates				
Bendiocarb	F	S-stag	Senegal	Lahr et al. 1995
Carbaryl	F	S-stag	USA (Ohio)	Havens 1994, 1995
–	F	S-stream	Canada (Maine)	Courtemanch and Gibbs 1980
Carbofuran	F	S-stag	Canada (Alberta)	Wayland 1991
–	F	S-stag	Canada (Alberta)	Wayland and Boag 1995

(∗) Studies do not meet all criteria but yield information on low exposure concentrations.
Test form: active ingredients (a.i.) were applied as a formulated product (F), or as a.i. in acetone (S), or as a.i. without a solvent (A). –: not reported. S-stag = single application in a stagnant system; S-stream = single application in a running system; M-stag = multiple applications in a stagnant system; M-stream = multiple applications in a running system; L-stag = prolonged constant exposure in a stagnant system; L-stream = prolonged constant exposure in a running system.

In most studies with pyrethroids, active ingredients were also applied by spraying onto, or injecting below, the water surface. In one study with the organophosphorous compound chlorpyrifos (Giddings et al., 1997) and three studies with pyrethroids [lambda-cyhalothrin (Hill et al., 1994b); tralomethrin (Mayasich et al., 1994); cyfluthrin (Johnson et al., 1994)], drift as well as runoff applications were performed in the same test system. In the case of runoff applications, the compound is brought into the systems bound to soil material. In the three pyrethroid studies specifically, it was not always clear whether the observed effects were caused by the drift or by the runoff application. This is to do with the fact that reported measured concentrations do not always tally, because of the high disappearance rate of pyrethroids from the water and variation in the first

Table 3. Experiments with synthetic pyrethroids included in this report

Active ingredient	Test form	Experiment	Location	Authors
Cyfluthrin	F	M-stag	USA (Texas)	Johnson et al. 1994, Morris et al. 1994
Cypermethrin	F	M-stag	UK	unpublished data
–	–	M-stag	UK	Farmer et al. 1995
–	F	M-stag1	USA (N. Carolina)	Hill 1985
–	F	M-stag2	USA (N. Carolina)	Hill 1985
Deltamethrin	F	S-stag	Senegal	Lahr et al. 1995
–	F	S-stag	Canada	Morill and Neal 1990
Esfenvalerate	F	M-stag	USA (Alabama)	Webber et al. 1992
–	S	M-stag	USA (Missouri)	Fairchild et al. 1992b
–	F	M-stag	USA (Minnesota)	Lozano et al. 1992, Tanner and Knuth 1996
–	A	S-stag	USA (lab)	Stay and Jarvinen 1995
–	F	S-stag	Denmark	Samsøe-Petersen et al. 2001
Fenvalerate	F	S-stag	Canada (Ontario)	Day et al. 1987
–	F	L-stream	USA (Iowa)	Breneman and Pontasch 1994
Lambda-cyhalothrin	–	M-stag	UK	Farmer et al. 1995
–	F	M-stag	USA (N. Carolina)	Hill et al. 1994b
–	F	M-stag	NL	Roessink et al. in prep
–	F	M-stag	NL	Van Wijngaarden et al. in prep a
Permethrin	S	S-stag	Canada (Ontario)	Kaushik et al. 1985
Tralomethrin	F	M-stag	USA (Texas)	Mayasich et al. 1994

Test form: active ingredients (a.i.) were applied as a formulated product (F), or as a.i. in acetone (S), or as a.i. without a solvent (A). –: not reported. S-stag = single application in a stagnant system; S-stream = single application in a running system; M-stag = multiple applications in a stagnant system; M-stream = multiple applications in a running system; L-stag = prolonged constant exposure in a stagnant system; L-stream = prolonged constant exposure in a running system.

sampling instance after spraying (less than 1 to 24 h). We therefore evaluated effects in these studies on the nominal concentration caused by drift application(s) only. In all cases, this is a worst-case approach since the observed effects may in part also be attributed to exposure via the runoff-emission route. The contaminated soil material of the runoff applications rapidly disappears from the water column by sedimentation, and bio-availability of the soil-bound pyrethroids is also lower (Hill, 1985, 1989; Maund et al., 1997, 1998). These factors are likely to mitigate the contribution of a runoff application to the effects of a combined spray and runoff application.

Particularly in drift simulating applications to stagnant waters, clear concentration gradients of insecticides can be found in the first hours post-treatment (Muir et al., 1992; Fairchild and Eidt, 1993; Crum and Brock, 1994; Farmer et al., 1995; Van Wijngaarden et al., 1996; Samsøe-Petersen et al., 2001). Shortly after drift applications, most of the active ingredient is then found in the superficial water layer. Also the influence of the type of formulation and/or additives on the dissipation mechanisms may play a role. Oil-based formulations are much more likely to retain high concentrations in superficial water layers than emulsifiable concentrate formulations which will dissipate more quickly throughout the water column.

Hence, superficially, initial concentrations may be considerably higher than the intended nominal concentrations. Simultaneously, exposure concentrations in subsurface water are then considerably lower than nominal concentrations. This implies that species, although they may be equally sensitive in the laboratory, may respond very differently in the field when they occupy different spatial niches in their natural environments. This is shown from a study with lambda-cyhalothrin (Hill et al., 1994b) in which surface bugs (Gerridae and Veliidae) reacted more sensitively than water bugs and beetles such as Notonectidae and Haliplidae.

In time, insecticides usually get mixed in the water column and often a considerable amount dissipates from the water. This disappearance, especially during the first days after application, is not only caused by physicochemical degradation

but also by the distribution of the active ingredient over different environmental compartments such as sediment, organic and inorganic particulate material, aquatic plants (e.g., Hill, 1989; Brock et al., 1993; Crum and Brock, 1994, Samsøe-Petersen et al., 2001; Hand et al., 2001) and volatilisation from the water (e.g., Larkin and Tjeerdma, 2000).

Initial half-life values of dissolved organophosphates and carbamates in the water of stagnant (model) ecosystems are in the order of less than 1–10 days (Crossland and Bennett, 1984; Hanazato and Yasuno, 1990; Lahr and Diallo, 1993; Crum and Brock, 1994; Tanner and Knuth, 1995; Wayland and Boag, 1995; Giddings et al., 1996). In the case of pyrethroids, initial half-life in the water columns are in the order of less than 1 h to 3 days (Stephenson et al., 1986; Heinis and Knuth, 1992; Fairchild et al., 1992b; Johnson et al., 1994; Farmer et al., 1995; Hand et al., 2001; Roessink et al., in press). Reported half-life of sediment-adsorbed pesticides is generally much longer (days to weeks) in the above-mentioned studies.

These spatio-temporal processes indicate that nominal concentrations cannot be directly converted into actual exposure concentrations for aquatic organisms in the field. The observed initial stratification of insecticides in the water column makes it likely that benthic organisms and those present in internal refugia, such as dense vegetations, are initially exposed to lower concentrations than organisms having niches and/or home ranges close to the water surface. In fact, spatio-temporal distribution of non-persistent insecticides forms a major issue in the discussion related to refinements of ecotoxicological risk assessments (Giesy et al., 1999; Hendley et al., 2001; Maund et al., 2001; Travis and Hendley, 2001).

Nevertheless, we have taken nominal concentration as a reference for describing the effects resulting from peak exposures because: (a) the applied nominal dose is given in almost all studies, (b) measured initial concentrations are not always comparable and/or reliable due to large differences in the first sampling instance after treatment (hours to days) in relation to the relatively high initial disappearance rate of most insecticides, (c) in registration policies the short-term exposure as a result of drift is calculated by assuming instantaneous mixing of the dose over the water column.

Effects on sensitive endpoints

Effects reported

A distinction between direct and indirect effects is frequently made in the reported effects of insecticides in microcosm and mesocosm experiments. However, a decrease in population density of a species after application of an insecticide cannot, in advance, be considered as a direct effect; it could also be the result of an indirect effect due to shifts in species interactions.

Reductions in population densities at relatively low insecticide concentrations are found especially in populations of crustaceans (cluster Amphipoda – Ostracoda/Anostraca in Tables 4 and 5), insects (cluster Trichoptera – Coleoptera) and fish (Pisces). Negative effects in these groups were observed below 1 TU_{mso} after single applications of acetylcholinesterase inhibitors (Table 4) and below 0.1 TU_{mso} after repeated applications of pyrethroids (Table 5). Reductions in numbers of Rotifera, Mollusca, Annelida and Turbellaria are only observed at relatively high exposure concentrations and in a limited number of studies. Negative effects on plants are only reported at exposure concentrations higher than 1–10 TU_{mso}.

When laboratory toxicity tests have been conducted with species that are found in microcosm and mesocosm experiments, the sensitivities among these species to insecticide exposures have been shown to be similar in both test systems (Dortland, 1980; Crossland, 1984; Van Wijngaarden et al., 1996; Lahr, 1998; Maund et al., 1998; Van den Brink et al., 2002a, b). In addition, responses found in the evaluated studies for specific taxonomic groups correspond well with those found in laboratory single-species toxicity tests with indigenous species from these groups (e.g., Crommentuijn et al., 1997; AQUIRE database, http://www.epa.gov/ecotox/). This makes it probable that in microcosm and mesocosm experiments, observed reductions in densities of crustaceans, insects and fish at low concentrations can generally be considered as direct toxic effects. One should, however, be aware that insects,

Table 4. Reported negative effects on various taxonomic groups as a result of single applications of acetylcholinesterase-inhibiting insecticides in aquatic microcosms and mesocosms

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

The effects are arranged according to toxic units (TU_{mso}) and expressed as a percentage of the cases ($n = x$) in which a reduction in numbers or biomass of one or more taxa within a taxonomic group was reported.
*Direct as well as indirect effects reported.
**Data do not allow clear conclusions as to whether or not effects occurred.
***Reported as indirect effects.

Table 5. Reported negative effects on various taxonomic groups as a result of repeated application of pyrethroids in aquatic microcosms and mesocosms

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

The effects are arranged according to toxic units (TU_{mso}) and expressed as a percentage of the cases ($n = x$) in which a reduction in numbers or biomass of one or more taxa within a taxonomic group was reported.

crustaceans and fish may also include relatively insensitive taxa (e.g., Dortland, 1980; Brock et al., 1992b; Lahr and Diallo, 1993; Giddings et al., 1996).

The categories 'Microcrustaceans', 'Macrocrustaceans', 'Insects' and 'Fish' include the sensitive organisms. The categories 'Rotifers', 'Other macroinvertebrates' and 'Algae & macrophytes' often include organisms that are indirectly affected but where the occurrence of direct effects cannot be excluded a priori.

Effects of acetylcholinesterase inhibitors

In stagnant test systems, clear effects (Classes 3, 4 and 5) are observed in the endpoint categories 'Microcrustaceans', 'Macrocrustaceans, 'Insects' and 'Fish' from about 0.1 TU_{mso} (Fig. 1a–d). Effects are hardly ever observed at insecticide concentrations below 0.1 TU_{mso}. One exception forms a study on a chronic exposure to chlorpyrifos (Van den Brink et al., 1995). For the previously mentioned four categories, more or less clear concentration-effect relationships are present (Fig. 1a–d). The data also show that single applications were studied most often (Fig. 1). Effects are more severe in studies with repeated or chronic applications (Fig. 1a, c).

Clear effects on 'Rotifers', 'Other macroinvertebrates', and 'Algae & macrophytes' generally occur from concentrations of 1 TU_{mso} and higher (Fig. 1e–g). Usually, effects in community metabolism endpoints were observed at concentrations around 10 TU_{mso} and higher (Fig. 1h). This indicates that the structure of the aquatic community is more sensitive to acetylcholinesterase inhibitors than functional characteristics of the ecosystem.

Few studies with acetylcholinesterase inhibitors have been done in running waters. Results are not incorporated in Fig. 1 because of the deviating exposure regimes. A pulse of 6 h with a concentration of 0.08 TU_{mso} chlorpyrifos had no effect on the abundance of fauna in experimental streams (Pusey et al., 1994). A clear effect on insect populations was observed in the same study for an equally long application of 3.85 TU_{mso}, after which recovery of the reduced populations occurred within 8 weeks. Courtemanch and Gibbs (1980) found a clear decrease in the abundance of Plecoptera and Ephemeroptera for carbaryl in streams at a nominal pulse concentration of 5.7 TU_{mso}. Morrison and Wells (1981) studied pulse applications of fenitrothion in streams. At 0.1 TU_{mso} they found no effect at all, and at 1.7 TU_{mso} only a slight effect, especially in the form of drift of insects. Thus, the results of the lotic systems do not seem to differ very much from that of lentic systems with regard to the direct impact of acetylcholinesterase-inhibitor concentrations.

Effects of synthetic pyrethroids

The microcosm and mesocosm studies with pyrethroids in particular, concern effects of repeated applications in stagnant water. Effects are observed in the categories 'Microcrustaceans' and 'Insects' from about 0.01 TU_{mso} and higher (Fig. 2a, c). In the range 0.01–0.1 TU_{mso} they relate especially to slight effects (Class 2). At higher exposure concentrations, in the range 0.1–1 TU_{mso}, clear effects (Classes 3, 4 and 5) are regularly reported for 'Microcrustaceans', 'Macrocrustaceans' and 'Insects', while for 'Fish' slight effects are reported in a limited number of studies (Fig. 2a–d). In some studies, clear effects at concentrations lower than 1 TU_{mso} are also reported for the category 'Rotifers (Fig. 2e). At concentrations higher than 1 TU_{mso}, effects can be observed in all categories of structural endpoints (Fig. 2a–g).

After repeated exposure to pyrethroids and at final peak concentrations higher than 0.1 TU_{mso}, long-term (> 8 weeks after last application) effects on – in particular – crustaceans and insects cannot be excluded (Fig. 2). The pyrethroid studies also indicated that the structure of the aquatic community is more sensitive to insecticides than functional characteristics of the ecosystem (Fig. 2a–g versus 2h).

Responses of the most sensitive endpoints

In a few cases, results clearly deviated from the general concentration-effect relationships for the sensitive endpoint categories ''Microcrustaceans'', ''Macrocrustaceans'', ''Insects'', and ''Fish'' (Figs. 1 and 2). For example, in the study of Lahr and Diallo (1993) with fenitrothion, macrocrustaceans responded by a factor of 10–100 times less sensitive

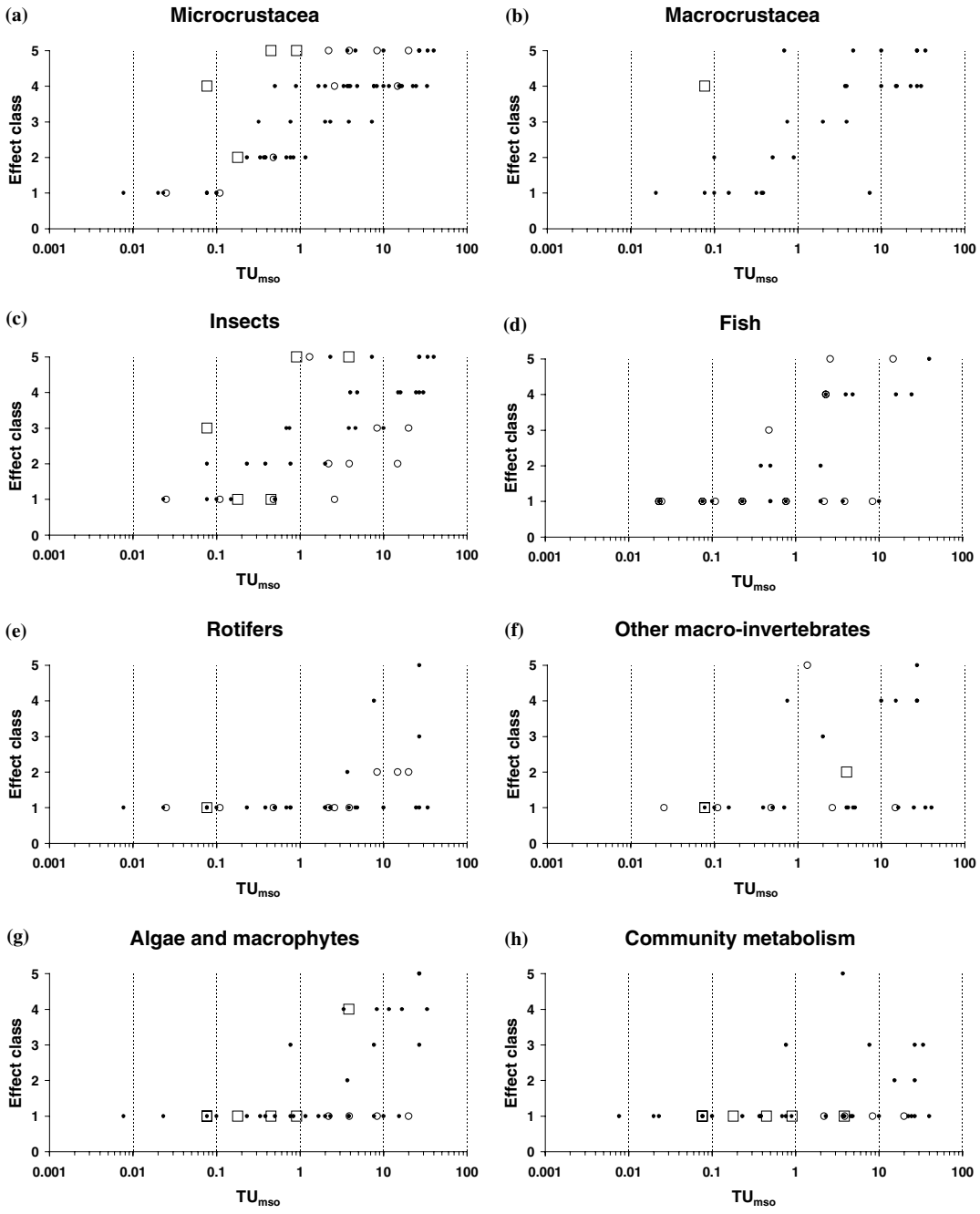

Figure 1. Effects of insecticides with an acetylcholinesterase-inhibiting mode of action in microcosm and mesocosm studies. The figure includes observations of studies in stagnant water (single and multiple applications), and of chronic applications in stagnant as well as running water test systems.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

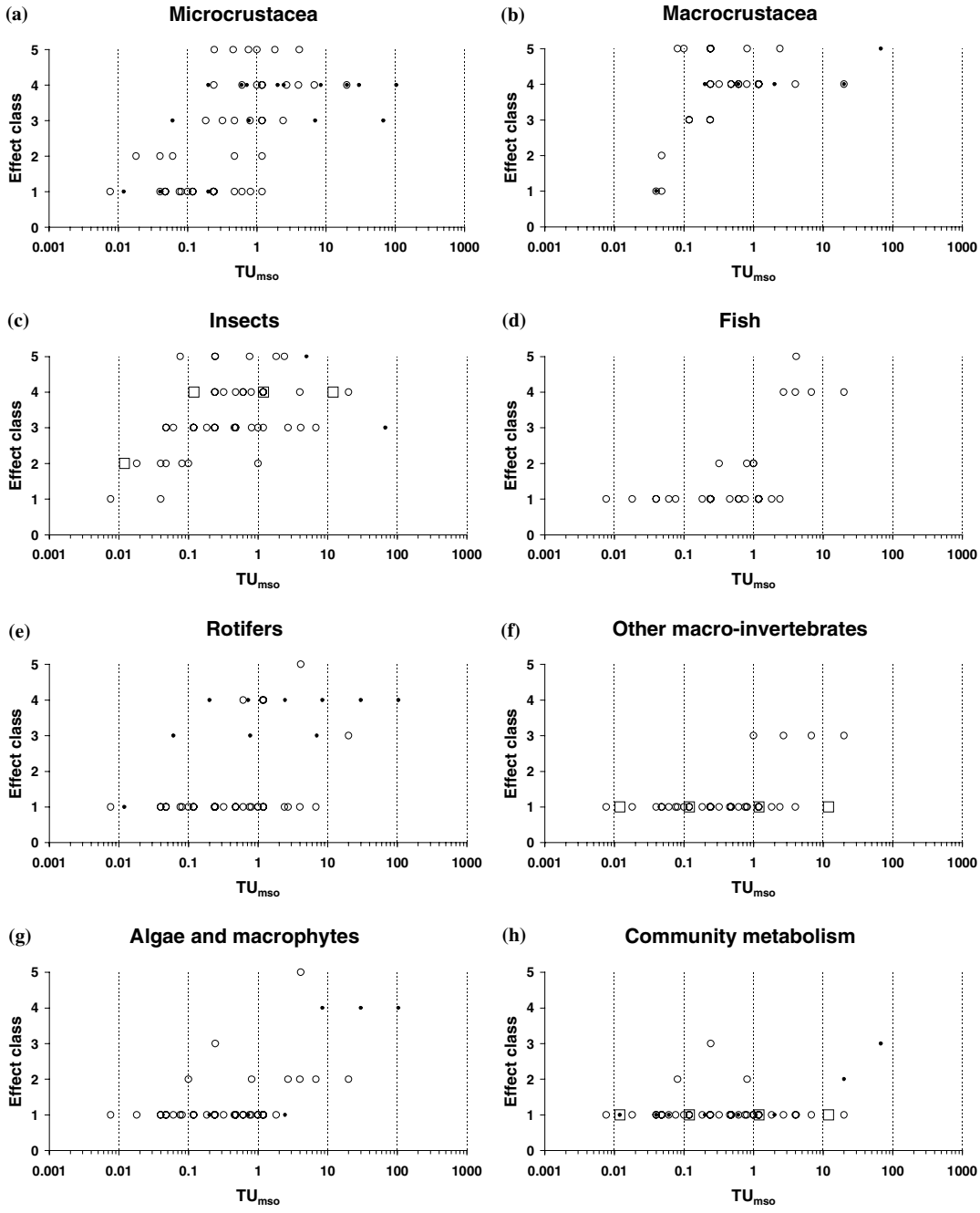

Figure 2. Effects of insecticides with synthetic pyrethroids in microcosm and mesocosm studies. The figure includes observations of studies in stagnant water (single and multiple applications), and of chronic applications in stagnant as well as running water test systems. Effects are classified into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

than in the other studies (No effects (Class 1) at 7.3 TU_{mso} in Fig. 1b). In this study, macrocrustaceans were only represented by the anostracan taxon *Streptocephalus* spp. which is relatively insensitive to fenitrothion. Overall, however, the study did not necessarily give deviating information on the ecological effects in the field because sensitive groups, in the form of insects and microcrustaceans, were still present. Lahr et al. (1995) studied the effects of a single application of deltamethrin at one relatively high concentration only (67.5 TU_{mso}). Here, Anostraca was shown to be the most sensitive group (Class 5, Fig. 2b) while the short-cyclic cladocerans (Class 3, Fig. 2a) and inflying hemiptera (Class 3, Fig. 2c) rapidly recolonized the treated natural ponds.

To reduce the emphasis on slight effects, and to focus on the realistic worst-case scenario of the effects observed in the microcosm and mesocosm studies, we selected the most sensitive endpoints of each study and plotted observed effects against studied concentrations (Fig. 3). In the case of single applications, effects on the most sensitive endpoints are not usually observed at concentrations of $\leq$ 0.1 TU_{mso} (Fig. 3a). At higher doses, slight to clear effects may be expected. In the case of microcosm and mesocosm studies, which typically simulate isolated water systems, there is a good chance that recovery of sensitive endpoints takes longer than 8 weeks (Class 5 effects) at single doses resulting in exposure concentrations of 1 TU_{mso} and higher (Fig. 3a).

For repeated and chronic exposures, concentrations below 0.01 TU_{mso} have rarely been the subject of studies (Fig. 3a and c). Nevertheless, the results show that below 0.01 TU_{mso}, it is unlikely for any clear effects to be expected. Within the concentration range 0.01–0.1 TU_{mso} mainly slight (Class 2) to short-term clear effects (Class 3) are reported for the most sensitive endpoints. Above 0.1 TU_{mso}, clear and prolonged effects (Class 5) are to be expected in test systems

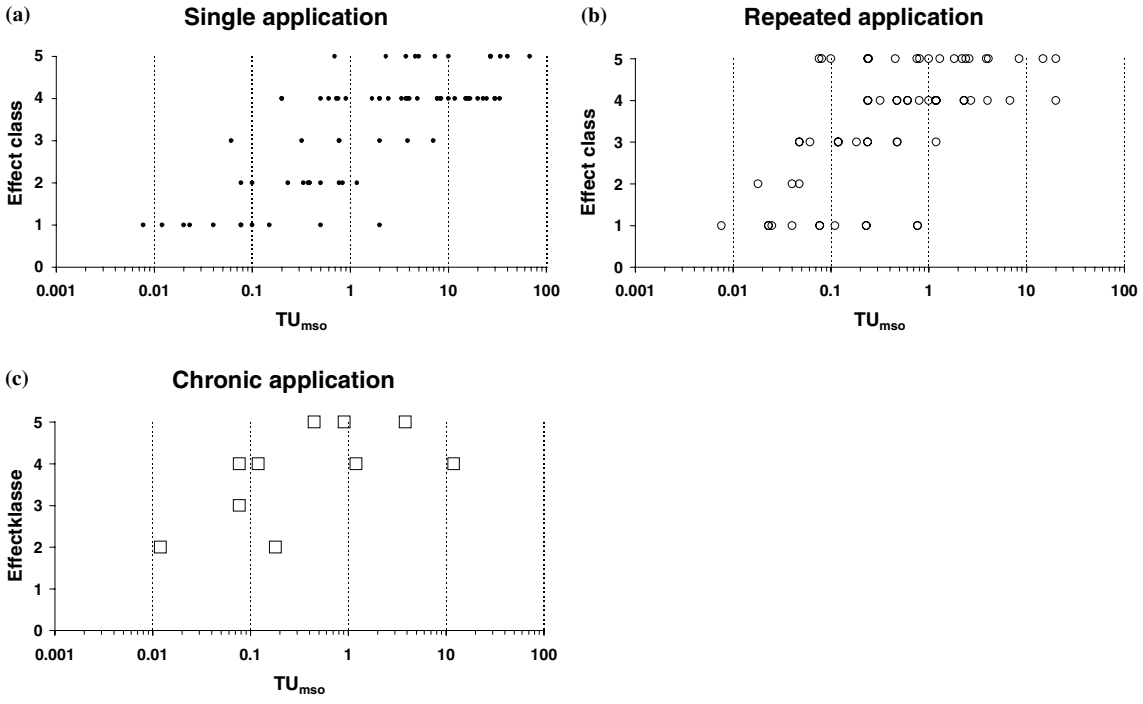

Figure 3. Responses of the most sensitive endpoint in microcosm and mesocosm studies performed with acetylcholinesterase inhibiting or pyrethroid insecticides, based on the data presented in Figs. 1 and 2. The effects on the most sensitive endpoints are presented for a single application (a), multiple applications (b), and chronic exposure (c). The effects are also classified (Effect class) according to magnitude and duration. 1 = no significant effect, 2 = slight effect, 3 = clear short-term effect ($<$8 weeks), 4 = clear effect in short-term study (recovery moment unknown), 5 = clear long-term effect ($>$8 weeks).

that are repeatedly or chronically stressed with insecticides.

Regression analysis indicates that when comparing Class 1 effects with Classes 3, 4 and 5 effects, single applications at concentration levels of 0.13 TU_{mso} can be expected to induce clear effects (Classes 3 to 5) in the field in 50% of cases (Table 6). There is a small probability (FEC-5%) that effects occur at concentrations below 0.05 TU_{mso} (FEC-5%: Field Effect Concentrations which will affect the most sensitive endpoints with a probability of 5%). There is a high probability (FEC-95%) that clear effects will occur in microcosm and mesocosm situations at concentrations of 0.34 TU_{mso} and higher.

For the situation where we include slight effects (Class 2) in the 'no-effect class', FEC-50% for single applications increases to 0.26 TU_{mso} (Table 6). FEC-5%, however, stays more or less at the same concentration level, i.e. 0.04 TU_{mso} against 0.05 TU_{mso} in the previous scenario.

Regarding multiple or chronic applications, effects can be expected to occur at lower concentrations (Table 6). FEC-50% levels were 16–33% of those for single applications. Differences between multiple and chronic exposures were less significant (Table 6). Probability calculations for chronic FECs, however, were less accurate since much less data were available (Table 6: no calculation possible; high range confidence limits). Nevertheless, it means that for an adequate risk analysis it is at least desirable to distinguish between exposure regimes resulting from single applications on the one hand, and that of multiple/chronic applications on the other.

Comparing $NOEC_{eco}$ with regulatory criteria

For the acetylcholinesterase inhibitors, most LOECs from the reviewed studies were in Classes 3 to 5 (Table 7). $NOEC_{eco}$s could be derived for five acetylcholinesterase inhibitors, and Class 2-$LOEC_{eco}$s for three compounds (Table 7). These usually concerned exposure regimes resulting from single applications. Comparing $NOEC_{eco}$s with first tier Uniform Principles (UP) criteria (EU, 1997) shows that these NOEC values were about a

Table 6. Field Effect Concentrations (FEC) as calculated by means of logistic regression

		Estimate	(95%-Confidence limits)
No effects versus clear effects			
Single	FEC5%	0.049	(0.016–0.154)
	FEC50%	0.130	(0.068–0.249)
	FEC95%	0.341	(0.093–1.257)
Multiple	FEC5%	0.016	(0.003–0.095)
	FEC50%	0.043	(0.020–0.094)
	FEC95%	0.118	(0.043–0.320)
Chronic	FEC5%	*x*	(*x*–*x*)
	FEC50%	*x*	(*x*–*x*)
	FEC95%	*x*	(*x*–*x*)
No slight effects versus clear effects			
Single	FEC5%	0.036	(0.007–0.198)
	FEC50%	0.261	(0.126–0.541)
	FEC95%	1.862	(0.502–6.914)
Multiple	FEC5%	0.023	(0.007–0.070)
	FEC50%	0.052	(0.032–0.085)
	FEC95%	0.119	(0.050–0.284)
Chronic	FEC5%	0.003	(0.000–4.868)
	FEC50%	0.043	(0.003–0.665)
	FEC95%	0.544	(0.010–29.01)

FECs, with 95%-confidence limits, are expressed in TU_{mso}. FECs were expressed as 5, 50 and 95 percentages of probability of effects occurring on the most sensitive endpoints for achetylcholinesterase inhibiting and pyrehtroid insecticides (Fig. 3). FECs were calculated for two scenarios; one where no effects are placed against clear effects (Effect Class 1 versus Effect Classes 3, 4 and 5) and one where no and slight effects are placed against clear effects (Classes 1 and 2 versus Classes 3, 4 and 5). Results were based on responses found in studies using single, multiple and chronic insecticide applications. x = no calculation possible due to a lack of data.

Table 7. $NOEC_{eco}$ and $LOEC_{eco}$ values (μg/l) for microcosm and mesocosm studies with single or multiple applications of acetylcholinesterase-inhibiting insecticides

Active ingredient	Dose	$NOEC_{eco}$ (Class 1)	$LOEC_{eco}$ (Class 2)	$LOEC_{eco}$ (Class 3, 4, 5)	Reference
Stagnant water systems					
Azinphos-methyl	Single	0.2	0.72	–	Stay and Jarvinen 1995
	Single	0.2	–	1.0	Knuth et al. 1992
	Single	–	–	1.0	Tanner and Knuth 1995
	Multiple	0.22	–	0.95	Giddings et al. 1994
Chlorpyrifos	Single	0.1	0.3	1.0	Biever et al. 1994
	Single	0.1	–	0.9	Van den Brink et al. 1996
	Single	0.1	–	1.0	Van Wijngaarden et al. subm. b
	Single	–	–	0.5	Brazner et al. 1989, Siefert et al. 1989, Brazner and Kline 1990
	Single	–	0.5	5	Stay et al. 1989
	Single	–	–	5	Brock et al. 1992a, b, 1993
	Single	–	–	10	Hughes et al. 1980
	Single	–	–	35	Van Donk et al. 1995, Brock et al. 1995b, Cuppen et al. 1995
	Continuous	–	–	0.1	Van den Brink et al. 1995
Diazinon	Multiple	–	–	2.4	Giddings et al. 1996
Fenitrothion	Single	–	–	80	Lahr and Diallo 1993
	Multiple	–	–	14.3	Fairchild and Eidt 1993
Parathion-ethyl	Continuous	0.2	–	0.5	Dortland 1980
Parathion-methyl	Single	–	–	10	Crossland 1988
	Single	–	–	100	Crossland 1984
Phorate	Single	–	–	23	Dieter et al. 1996
Bendiocarb	Single	–	–	24	Lahr et al. 1995
Carbaryl	Single	–	2	20	Havens 1994, 1995
Carbofuran	Single	5	–	25	Wayland 1991
Running water systems					
Chlorpyrifos	Single	0.1	–	5	Pusey et al. 1994
	Continuous	–	–	0.1	Ward et al. 1995
Fenitrothion	Single	1.1	–	18.7	Morrison and Wells 1981
	Single	–	–	30.8	Poirier and Surgeoner 1988
	Single	–	–	460	Yasuno et al. 1981
Carbaryl	Single	–	–	34	Courtemanch and Gibbs 1980

$LOEC_{eco}$ values are divided into slight effects (Class 2) and more severe effects (Classes 3–5). $NOEC_{eco}$ represents the 'no effect class' (Class 1).

factor of 10 or more, higher than set acceptable concentrations (Table 8).

Most of the pyrethroid studies also yielded Classes 3 to 5-$LOEC_{eco}$ values only (Table 9). A $NOEC_{eco}$ could be derived for three pyrethroids. These NOECs did not deviate much from the first tier UP criteria (Table 10). NOECs were equal to, or less than, a factor of five higher than set safety criteria. Hence, the margin between UP criteria and $NOEC_{eco}$s observed in the field was less for synthetic pyrethroids than for acethylcholinesterase inhibitors. This can be explained by the fact that some non-target organisms in the field are relatively more sensitive to pyrethroids than to acetylcholinesterase inhibitors, at least when compared with the standard test species of *Daphnia* and fish (Schroer et al., 2004).

Overall, the established $NOEC_{eco}$s indicate that set safety factors and criteria for protecting aquatic organisms as described in the EU Uniform Principles seem to be adequate for both groups of insecticides, and possibly over-protective for single applications acetylcholinesterase inhibitors.

In this paper we specifically focussed on the regulatory implications of the outcome of model ecosystem studies for first tier risk assessment procedures as applied in the EU. Like the EU-member states, many other countries from all

Table 8. Summarised $NOEC_{eco}$ and $LOEC_{eco}$ values for acetylcholinesterase-inhibiting insecticides in microcosm and mesocosm studies

			Actual nominal concentrations			TUR		
Active ingredient	Exposure regime	UP	$NOEC_{eco}$ (Cl 1)	$LOEC_{eco}$ (Cl 2)	$LOEC_{eco}$ (Cl 3–5)	$NOEC_{eco}$ (Cl 1)	$LOEC_{eco}$ (Cl 2)	$LOEC_{eco}$ (Cl 3–5)
Stagnant water systems								
Azinphos-methyl	Single	0.02	0.2	0.72	1	10	36	50
	Multiple	0.02	0.22	–	0.95	11	–	48
Chlorpyrifos	Single	0.013	0.1	0.3	0.5	7.7	23.1	38.5
	Continuous	0.013	–	–	0.1	–	–	7.7
Diazinon	Multiple	0.01	–	–	2.4	–	–	240
Fenitrothion	Single	0.11	–	–	80	–	–	727
	Multiple	0.11	–	–	14.3	–	–	130
Parathion	Continuous	0.011	0.2	–	0.5	18	–	45.5
Parathion-methyl	Single	0.014	–	–	10	–	–	714
Phorate	Single	0.015	–	–	23	–	–	1533
Bendiocarb	Single	0.74	–	–	24	–	–	32.4
Carbaryl	Single	0.056	–	2	20	–	35.7	357
Carbofuran	Single	0.33	5	–	25	15	–	76
Running water systems								
Chlorpyrifos	Single	0.013	0.1	–	5	7.7	–	385
	Continuous	0.013	–	–	0.1	–	–	7.7
Fenitrothion	Single	0.11	1.1	–	18.7	10	–	17
Carbaryl	Single	0.056	–	–	34	–	–	607

Concentrations in μg/l. First tier acceptable concentrations (UP) were derived from the EU-Uniform Principles (Table 2). $LOEC_{eco}$ values are divided into slight effects (Class 2) and more severe effects (Classes 3 to 5). $NOEC_{eco}$ represents the 'no effect class' (Class 1). Cl = Class. TUR shows the $NOEC_{eco}$ or $LOEC_{eco}$ –first tier acceptable concentration ratio (Toxicity–UP Ratio).

over the world use OECD guidelines for toxicity testing and apply safety factors in one way or another as a first step in aquatic risk assessment (e.g., US-EPA, 1998). In the case of acetylcholinesterase inhibitors and synthetic pyrethroids, the OECD standard test species *D. magna* and standard test fishes were good representatives of sensitive species in the field. When one accepts to rank toxicity of acetylcholinesterase inhibitors and synthetic pyrethroids to these standard species, then exposure–toxicity ratio methods like for example applied in the USA (hazard quotient method (Urban and Cook 1986)), also seem to be protective towards aquatic ecosystems.

General discussion and conclusions

The ecological risk of 18 insecticides in freshwater ecosystems is discussed in this paper. They form 15% of the 123 pesticides with similar modes of action that are, or were, available on the market for agricultural pest management programmes (Tomlin, 2000). Nevertheless, given the range of responses reported among these pesticide studies, they appear to represent general ecological effects for acetylcholinesterase inhibitors and synthetic pyrethroids in aquatic ecosystems (Fig. 3).

Normalisation of reported field concentrations to TU_{mso} enables a comparison to be made between studies with insecticides that have working mechanisms in common. The use of TU_{mso} has been shown to be an adequate reference for estimating field responses due to direct toxic effects. It should be kept in mind, however, that for these compounds standard species are relatively good representatives of sensitive species. If standard species are not representative of the sensitive taxonomic groups, then the choice of TU_{mso} will be less successful.

The studies were done in various parts of the world and under various experimental conditions. However, $NOEC_{eco}$s and Class 2-$LOEC_{eco}$s were still shown to be very consistent regardless of study location, at least when similar exposure regimes are considered (Table 11). Leeuwangh (1994)

Table 9. $NOEC_{eco}$ and $LOEC_{eco}$ values (μg/l) for microcosm and mesocosm studies with single or multiple applications of a pyrethroid insecticide

Active ingredient	Dose	$NOEC_{eco}$	$LOEC_{eco}$ (Class 2)	$LOEC_{eco}$ (Class 3,4,5)	Reference
Stagnant water systems					
Cyfluthrin	Multiple	–	–	0.036	Johnson et al. 1994, Morris et al. 1994
Cypermethrin	Multiple	–	–	0.07	Farmer et al. 1995
	Multiple	–	–	0.16	Hill 1985
Deltamethrin	Single	–	–	0.2	Morrill and Neal 1990
	Single	–	–	2.7	Lahr et al. 1995
Esfenvalerate	Single	0.01	0.05	0.15	Stay and Jarvinen 1995
	Multiple	0.01	–	0.25	Webber et al. 1992
	Multiple	–	0.01	0.08	Lozano et al. 1992
	Multiple	–	–	0.25	Fairchild et al. 1992b
Fenvalerate	Single	0.01	–	0.05	Day et al. 1987
Lambda-cyhalothrin	Multiple	0.0016	–	0.016	Hill et al. 1994b
	Multiple			0.017	Farmer et al. 1995
	Multiple	–	0.01*	0.025	Roessink et al. in press Van Wijngaarden et al. subm. a
	Multiple	–	0.01*	0.025	Roessink et al. in press
	Multiple	–	0.01*	0.025	Van Wijngaarden et al. subm. a
Permethrin	Single	–	–	0.5	Kaushik et al. 1985
Tralomethrin	Multiple	–	0.0027	0.0092	Mayasich et al. 1994
Running water systems					
Fenvalerate	Continuous	–	0.01	0.1	Breneman and Pontasch 1994

$LOEC_{eco}$ values are divided into slight effects (Class 2) and more severe effects (Classes 3 to 5).
$NOEC_{eco}$ represents the 'no effect class' (Class 1).
*Longer-term effects on one pre-dominant species. For the community as a whole, NOECs calculated were 0.01 μg/l.

Table 10. Summarised $NOEC_{eco}$ and $LOEC_{eco}$ values from studies with pyrethroids in microcosm and mesocosm experiments

			Actual nominal concentrations			TUR		
Active ingredient	Exposure regime	UP	$NOEC_{eco}$ (Cl 1)	$LOEC_{eco}$ (Cl 2)	$LOEC_{eco}$ (Cl 3–5)	$NOEC_{eco}$ (Cl 1)	$LOEC_{eco}$ (Cl 2)	$LOEC_{eco}$ (Cl 3–5)
Stagnant water systems								
Cyfluthrin	Multiple	0.0015	–	–	0.036	–	–	24
Cypermethrin	Multiple	0.0068	–	–	0.07	–	–	10
Deltamethrin	Single	0.0004	–	–	0.2	–	–	500
Esfenvalerate	Single	0.0025	0.01	0.05	0.15	4	20	60
	Multiple	0.0025	0.01	0.01	0. 08	4	4	32
Fenvalerate	Single	0.008	0.01	–	0.05	1.25	–	6.25
Lamda-cyhalothrin	Multiple	0.0021	0.0016	0.01	0.025	0.76	4.8	11.9
Permethrin	single	0.0065	–	–	0.5	–	–	77
Tralomethrin	Multiple	0.0015	–	0.0027	0.0092	–	1.8	6.1
Running water systems								
Fenvalerate	Continuous	0.008	–	0.01	0.1	–	1.25	12.5

Concentrations in μg/l. First tier acceptable concentrations (UP) were derived from the EU-Uniform Principles (Table 2). $LOEC_{eco}$ values are divided into slight effects (Class 2) and more severe effects (Classes 3 to 5). $NOEC_{eco}$ represents the 'no effect class' (Class 1). Cl = Class. TUR shows the $NOEC_{eco}$ or $LOEC_{eco}$ – first tier acceptable concentration ratio (Toxicity–UP Ratio).

compared the outcome of various microcosm and mesocosm studies done with chlorpyrifos. He concluded that direct effects on susceptible species are often concentration-related and not dependent on system scale or geographical location. Considering the consistency of the threshold values of several compounds (Table 11) this conclusion seems to be applicable to other pesticides as well.

Table 11. Threshold concentrations ($NOEC_{eco}$/Class2-$LOEC_{eco}$) in relation to experimental set-ups and locations of model ecosystem studies with several insecticides

Compound	Dose	Experiment	Location	$NOEC_{eco}$ or Class 2-$LOEC_{eco}$ (µg/l)	References
Azinphos-methyl	Single	Microcosms	Lab	0.2	1
Azinphos-methyl	Single	Littoral enclosures	USA Minnesota	0.2	2
Chlorpyrifos	Single	Outdoor microcosms	USA Kansas	0.1	3
Chlorpyrifos	Single	Experimental ditches	NL	0.1	4
Chlorpyrifos	Single	Microcosms simulating Mediterranean conditions	lab	0.1	5
Esfenvalerate	Multiple	Outdoor mesocosms	USA Alabama	0.01	6
Esfenvalerate	Multiple	Littoral enclosures	USA Minnesota	0.01	7
Lambda-cyhalothrin	Multiple	Outdoor mesocosms	USA N-Carolina	0.002	8
Lambda-cyhalothrin	Multiple	Plankton-dominated enclosures	NL	0.01	9
Lambda-cyhalothrin	Multiple	Macrophyte-dominated enclosures	NL	0.01	9
Lambda-cyhalothrin	Multiple	Enclosures, spring versus late-summer	NL	0.01	10

1: Stay and Jarvinen (1995). 2: Tanner and Knuth (1995). 3: Biever et al. (1994). 4: Van den Brink et al. (1996). 5: Van Wijngaarden et al. (submitted b). 6: Webber et al. (1992). 7: Lozano et al. (1992). 8: Hill et al. (1994b). 9: Roessink et al. (in press). 10: Van Wijngaarden et al. (submitted a).

Table 12. Indirect effects summarised from studies in stagnant waters after a single application of an organophosphorous insecticide, a carbamate, or a pyrethroid

	Structural aspects		Functional aspects	
Range TU_{mso}	Shifts in animal populations	Shifts in algae and higher plants	Decrease in decomposition	Shifts in community metabolism
10–100	$X^{1,3,4,5,8,9,10}$	$X^{4,5,8,10}$	$X^{3,4,5}$	$X^{3,4}$
1–10	$X^{1,2,3,4,6,7,10,13,14}$	$X^{1,10,\ 14}$		X^{14}
0.1–1	$X^{1,2,11,13,14}$	$X^{1,14}$		X^{14}
0.01–0.1	X^{12}			

The nominal concentrations reported in the studies are expressed in TU_{mso}.
Organophosphorous compounds: [1]Siefert et al. (1989), Brazner and Kline (1990), [2]Biever et al. (1994), [3]Van den Brink et al. (1996), Kersting and Van denBrink (1997), Brock et al. (1992a), (1993b), [5]Van Donk et al. (1995), Brock et al. (1995b), Cuppen et al. (1995), [6]Hughes et al. (1980), [7]Fairchild and Eidt (1993), [8]Crossland (1984), [9]Crossland (1988), [14]Van Wijngaarden et al. (subm. b).
Carbamates: [10]Havens (1995), [11]Wayland (1991).
Pyrethroids: [12]Day et al. (1987), [13]Kaushik et al. (1985).

In the case of acetylcholinesterase inhibitors and pyrethroids, Arthropoda contain the species most sensitive to these compounds. In the different types of ecosystems, both natural and model, sensitive representatives of this group are usually available and generally form a predominant part of aquatic communities. This overall presence of one or a few sensitive taxa in microcosm and mesocosm studies carried out with these types of insecticides, explains why such studies have a certain robustness and a general predictive value for ecological risk assessment in the field.

Above threshold levels, studied endpoints show wide concentration ranges (in TU) per effect class between experiments. For example, concentrations inducing Class 3 effects ranged over approximately two orders of magnitude in TU for the frequently measured endpoints 'Microcrustaceans' and 'Insects' (Figs. 1 and 2). This high variability relates to ecological properties of the test systems, the experimental set-up and frequency of observations used, organisms studied and taxonomic level of identification, and ecotoxicological profile of the insecticides. Differences in environmental behaviour of the insecticides, resulting in differences in bioavailability, can be expected to be another source of observed variation in response concentrations.

Only a limited number of studies appeared to be suitable for validation of the first tier risk

assessment criteria. $NOEC_{eco}$ values could be established for eight compounds only. Many of the studies were simply not designed to give this type of information. Obtained $NOEC_{eco}$s and Class 2-$LOEC_{eco}$ data, however, suggest that the safety factors as calculated in this paper generally offer aquatic organisms and ecosystem functions adequate protection against adverse effects related to usage of organophosphorous and pyrethroid insecticides. These studies also show that it seems to be significant to distinguish between exposure regimes; for a single application of non-persistent insecticides it seems possible to be a factor of ten more lenient than for repeated and chronic exposures to the same chemicals.

The most sensitive endpoints for direct effects of the insecticides studied were structural ecosystem characteristics and usually concerned population densities of crustaceans and insects. These direct effects can generally be well predicted on the basis of laboratory tests with similar species as studied in the microcosm and mesocosm experiments (e.g., Crossland and Wolff, 1985; Fairchild et al., 1992a; Van Wijngaarden et al., 1996; Maund et al., 1998; Sheratt et al., 1999; Schroer et al., 2004). Different studies conducted with the same insecticide (e.g., chlorpyrifos, esfenvalerate, lambda-cyhalothrin) also yield similar critical threshold values (Tables 7 and 9). This may imply that $NOEC_{eco}$s and Class 2-LOECs of adequate model ecosystem studies can be used to validate the cut-off-values such as the HC_5 or HC_{10} values of Species Sensitivity Distribution curves (Solomon et al., 2001; Van den Brink et al., 2002a; Postuma et al., 2002) based on laboratory tests with standard and additional species. As it cannot be excluded that taxa that may be sensitive to a pesticide in a natural system are not screened in the laboratory because they are not easily cultured, held or tested.

Indirect effects of insecticides seem to be much more variable (e.g., Leeuwangh, 1994; Brock et al., 1992b, 2000b). Such types of effects are steered more by experimental conditions and stochastic processes than in the case of direct effects. However, when indirect effects were summarised, general response patterns could be recognised (Table 12). The studies show that the frequency of reported indirect effects increased with increasing concentrations. Indirect effects on functional endpoints were less frequently reported, which on the one hand supports the idea that functional aspects of the ecosystems are less sensitive to toxic stress by compounds studied. On the other hand, however, it cannot be excluded that functional endpoints have been less frequently reported because they are not often measured in these types of studies. Indirect effects on structural endpoints are to be expected from exposure concentrations in the range of 0.1–1 TU_{mso} and higher (Table 12). Although it seems difficult to predict accurately which specific species will suffer indirect effects due to insecticide stress, aggregation of biological taxa into functional groups allows food-web modelling and the prediction of overall ecological responses that will follow direct toxic effects (Traas et al., 1998; Baird et al., 2001).

Many of the studies evaluated were stopped before recovery times of sensitive populations could be established (Class 4 observations in Figs. 1 and 2). Nevertheless, on the basis of the remaining studies, a general picture of the recovery of sensitive invertebrates can be given. In stagnant waters sensitive species having short life-cycles (microcrustaceans), usually recovered within 8 weeks after a single exposure of less than 10 TU_{mso} (Fig. 1a). Fewer data are available on the recovery rate in systems that are repeatedly exposed. Figure 2a, however, suggests that also after repeated applications, recovery generally occurs within 8 weeks of the last application as long as this last application was less than 10 TU_{mso}.

Duration of effects and recovery of stressed ecosystems is an important issue in higher tier risk evaluation (Campbell et al., 1999). Testing pesticides in outdoor (model) ecosystems has the advantage that this type of research may provide information on the recovery of the systems after pesticide contamination has ceased. Actual recovery of sensitive populations depends on the instant that concentrations reach non-toxic levels again, in combination with an array of biological and ecological characteristics (e.g., Giesy et al., 1999; Brock and Budde, 1994; Lahr et al., 2000). The microcosm and mesocosm studies demonstrate that recovery after pesticide contamination is expected to be rapid in the real world when (a) the compound is not persistent, (b) the physicochemical environment is not altered, or is quickly restored, (c) the generation

times of vulnerable populations are short, and/or (d) when there is immigration from residual populations in nearby unaffected areas.

Model ecosystems are generally of smaller dimensions than the aquatic ecosystems they aim to simulate. In addition, model ecosystem studies are restricted in duration (of the 51 evaluated studies, 26 lasted from 2 to 6 months, three made observations in the following growing season, and the rest lasted for less than 2 months after the (last) treatment). Because of these characteristics, it may be expected that organisms on the microscale (e.g., plankton) are better adapted to dimensions and time-scale of the experiments than organisms on the macroscale (e.g., macroinvertebrates, fish). Hence, predominantly small-sized species, especially when they also have short generation times (e.g., plankton, multivoltine invertebrates), have an ecological advantage over other life history traits in these types of studies. It should therefore be taken into account in the interpretation of effects and recovery of species from model ecosystem studies whether or not the experimental circumstances provide unrestricted conditions for studying the effects and recovery of species of interest.

This review of ecological effects studied under quasi-natural conditions shows that some estimations of direct effects in the field can be made by taking the acute EC_{50} of the most sensitive standard test species (TU_{mso}) as a reference concentration, and by classifying the effects. Modelling the observed responses of the most sensitive endpoints (Fig. 3) provides a way of extrapolating results of microcosm and mesocosm observations to probabilities of effect occurrences in the field at predicted or measured environmental concentrations (Table 6). Using the same regression model, the outcome of low risk concentration values can be varied by choosing either a strict, or a more lenient, scenario (considering Effect Class 1 only or, considering Effect Classes 1 and 2 as the 'no-effect' classes). Other options are to choose higher or lower probability levels (e.g. FEC5% or FEC10%) as a criterion and/or take the lower 95% confidence limit into account to set safe concentrations.

Recently, this approach has been further explored by developing the empirical model PERPEST (Van den Brink et al., 2002b). PERPEST makes use of the database described in this paper, and that of microcosm and mesocosm data of insecticides that have other modes of action (Brock et al., 2000b), plus that of herbicides (Brock et al., 2000a), to predict ecological effects of pesticides on freshwater ecosystems. The PERPEST model searches for situations in the database that are analogous to a case in question.

Our effect classification system was shown to be helpful in evaluating treatment-related effects of different insecticides as observed in various ecosystem experiments that were made available in the open literature. The effect classification system can be equally well-applied in future higher tier risk evaluations. Recently, it was advocated that protection goals should be formulated more specifically and to specify more clearly what must be considered as 'unacceptable damage' to the ecosystem (Van Dijk et al., 2000; Giddings et al., 2002). When site-specific protection goals, and consequently target images become available, the effect classification system can be of help in the decision-making process. In this context, the classification system may be used to derive more than one 'regulatory acceptable concentration'. Eco-ethical principles may be used to derive acceptable concentrations in a landscape-ecological context, e.g., dependent on the functionality and vulnerability of the freshwater ecosystem concerned (see, e.g., Brock, 2001). Defining effect classes and differentiated protection goals, also has the advantage that the different stake-holders involved in the process of authorising pesticides, can discuss more transparently the decision-making of 'Ecologically Acceptable Concentrations (EACs)' from model ecosystem studies.

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