

## Ecological risks of pesticides in freshwater ecosystems

### *Part 1: Herbicides*

The research presented in this report is financially supported by STOWA and The Netherlands Ministry of Agriculture, Nature Management and Fisheries. The translation of the report from Dutch into English was sponsored by the European Crop Protection Association.

# **Ecological risks of pesticides in freshwater ecosystems**

## ***Part 1: Herbicides***

**T.C.M. Brock, J. Lahr and P.J. Van den Brink**

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## ABSTRACT

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A literature review of freshwater model ecosystem studies with herbicides was performed 1) to assess the NOEC<sub>ecosystem</sub> for individual compounds, 2) to compare these threshold levels with water quality standards, and 3) to evaluate the ecological consequences of exceeding these standards. Studies were judged appropriate for this purpose when 1) the test systems simulated a realistic freshwater community, 2) the experimental design was generally sound (ANOVA or regression design; exposure concentrations described) and 3) when published in 1980 and later. Almost half of the collected papers did not meet these selection criteria. Effects were classified according to their magnitude and duration. The most sensitive endpoints for photosynthesis inhibitors, the most widely studied group of herbicides, were responses related to community metabolism and the structure of phytoplankton, periphyton and macrophytes. These endpoints showed a clear dose-response relationship. The criteria as set by the Uniform Principles appeared to provide sufficient protection for aquatic ecosystems against herbicides. Possible exceptions are the herbicides with an auxin-simulating mode of action, because aquatic macrophytes appeared to be more sensitive to these substances than algae. Functional responses of communities in phytoplankton-dominated ecosystems sometimes recovered rapidly through shifts in algae species composition and adaptation. Indirect effects on the zooplankton in such systems generally occurred at higher concentrations than primary effects. Adequate studies in macrophyte-dominated systems were rare, but in several experiments a pronounced long-term decline of macrophytes was observed at chronic concentrations only slightly above the NOEC<sub>ecosystem</sub>. This may result in considerable indirect effects on the macrophyte-associated fauna. The most important modifying factors with respect to types of effects and recovery rates following the application of herbicides to freshwater ecosystems are also discussed.

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

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## LIST OF ABBREVIATIONS

ASTM	American Society for Testing and Materials, Philadelphia
<sup>14</sup> C	(radioactive) carbon-14
CAB	Chemical Abstracts
2,4-D	2,4-dichlorophenoxy acetic acid
2,4-D BBE	butoxyl ester of 2,4-D
2,4-DP	2,4-dichlorophenol
DLO	Agricultural Research Department, Wageningen, The Netherlands
DMF	dimethylformamide
DMSO	dimethylsulphoxide acid
DNOC	dinitro-orthocresol
DO	dissolved oxygen
DOC	dissolved organic carbon
DT <sub>50</sub>	half-life value for degradation
EC <sub>50</sub>	concentration at which effects occur in 50% of the number of test organisms
EPA	Environmental Protection Agency (USA)
EU	European Union, Brussels
gm-EC <sub>50</sub>	geometric mean of different EC <sub>50</sub> values of the same test species
HRAC	Herbicide Resistance Action Committee, Leverkusen
LOEC	lowest concentration at which an effect is observed
LOEC <sub>eco</sub>	LOEC for the most sensitive endpoint studied in the ecosystem
LC <sub>50</sub>	concentration at which mortality occurs in 50% of the number of test organisms
LNV	Ministry of Agriculture, Nature Management and Fisheries, The Netherlands
MCPA	methoxychlorophenoxy acetic acid
MCPP	mecoprop
MPC	Maximum Permissible Concentration
MSMA	monosodiummethyl arsenate
Nefyto	Dutch Association of Agrochemical Industries, The Hague
NOEC	highest concentration at which no effect is observed
NOEC <sub>eco</sub>	NOEC for the most sensitive endpoint studied in the ecosystem
NW4	Fourth Memorandum Water Management
OECD	Organisation for Economic Co-operation and Development, Paris
PCB	polychlorobiphenyl
PD	Plant Protection Service, Wageningen, The Netherlands
POC	Particulate Organic Carbon
RIKZ	National Institute for Coastal and Marine Management, Middelburg, The Netherlands
RIVM	National Institute of Public Health and the Environment, Bilthoven, The Netherlands
RIZA	National Institute for Inland Water Management and Waste Water Management, Lelystad, The Netherlands
STOWA	Foundation for Applied Water Research, Utrecht
2,4,5-T	2,4,5-trichlorophenoxy acetic acid
TU <sub>gsa</sub>	Toxic Units on the basis of the most sensitive standard alga; concentration active ingredient in the water (C <sub>w</sub> ) divided by the gm-EC <sub>50</sub> of the most sensitive standard alga
UP	Uniform Principles (registration criteria for crop protection products according to the EU)
WU	Wageningen University





## **PREFACE**

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

Lahr, J., van den Brink, P.J. & Brock, T.C.M. (1998): Ecologische risico's van bestrijdingsmiddelen in zoetwater ecosystemen, deel 1: herbiciden. STOWA publicatie 98-30, Utrecht.

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

During the past years, researchers of Alterra - in collaboration with Wageningen University (WU), the National Institute of Public Health and the Environment (RIVM), and the National Institute for Inland Water Management and Waste Water Management (RIZA) - conducted experiments in artificial ecosystems with the objective to validate the water quality criteria for pesticides. Other (foreign) research institutes also conducted experiments of which the results have been published in the scientific literature. This information can also be used to establish ecological threshold values for pesticides in surface water.

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

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



# 1 INTRODUCTION

This report presents an analysis of the actual ecological risks of herbicides in freshwater ecosystems. Actual risks are understood to be risks that have been estimated on the basis of experimental observations in (semi) field experiments. In The Netherlands, various reports have been published in which the aquatic ecotoxicology of pesticides are discussed (e.g., Ordelman *et al.*, 1993; Crommentuijn *et al.*, 1997; Teunissen-Ordelman *et al.*, 1997). These reports present information on physico-chemical properties, presence in surface water, toxicity to aquatic organisms, and criteria-setting. Results of controlled (semi) field experiments with herbicides have, however, hardly been included in these reports. A comparison of the sensitivity of aquatic species between laboratory tests and micro/mesocosm experiments has been presented by Emans *et al.* (1992) and Jak *et al.* (1994) for a limited number of herbicides. A recent overview of the ecological impact of herbicides in freshwater ecosystems, however, is lacking. This report attempts to fill this gap by presenting a review of the available information on ecological effects of herbicides in freshwater ecosystems.

The available literature shows that descriptive hydrobiological field research into effects of herbicides is scarce, and that such field research is often difficult to interpret due to the spatial and temporal variation in environmental conditions and the lack of a well-described, untreated reference system. This is the reason why the data presented in this report are mainly based on experiments in aquatic model ecosystems, also called –depending on their dimensions- microcosms (relatively small) or mesocosms (relatively large). An advantage of these experimental ecosystems constructed by the researcher is that they can be replicated. This offers the possibility to do research at the ecosystem level under controlled conditions. These systems also have the advantage that several concentrations of a pollutant can be tested at the same time, and that responses in treated systems can be compared with controls.

Microcosms and mesocosms are constructed by collecting parts of natural ecosystems and bringing these into an artificial housing or by enclosing parts of existing ecosystems in the field (enclosures). These test systems are considered as an experimental tool bridging the gap between controlled laboratory experiments and the variable and complex conditions in the field. In other words they link true experimental reproducibility and ecological realism (Figure 1). For a discussion of the advantages and shortcomings of such systems in comparison with natural aquatic ecosystems we refer to Brock *et al.* (1993a; 1995).

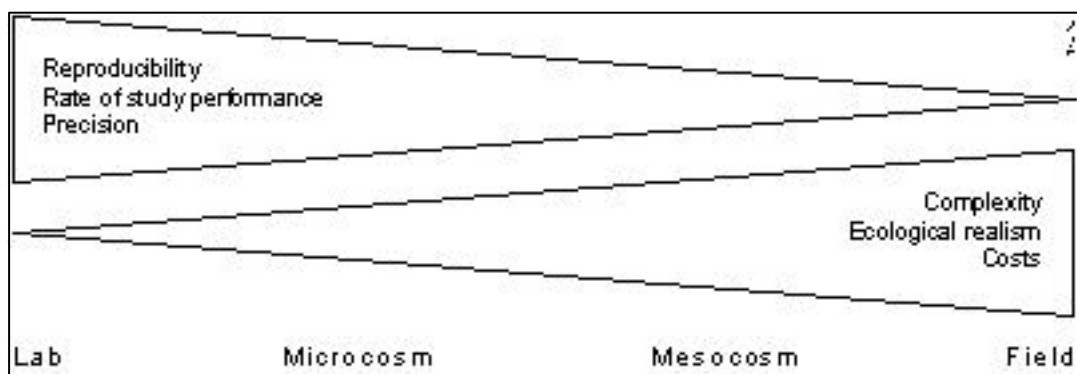
The objectives of the literature review presented in this report are:

- a) compiling an inventory of the NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values for individual herbicides in surface water as these have been established experimentally by means of freshwater model ecosystems (microcosms, mesocosms) or adequate

field studies. The  $NOEC_{eco}$  is the highest tested concentration at which no, or hardly, effects on the structure and the functioning of the studied (model) ecosystem are observed; the  $LOEC_{eco}$  is the lowest tested concentration at which clear effects occur. It is also investigated whether it is necessary to make a distinction between exposure regime and application frequency (single versus multiple);

- b) comparison of these  $NOEC_{eco}$ 's with registration and water quality criteria derived for herbicides in surface water;
- c) evaluation of the ecological consequences of criteria being exceeded, including indirect (secondary) effects and recovery time.

Sales of herbicides in The Netherlands in 1995 amounted to 3 070 000 kg active ingredient, about 28% of the total amount of pesticides sold in The Netherlands (Nefyto, 1996). Herbicides are classified into a number of chemical groups with different modes of action. The anilids (212 000 kg), dinitroalkyl phenols (151 000 kg), phenoxyacetic acids (phenoxyacetic acids and phenoxypropionic acids and esters: 414 000 kg), thiocarbamates (196 000 kg), triazines and triazinones (412 000 kg), urea compounds (349 000 kg) and aminophosphonates (344 000 kg) are the most frequently used herbicides in The Netherlands (Nefyto, 1996). For this study we have classified the herbicides into three groups on the basis of differences in mode of action, i.e.: photosynthesis inhibitors, auxin simulators and other substances. This last group can also be considered as growth inhibitors because the mechanism of most compounds in this group can broadly be considered as such.



**Figure 1.** Experimental ecosystems as bridge to the field.

## **2 MATERIAL AND METHODS**

### **2.1 Collected literature**

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

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

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

1. The test system is representative of a realistic freshwater community (organisms represent various trophic levels, primary producers are most important in case of herbicides, but consumers and decomposers are preferably also present).
2. The description of the experimental set-up is adequate and unambiguous.
3. The exposure concentrations that are relevant for the study can be derived (at least the nominal concentrations are known) and harmful solvents have not been used to apply the herbicide.
4. The investigated 'endpoints' (parameters selected as measuring target) are sensitive to the substance and the effects can reasonably be expected to be related to the modes of action of herbicides. Especially primary producers (such as phytoplankton, periphyton, macrophytes) are considered as sensitive endpoints for herbicides.
5. The effects are statistically significant and show an unambiguous dose-effect relationship, or the observed effects are in agreement with a dose effect relationship from additional studies.
6. For the establishment of a  $NOEC_{eco}$ , at least the lowest test concentration within a study should not show a consistent effect that can be attributed to the treatment; the concentration above the  $NOEC_{eco}$  shows a clear effect ( $LOEC_{eco}$ ).
7. Toxicity data of standard test organisms (at least one algal species) and/or water quality criteria (MPC's) should be known for the comparison of field concentrations with criteria concentrations.

8. The study was published in 1980 or later.

Subsequently, the selected studies were classified according to mode of action of the herbicides (photosynthesis inhibitors, auxin simulators, growth inhibitors), exposure regime (single, multiple, or continuous exposure), and type of test system (stagnant or running). Many of the evaluated studies in running water were conducted in recirculating systems, which means that the exposure is in fact comparable to that in a stagnant system. Studies with dosing of a herbicide in (non-recirculating) running water were only sporadically found.

### **2.3 Endpoints and recovery**

Besides effects on structural endpoints (such as densities of algae and biomass of aquatic plants), important effects on functional endpoints are also to be expected in experiments with herbicides. This especially concerns effects on primary production. This can be measured indirectly as a decrease of DO (the amount of dissolved oxygen) and pH. Another possibility is the direct measurement of primary production and respiration by incubation of water samples or substrates with periphyton or plants and by measuring the dynamics of  $^{14}\text{C}$ .

An advantage of experiments conducted outdoors in comparison with model ecosystem experiments conducted in the laboratory is that more realistic information can be obtained about the recovery of disturbed populations and ecosystem functions after termination of the stress. We consider an endpoint in a stressed system recovered if this, after a significant increase or decrease as direct or indirect result of the treatment, becomes consistent again with the normal range of the control systems. On theoretical grounds, the recovery of affected endpoints in (semi) field situations may occur if:

- the toxic substance disappears and/or the biological availability of the substance decreases to such an extent that the critical threshold values are 'underceeded', and;
- the other relevant environmental conditions (such as food supply, nutrients, temperature) do still, or again, meet the requirements of the affected populations, and;
- the generation time of the affected species is shorter than the duration of the study, and;
- recolonisation from outside the system can take place in case of complete disappearance of species.

Microcosm studies that have been conducted in the laboratory do in many cases not meet the two last-mentioned boundary conditions. These experiments generally only yield information on recovery of populations which possess resistant life stages and which can complete their life cycle in a short period in the microcosm.

Adaptation is regularly observed in studies with herbicides (see references Figure 12). This is in particular reported for phytoplankton and periphyton, probably due to

their short generation time. There are two types of adaptation. In the first case, the most sensitive algae disappear but less sensitive species increase in number due to decreased competition for nutrients, CO<sub>2</sub> etc. (adaptation of the community). Adaptation may, however, also occur within a population of a single species, in which case the resistant individuals reproduce until the population recovers. Functional endpoints, such as primary production, may, in case of continuous exposure to the herbicide, recover as a result of adaptation, despite a prolonged change in species competition. In most studies, adaptation is not directly measured (e.g. by an increased tolerance towards the pesticide), but the occurrence of the phenomenon is also deduced from the fact that functional parameters and/or individual populations recover while the substance remains present in the system for a long time.

## **2.4 Criteria for the classification of effect classes**

The effects of treatments with herbicides described in the literature are classified according to sensitivity of the response of the studied endpoints. The endpoints are subdivided into eight groups: community metabolism, phytoplankton, periphyton, macrophytes, zooplankton, macrocrustaceans and insects, molluscs, and fish and amphibians. The effects reported on these endpoint are classified into five effects classes that are based on the following criteria:

### **Class 1: 'effect could not be demonstrated'**

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

### **Class 2: 'slight effect'**

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

### **Class 3: 'pronounced short-term effect'**

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

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

- clear effects (such as strong reductions of functional endpoints and elimination of sensitive species) observed, but the study is too short to demonstrate complete recovery within 8 weeks after (the last) application of the pesticide.

### **Class 5: 'pronounced long-term effect'**

- clear response of sensitive endpoints and recovery time of sensitive endpoints is longer than 8 weeks after the last application, and
- effects reported as 'long-term effects on many sensitive species/endpoints'; 'elimination sensitive species'; 'effects on less sensitive species/endpoints' and/or other similar descriptions, and
- effects observed at various subsequent samplings.

For all eight groups of endpoints, it was established for each studied concentration of each study into which effect class the response could be classified. Plotting these results against the tested (nominal) concentrations yields a picture of the reported effects and at which concentrations these occur (see Figure 3 as example).

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

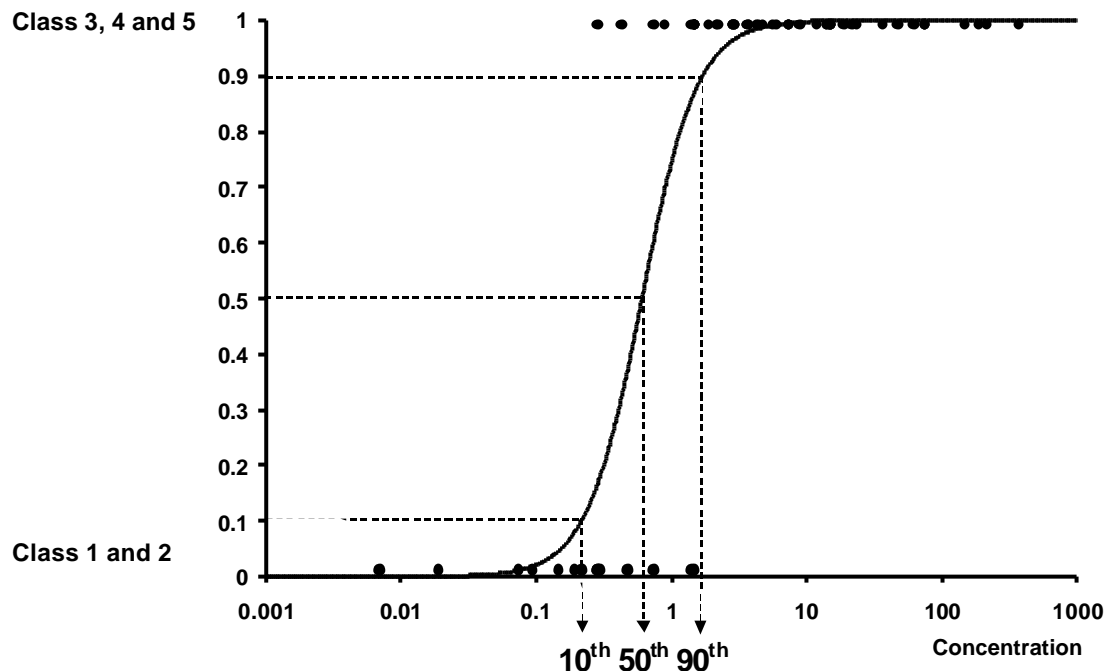
$$y = \frac{1}{1 + e^{b(\ln(x)-a)}} ,$$

in which  $y$  is the response variable (yes/no effect; yes/no recovery),  $x$  is the concentration expressed in  $TU_{gsa}$ ,  $a$  is the concentration at which for 50% of the studies an effect or no recovery has been reported, and  $b$  is the slope of the sigmoid curve at this concentration.

The 10-, 50- and 90-percentiles have been calculated by means of this function; i.e., those fitted concentrations (expressed in  $TU_{gsa}$ ; for calculation see Section 2.5) for which it is predicted that in 10, 50 and 90%, respectively, of the studies an effect or no recovery occurs. The 95% confidence intervals for these percentiles have also been calculated. A distinction has been made between functional and structural endpoints. The response of community metabolism has been used as functional endpoint, the most sensitive measured response of the primary producers as structural endpoint. The calculations have been performed with the GENSTAT statistical programme (Payne & Lane, 1993).



Figure 2 presents an example of an analysis of class 1 and 2 effects versus class 3, 4 and 5 effects. The 50-percentiles are used for the comparison of the results because the results of the 10- and 90- percentiles are more sensitive to the chosen model function.



**Figure 2.** Example of the calculation of 10-, 50- and 90-percentiles from the data of the microcosm and mesocosm studies.

## 2.5 Comparison of herbicides

To enable a good comparison between studies with different herbicides, the reported field concentrations have been ‘normalised’ by dividing these by the  $EC_{50}$  of the most sensitive standard algal species recommended by the OECD (1984): *Scenedesmus subspicatus*, *Selenastrum capricornutum* or *Chlorella vulgaris*. All three species belong to the green algae (Chlorophyta). The species *Selenastrum capricornutum* has recently been renamed to *Raphidocelis subspicata*, but because this name is not yet generally adopted, the old name is still used in this report. In most cases *S. subspicatus* or *S. capricornutum* have been used for the assessed herbicides. Data on *C. vulgaris* were scarce.

The related species *Chlorella pyrenoidosa* is more frequently used in toxicity tests.

*Chlorella*, however, was found to be less sensitive than the other two standard species in all studied cases. In one case (fluridon), the  $EC_{50}$  for a blue-green alga (Cyanophyta or Cyanobacteria) was used because data on green algae were not available. A suitable (semi) field study was found for MSMA, but no suitable algae data.

The  $EC_{50}$  in standard tests with algae (OECD, 1984) can be calculated in two ways: on the basis of growth or on the basis of growth rate. Algae populations in the ‘log phase’ of growth are for 72 to 120 hours exposed to different concentrations of a

substance. The measured endpoints may range from number of cells and biovolume to wet or dry weight or chlorophyll-a content of the population. In the case of growth, the  $EC_{50}$  is calculated as the concentration at which there is a 50% reduction of one of these parameters in comparison with the control after 72-120 hours. In the case of growth rate, the  $EC_{50}$  is derived from the slope of the growth curves. Both values are not necessarily the same.

The publications of Crommentuijn *et al.* (1997), Solomon *et al.* (1966) (atrazine), Fairchild *et al.* (1977) and the references from the papers on the evaluated (semi) field studies were used as first information source of the toxicity data, followed by a search in on-line literature data bases for some substances. In case more  $EC_{50}$ 's were available for algae, the geometric mean of these was calculated for the most sensitive species. This procedure was followed because deviating  $EC_{50}$  values (if any) are thus given less weight. The geometric mean of available  $EC_{50}$  values for the selected standard test species is in the following of this report referred to as 'gm- $EC_{50}$ '. When establishing the gm- $EC_{50}$ , no distinction has been made between those based on growth or on growth rate because the variation between the  $EC_{50}$ 's per alga species due to the different sources was larger than the variation due to the applied calculation method and/or endpoints.

One of the objectives of this report is the mutual comparison of experiments with different herbicides, for which the tested concentrations have been normalised for their toxicity to the most sensitive standard test alga; the water concentration as tested in the different experiments has been divided by the gm- $EC_{50}$  of the most sensitive standard alga. The unit of the resulting variable is defined as  $TU_{gsa}$  (Toxic Unit of the most sensitive standard alga).

Besides the  $EC_{50}$  values for standard algae, the available data for duckweed, *Lemna spp.*, were collected. This concerned tests with different species for which OECD protocols do not yet exist. The duration of these tests ranges from 4 days to 3 weeks. Seven days is recommended in a provisional document of the ASTM (1997).

All collected toxicity data for standard algae and duckweed are included in the appendices. Only the gm- $EC_{50}$ 's or the water concentrations expressed in  $TU_{gsa}$  are presented in the following of the main report.

Although it is likely that algae are the most sensitive test species for herbicides, attention has also been paid to the toxicity of the evaluated substances to *Daphnia* and fish. For one substance, triallate, the acute 48-h  $LC_{50}$  value for *Daphnia* is similar to that for standard algae. This value is also reported in Appendix 18. Fish are –for all studied substances- less sensitive than standard algae.

## **2.6 Comparison of ecological threshold values with criteria**

The ecological threshold values ( $NOEC_{eco}$ 's) obtained from (semi) field studies are compared with the criteria applied in The Netherlands. For pesticides in surface

water, a distinction can be made between registration criteria and water quality criteria. The water quality criteria are based on the Maximum Permissible Concentration (MPC). In case more than 4 adequate chronic toxicity data for aquatic organisms are available, the MPC is established according to the method described by Aldenberg & Slob (1993). In case less than 4 chronic NOEC's are available, the MPC is determined according to the modified EPA-method as described by Crommentuijn *et al.* (1997).

Registration criteria are based on the criteria described in the Uniform Principles (EU, 1997). According to the Uniform Principles, the concentration of a pesticide in surface water may not be higher than 0.01 x the acute L(E)C<sub>50</sub> for fish or *Daphnia* and 0.1 x the EC<sub>50</sub> for algae in the first tier of the risk assessment. In addition, the average exposure concentration may not be higher than 0.1 x the chronic NOEC for *Daphnia* (21 days) and fish (28 days) in case of prolonged exposure. Within the Dutch legal framework, however, for algae the first-tier criterion of 0.1 x NOEC is applied. In the second tier there may be a deviation from the above-mentioned registration criteria provided that it is demonstrated by means of an adequate risk assessment that the actual risk to aquatic organisms is acceptable.

Criteria according to the Uniform Principles (UP criterion) are in this report established on the basis of toxicity data for algae species according to OECD protocols. These were in all cases the most sensitive group of standard test species. We define a liberal and a conservative UP criterion. The lowest EC<sub>50</sub> value reported in the literature for one of the above-mentioned standard algae is taken as a basis for the calculation of the conservative UB criterion by dividing this value by a factor 10. The liberal UP criterion is established by dividing the gm-EC<sub>50</sub> described in the preceding section by a factor 10.



## **3 AVAILABLE INFORMATION**

### **3.1 Used studies**

A number of 124 studies describing the effects of herbicides on an aquatic system were found in the literature, of which 29 had to be dropped immediately because the studies had not been conducted in (entirely) fresh water or because they had been published before 1980, and their experimental design usually was not in line with recent guidelines. Of the remaining 95 studies, 39 (41%) did not meet the evaluation criteria described in Section 2.2 of this report. An important reason for this was in many cases an inadequate statistical basis of the data. This was in particular found in experiments with a so-called regression design, and in which the various treatments were not replicated. But the quality of the statistical analyses of the replicated studies was also quite often below standard. Most publications of this last group, however, have been included in the review. In some cases, the effects in unreplicated studies were so clear that they could in the end still be used. The observed effects should in such cases, however, be made plausible by the results of better conducted and comparable experiments by other authors or by a good correlation with acute toxicity data.

First, extensive summaries have been prepared of the selected studies; these were then included in a spreadsheet where each study was allocated a study number. A concise version of these summaries is presented in Appendices 1-20 of this report, where the herbicides are arranged per individual compound in the order of increasing concentrations.

### **3.2 Photosynthesis inhibitors**

This group includes the triazines/triazinones and the urea compounds. These substances block the electron transport in the Hill reaction of photosystem II, which means that primary producers such as higher plants and algae can no longer supply their energy need (Van Rijn *et al.*, 1995). Important representatives of the triazines are atrazine and simazine, and representatives of the urea compounds are isoproturon, linuron, monolinuron and diuron. Most of these products are used as herbicides in arable farming and horticulture; they are applied as residual (soil-applied) herbicides.

Table 1 presents a review of the 37 studies with photosynthesis inhibitors that were found to meet the criteria. The studies include 7 active ingredients of which atrazine was by far the most elaborately studied compound (over half of the number of studies in the table). Most of the experiments were conducted in stagnant experimental systems. Hexazinone is the only mentioned substance that is not registered in The Netherlands.

Products from the group of the photosynthesis inhibitors are very frequently found in fresh water in The Netherlands. In a study of large surface waters Phernambucq *et*

*al.* (1996) found traces of atrazine and simazine in just about 100% of all samples. Diuron and isoproturon were found in more than 50% of the cases. The highest triazine concentrations that were found during various monitoring studies were 14 µg/L atrazine, 2.5 µg/L simazine and 1.1 µg/L terbutryn (Ordelman *et al.*, 1993). This in particular concerned regional waters. Maximum concentrations in the larger national waters were lower. The highest urea compound concentrations in national waters were 43, 4.0 and 2.2 µg/L for diuron, isoproturon and linuron, respectively (Teunissen-Ordelman *et al.*, 1997). It can, however, not be excluded that the maximum concentrations of these substances in the regional areas will be higher but the particular report does not include data on this.

### **3.3 Auxin simulators**

The group of auxin simulators includes different groups of phenoxy-carbon acids. These are taken up by the roots or the leaves of plants and imitate the action of the auxin hormone that regulates the growth of plants. Because phenoxy-carbon acids do not decompose in plants, these are literally growing to death (Van Rijn *et al.*, 1995). The effects of phenoxyacetic acid 2,4-D were studied in half of the 8 adequate studies (Table 2). Studies with 2,4,5-T, the pyridine compounds picloram and clopyralid, and the pyridyloxy acetic acid trichlopyr were also found. Many experiments with substances from this group were published before 1980 and/or were found to be inadequate for other reasons. Studies with the well-known hormone-type herbicides such as the frequently used products MCPA, mecoprop (MCP) and dichlorprop (2,4-DP) could therefore not be evaluated in the context of this review. Of the substances in Table 2, 2,4-D, clopyralid and trichlopyr are registered in The Netherlands.

Phernambucq *et al.* (1996) found 2,4-D in over 10% of the samples from national waters in The Netherlands; 2,4,5-T was found in about 4% of the cases. The maximum 2,4-D concentration found in national waters was 23 µg/L.

### **3.4 Other herbicides (growth inhibitors)**

The group of other herbicides in the context of this report includes all products that have no direct photosynthesis-inhibiting or hormone-simulating effect. Most of these substances have a growth- or cell-division-inhibiting effect by disrupting biosynthesis and growth processes in the cell. The number of suitable studies in this category was 11. Diquat was the most frequently studied compound. The studies are presented in Table 3.

The bipiridyl compounds diquat and paraquat disrupt the plant's photosystem-I by forming harmful oxygen radicals (superoxides) that affect plant tissue. Fluridon is a 4-pyridon that disrupts carotene synthesis. Metsulfuron-methyl is a sulfonyl-urea which, contrary to the related urea compounds, does not disrupt photosynthesis but inhibits amino acid synthesis and cell division. Alachlor is one of the anilids. This group probably affects protein and fatty acid synthesis and decreases cell division and root elongation in plants. Triallate is one of the thiocarbamates and trifluralin is a

dinitroanilin. Both compounds also inhibit cell division and elongation. Finally, the mode of action of MSMA, an organo-arsenic compound, is not known.

**Table 1.** Ecosystem experiments with photosynthesis-inhibiting herbicides included in this report.

Active ingredient	Test system	Water regime	Dose	Location	Reference(s); [Study no.]
atrazine	microcosms, lab.	stagnant	single	USA, Georgia	Brockway <i>et al.</i> , 1984; [11]I
atrazine	exp. ponds	stagnant	single	France, Paris	Baturo <i>et al.</i> , 1995; [6]
atrazine	microcosms, lab.	stagnant	single	USA, Missouri	Johnson, 1986; [7]
atrazine	microcosms, lab.	stagnant	single	USA, Oregon	Stay <i>et al.</i> , 1989; [11]
atrazine	exp. streams, lab.	recirculating	single	USA, Vermont	Guessner <i>et al.</i> , 1996; [5]
atrazine	exp. streams, outd.	recirculating	single	USA, Texas	Moorhead <i>et al.</i> , 1986; [17]
atrazine	exp. ponds	stagnant	single	USA, Montana	Fairchild <i>et al.</i> , 1994; [13]
atrazine	microcosms, lab.	stagnant	single	USA, Oregon	Stay <i>et al.</i> , 1985; [14]
atrazine	natural stream	flow-through	two pulses	USA, Nebraska	Jurgensen <i>et al.</i> , 1990; [19]
atrazine	exp. ponds	stagnant	multiple	USA, Kansas	DeNoyelles <i>et al.</i> , 1982; [10] Dewey, 1986 Kettle <i>et al.</i> , 1987 DeNoyelles <i>et al.</i> , 1989 DeNoyelles <i>et al.</i> , 1994 Hamilton <i>et al.</i> , 1987; [15]
atrazine	enclosures in lake	stagnant	multiple additive & single	Canada, Ontario	
atrazine	microcosms, lab.	flow-through	constant	USA, Georgia	Brockway <i>et al.</i> , 1984; [11]II
atrazine	microcosms, lab.	flow-through	constant	USA, Virginia	Pratt <i>et al.</i> , 1988; [2]
atrazine	enclosures in pond	stagnant	constant	Germany, Bayern	Jüttner <i>et al.</i> , 1995; [3]
atrazine	microcosms, lab.	stagnant	constant	Netherl, Wageningen	Van den Brink <i>et al.</i> , 1995; [4]
atrazine	enclosures in lake	stagnant	multiple additive	Canada, Ontario	Herman <i>et al.</i> , 1986; [18] Hamilton <i>et al.</i> , 1988 Hamilton <i>et al.</i> , 1989
atrazine	exp. streams, outd.	recirculating	constant	USA, Texas	Kosinski, 1984; [8] Kosinski & Merkle, 1984
atrazine	exp. swamp	flow-through	constant	USA, Minnesota	Detenbeck <i>et al.</i> , 1996; [9]
atrazine	exp. streams, outd?	recirculating	constant	USA, Ohio	Krieger <i>et al.</i> , 1988; [12]
atrazine	microcosms, lab.	flow-through	constant	USA, Georgia	Hamala & Kollig, 1985; [16]
simazine	enclosures in swamp	stagnant	single	Canada, Manitoba	Goldsborough <i>et al.</i> '83; [21] Goldsborough <i>et al.</i> 1986 Goldsborough <i>et al.</i> '85; [22]
simazine	enclosures in pond	stagnant	single	Canada, Manitoba	Gumey <i>et al.</i> , 1989; [23]
simazine	microcosms in pond	stagnant	single	USA, Virginia	Jenkins <i>et al.</i> , 1990; [24]
terbutryn	enclosures in swamp	stagnant	single	Canada, Manitoba	Goldsborough <i>et al.</i> '83; [21] Goldsborough <i>et al.</i> 1986 Gumey <i>et al.</i> , 1989; [23]
terbutryn	enclosures in pond	stagnant	single	Canada, Manitoba	Struve <i>et al.</i> , 1991; [25]
terbutryn	microcosms, lab.	flow-through	pulse	UK, Bristol	Paterson <i>et al.</i> , 1987; [38]
hexazinone	enclosures in lake	stagnant	single	Canada, Ontario	Thompson <i>et al.</i> , 1993a; [28] Thompson <i>et al.</i> , 1993 a
hexazinone	exp. streams, lab. & exp. streams, outd.	flow-through	pulse	Canada, Ontario	Kreutzweiser <i>et al.</i> , 1995; [26]
hexazinone	exp. streams, outd.	flow-through	pulse	Canada, Ontario	Kreutzweiser <i>et al.</i> , 1992; [27]
linuron	enclosures in pond	stagnant	single	UK, Kent	Stephenson <i>et al.</i> , 1984; [29]
linuron	microcosms, lab.	stagnant	constant	Netherl., Wageningen	Van den Brink <i>et al.</i> , '97; [30] Cuppen <i>et al.</i> , 1997
isoproturon	microcosms, lab.	stagnant	single	France, Bordeaux	Feurtet-Mazel <i>et al.</i> , '96; [31]
isoproturon	microcosms, lab.	stagnant	single	France, Bordeaux	Pérès <i>et al.</i> , 1996; [32]
isoproturon	microcosms, lab.	stagnant	single	Germany, München	Traunspurger <i>et al.</i> , '96; [42]
diuron	microcosms, lab.	stagnant	single	USA, Minnesota	Flum & Shannon, 1987; [20]

**Table 2.** Ecosystem experiments with auxin-simulating herbicides included in this report.

Active ingredient	Test system	Water regime	Dose	Location	Reference(s); [Study no]
2,4-D	exp. ponds	stagnant	single	not mentioned	Boyle, 1980; [33]
2,4-D	enclosures in pond	stagnant	single	Can., Saskatchewan	Forsyth <i>et al.</i> , 1997; [34]
2,4-D	microcosms & lake	stagnant	single	USA, Kentucky	Kobriae & White, 1996; [35]
2,4-D	exp. ponds	stagnant	single	Canada, Ontario	Scott <i>et al.</i> , 1981; [44] Stephenson & Mackie, 1986 Sherry, 1994
2,4,5-T	microcosms, lab.	stagnant	single	Japan	Sugiura, 1992; [46]
picloram	enclosures in pond	stagnant	single	Can., Saskatchewan	Forsyth <i>et al.</i> , 1997; [34]
clopyralid	enclosures in pond	stagnant	single	Can., Saskatchewan	Forsyth <i>et al.</i> , 1997; [34]
trichlopyr	exp. streams, outd.	flow-through	pulse	Can., Ontario	Kreutzweiser <i>et al.</i> , '92; [27]

**Table 3.** Ecosystem experiments with other groups of herbicides included in this report. Most substances have a growth-inhibiting effect.

Active ingredient	Test system	Water regime	Dose	Location	Reference(s); [Study no]
diquat	microcosms, lab.	stagnant	constant & single	Germany	Draxl <i>et al.</i> , 1991; [36]
diquat	microcosms, lab.	stagnant	single	USA, Pennsylvania	Barreiro <i>et al.</i> 1994; [37]
diquat	microcosms, lab.	stagnant	single	USA, Pennsylvania	Pratt <i>et al.</i> , 1990; [45]
diquat	microcosms, lab.	flow-through	pulse	UK, Bristol	Paterson <i>et al.</i> , 1987; [38]
paraquat	exp. streams, outd.	recirculating	constant	USA, Texas	Kosinski, 1984; [8] Kosinski & Merkle, 1984
fluridon	enclosures in pond	stagnant	single	USA, Alabama	Struve <i>et al.</i> , 1991; [25]
metsulfuron-methyl	enclosures in lake	stagnant	single	Canada, Ontario	Thompson <i>et al.</i> , '93a; [28] Thompson <i>et al.</i> , 1993
alachlor	exp. streams, lab.	recirculating	single	USA, Nebraska	Spawn <i>et al.</i> , 1997; [39]
trallate	microcosms, lab.	stagnant	single	USA, Missouri	Johnson, 1986; [7]
trifluralin	microcosms, lab.	stagnant	single	USA, Missouri	Johnson, 1986; [7]
MSMA	exp. streams, outd.	recirculating	constant	USA, Texas	Kosinski, 1984; [8] Kosinski & Merkle, 1984

The nomenclature and classification of the above-mentioned products tend to show differences; we have followed the Nederlandse Gewasbeschermingsgids (Dutch Crop Protection Guide, PD, 1996). Publications by Dejonckheere & Steurbaut (1966), Tomlin (1994), Van Rijn *et al.*, (1995) and HRAC (1996) were used to describe the modes of action. Metsulfuron-methyl and triallate are registered in The Netherlands. No suitable studies were found for the frequently applied product glyphosate (Roundup), an amino-phosphonate that inhibits the amino acid synthesis of the plant.

From this group of other compounds trifluralin was found in waters in The Netherlands (Phernambuq *et al.*, 1996). The compound was, however, only found in some of the measurements; the maximum was 0.01 µg/L.



## 4 APPLICATION METHOD AND BEHAVIOUR IN SURFACE WATER

The solubility of most herbicides in water is too low to be able to prepare a very concentrated aqueous solution. Concentrated solutions are, however, often desirable for the experimental application of a herbicide in micro- and mesocosms, in which case organic solvents are used to prepare these. It is, however, known for most solvents used (ethanol, methanol, acetone) that these may be toxic to algae; they are, e.g., also used for the extraction of photopigments, such as chlorophyll, from plant material. Hess (1980) found that cells of the alga *Clamydomonas eugametos* were damaged at more than 10 mL/L (1% v/v) ethanol or dimethylsulfoxide (DMSO); the limit for acetone was 5 mL/L (0.5% v/v). St. Laurent *et al.* (1992) reported NOEC values of the same order of magnitude for *Selenastrum capricornutum* for methanol and acetone of 6.8 and 2.3 mL/L, respectively. Bérard (1996) observed shifts in species in natural phytoplankton communities at 0.5 mL/L ethanol, methanol, DMSO and dimethylformamide (DMF). Ethanol also had an inhibiting effect on chlorophyll-a in the same experiment.

It is not clear whether direct toxicity to algae may also be the cause of sometimes enormous effects on the oxygen concentrations observed at still lower ethanol concentrations. Feurtet-Mazel *et al.* (1996), e.g., found clear effects of ethanol at 0.5 mL/L and below in microcosms. And it can be derived from other experiments that ethanol may reduce oxygen concentrations to almost zero even at concentrations of 2.0-2.9  $\mu\text{L/L}$  (Lay *et al.*, 1984; Peichl *et al.*, 1984, 1985; Lampert *et al.*, 1989; Neugebauer, 1990). At the same time, these experiments show a strong pH decrease and in some cases zooplankton even disappears. Lynch *et al.* (1985) dosed experimental streams with atrazine and a PCB, for which the solvents acetone and DMSO were used, both at a concentration of 69  $\mu\text{L/L}$ . Strong reductions of gross primary production and community respiration (periphyton) were observed in the treated streams as well as in the streams only dosed with the solvent. Because the calculation of these parameters was based on the course of the oxygen concentration in the systems, it is likely that also here reductions in the amount of dissolved oxygen were the cause of the reductions in production and respiration. The drift of macroinvertebrates in the controls of this experiment also increased as a result of solvents. A possible explanation for the reductions in the oxygen concentrations by organic solvents is suggested by Feurtet-Mazel *et al.* (1996), i.e., a direct or indirect effect on the activity of (heterotrophic) bacteria. Without further additional research, however, it cannot be fully excluded that there is a direct toxic effect on algae under (temporary) reduction of the oxygen production.

It will be clear from the above that alternative, non-toxic solvents and methods of application are to be preferred in experiments with herbicides. It can only be concluded beyond doubt whether these carriers do or do not affect the results when the experiments include controls with as well as without organic solvents. All studies

of which we suspected that the solvent could have caused or masked the results (see the above-mentioned syndrome of effects) have been excluded from this report.

In most (semi) field studies evaluated in this report, the products were directly mixed into the water column. Injection and pouring on the water surface were the most frequently used methods for stagnant systems. Dosing in running systems usually took place via the main tank that fed the system with water or via dripping into the stream itself. In four studies the herbicide was applied with sprayers and in two studies leaching was simulated by mixing the compounds with wet soil before pouring this slurry into the system. Pellets of 2,4-D BBE were used in the study of Scott *et al.* (1988).

Half life values of several weeks to some months were measured for the triazine and urea compounds in the studies included in this report. Atrazine is the most persistent compound with half life values ( $DT_{50}$ 's) up to more than 6 months (DeNoyelles *et al.*, 1982). The other herbicides are also relatively persistent. The reported  $DT_{50}$  values are usually higher than two weeks. According to the classification of Van Rijn *et al.* (1995) this means that the studied herbicides are 'moderately degradable' to 'very persistent'.

The studies are classified into three types: stagnant systems, recirculating (running water) systems and running water (non-recirculating) systems. The first two types have been combined because the exposure regime was the same; the substance remained in the system and was not discharged. Studies conducted in running water systems, in which the contaminated water was not again circulated, are, however, treated as a separate category. These systems were dosed in the form of pulses, where besides the nominal pulse concentration the duration of the pulse is an important factor. There were only a few of such studies; the results of these are only briefly discussed.

In most cases we used the reported nominal concentrations in the test systems as these are given by the authors. In the studies where these concentrations were verified by chemical analyses, they showed good correspondence with the found values. We used the maximum measured peak concentration in case of single and multiple dosing in stagnant and recirculating systems. The experiments are, in as far as exposure is concerned, classified into two groups: a single dose on the one hand and a multiple or constant exposure on the other. Experiments of the last group are in the following of this report referred to as studies with multiple application.

## 5 SENSITIVE ENDPOINTS

The measured endpoints in the studies are subdivided into eight groups, where a distinction is made between functional and structural endpoints. Most measured functional endpoints concerned the metabolism of the communities present. These include reductions of the amount of dissolved oxygen by direct inhibition of primary production ( $^{14}\text{C}$  uptake) or indirectly as a result of the disappearance of the primary producers themselves. In many studies, community respiration was also measured or derived. Besides primary producers, the heterotrophic community plays an important role in processes related to respiration. There were, however, hardly studies in which these organisms were investigated explicitly. Effects on nutrients, minerals, pH, alkalinity, conductivity and organic matter are discussed under the indirect effects in Chapter 6. Other functional endpoints, such as organic matter degradation (decomposition) and microbial activity, were hardly measured in these studies. Kersting (1994) gives a review of responses of functional endpoints in pesticides-stressed freshwater ecosystems.

The consulted studies show large differences in taxonomic level of the presented biological data. Algae and periphyton, e.g., are only studied at species level in a third of the publications. Most studies are limited to the larger taxonomic units (Chlorophyta, Bacillariophyta, Cyanophyta etc.), or chlorophyll-a or metabolism are measured. Negative effects on Chlorophyta, the most frequently occurring and most frequently studied group of algae, are reported in many cases, but Bacillariophyta, Cyanophyta and Pyrrophyta are also found to be sensitive in many studies. It cannot be established unambiguously which groups of algae or aquatic plants are most sensitive to herbicides. This is one of the reasons why the structural endpoints for a generic picture are divided into seven fairly broad groups: phytoplankton, periphyton, macrophytes, zooplankton, macrocrustaceans and aquatic insects, molluscs, and finally the fish and amphibians (tadpoles). This classification broadly corresponds with the review by Brock & Budde (1994). Structural properties include parameters such as abundance (numbers, density, cover by aquatic plants etc.), species composition, biomass, diversity of a group of organisms, and chlorophyll-a concentrations (measure of the biomass of primary producers). The biomass of fish (weight) is also considered as a structural property of the fish population. Effects of herbicides on microorganisms were studied so infrequently that these have not been included in the review.

### 5.1 Hormesis

Hormesis is the phenomenon that toxic substances may at low, sub-lethal concentrations have a stimulating effect on organisms, especially on growth. It is a fairly common phenomenon that can be observed in a wide range of toxicants and organisms (Stebbing, 1982). There is no univocal explanation for hormesis but it could signify regulatory overcorrections by the mechanisms that control biosynthesis (Stebbing, 1982).

Hormesis was observed in several cases in the herbicide studies reported here, in particular for substances with an auxin-s(t)imulating effect (Kobrija & White, 1996; Forsyth *et al.*, 1997). This is not difficult to explain because the effect of these compounds is based on the (over)stimulation of growth. For these substances the diagnosis of hormesis also strongly depends on the time of the observations. A substance such as 2,4-D initially causes an increase in growth of (aquatic) plants, later causing the plant to die. Hormesis was also observed in some studies with photosynthesis inhibitors and the group of other herbicides: atrazine and periphyton (Pratt *et al.*, 1988), atrazine and phytoplankton (Jüttner *et al.*, 1995), diquat and periphyton (Paterson & Wright, 1987), and triallate and primary production (Johnson, 1986).

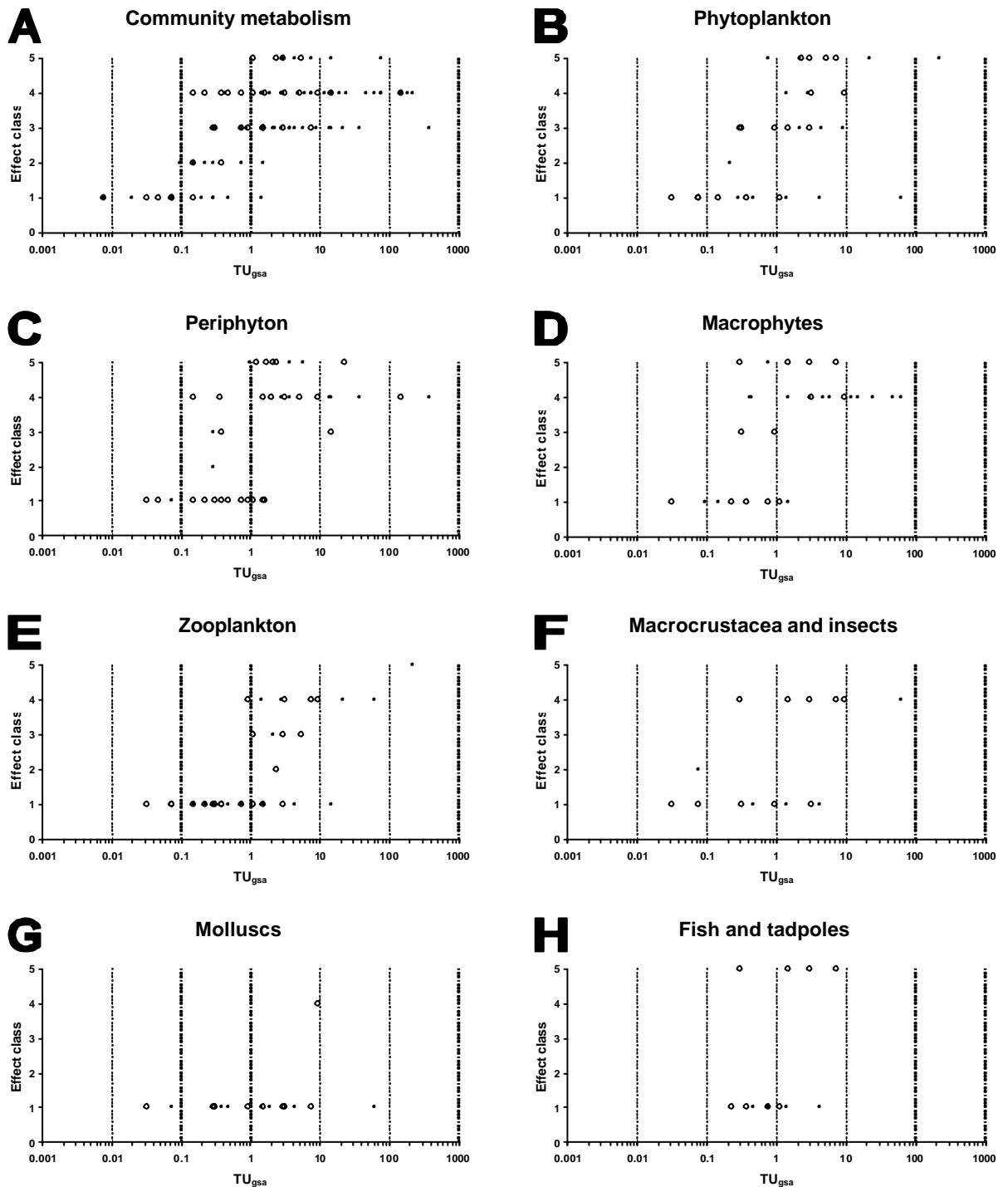
None of the consulted studies, however, was explicitly aimed at the detection of hormesis. The examples that were found usually concerned a slight and temporary effect. An additional factor is that it is very hard to distinguish hormesis from indirect positive effects within the complex communities of (semi) field experiments. Also because little is known about the ecological relevance of the phenomenon, we did not include hormesis as an important ecological effect in this evaluation. At concentrations at which there were indications of hormesis, we assumed that this had no negative effect on the community and the ecosystem. These effects are therefore classified into Class 1.

## 5.2 Photosynthesis inhibitors

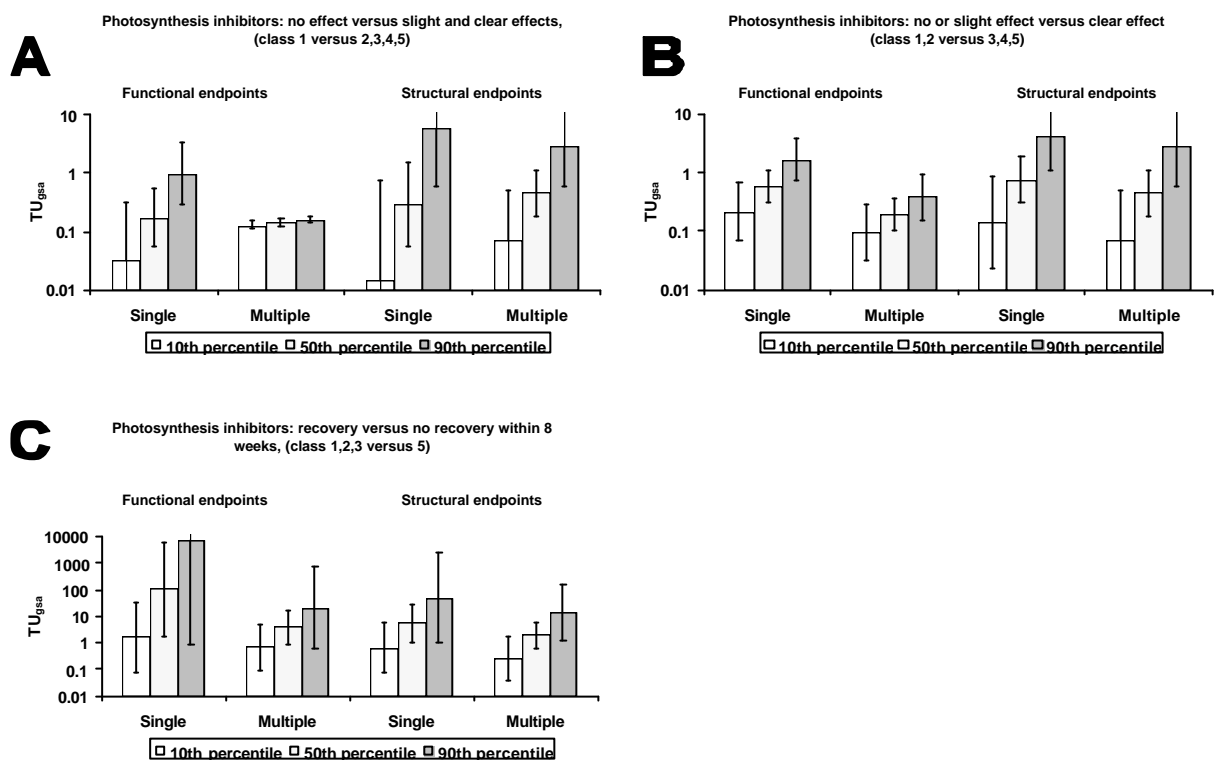
Figure 3 summarises the observed effects (expressed in classes) as these were found in the different studies, and at which concentrations (expressed in  $TU_{gsa}$ ) these occurred. The most sensitive endpoints for photosynthesis inhibitors in stagnant or recirculating test systems are community metabolism and the structure of the phytoplankton, periphyton and macrophyte populations. Clear effects are observed from about 0.1  $TU_{gsa}$  (Figure 3 A-D); no effects are observed at lower levels. The figure also shows a clear dose-effect relationship for these endpoints. The number of studies in which negative effects are reported as well as the class of the effects (Figure 3 A-D) increase with increasing concentrations. The figure also shows that the effects classified into class 4 and 5 start to occur at lower normalised concentrations ( $TU_{gsa}$ ) in the multiple or chronically dosed ecosystems than in the single dosed systems.

Figure 4 shows that the logistic regression in most cases yields 10- and 90-percentile values with a wide variation. The 10-percentile for, e.g., no or slight versus clear effect of functional endpoints is 0.216  $TU_{gsa}$  (0.070-0.662) for a single load and 0.095  $TU_{gsa}$  (0.032-0.280) for multiple applications. Because the 50-percentiles are the parameters with the lowest uncertainty, these are used for the comparison of the results of functional and structural endpoints and of single and multiple applications.

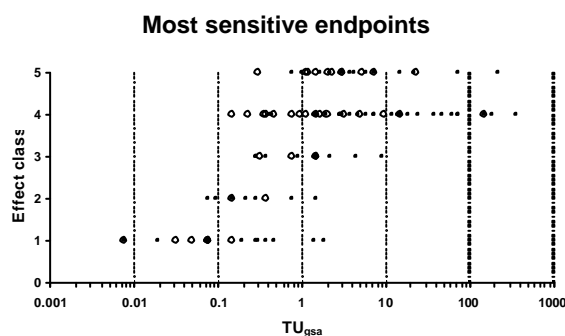
The functional endpoints react somewhat stronger to a single and multiple application of a photosynthesis inhibitor than the structural endpoints related to primary production (Figure 3A-D; Figure 4A and B). This may be because these parameters often have a small ecological variation and can be measured fairly accurately.



**Figure 3.** Classified effects of herbicides with a photosynthesis-inhibiting effect in (semi) field studies with stagnant or recirculating test systems. The effects are classified into a functional category (community metabolism; A) and into several categories structural endpoints (B-H). The effects are also classified according to magnitude and duration. 1 = no 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). For an extensive description of the effect classes we refer to Section 2.4. The small closed circles (●) indicate the experiments with a single application. The large open circles (○) indicate the experiments with multiple or continuous exposure. TU<sub>gsa</sub>: Toxic Unit on basis of the EC<sub>50</sub> of the most sensitive standard alga (see Section 2.5).



**Figure 4.** 10-, 50- and 90-percentile values (expressed in  $TU_{gsa}$  with 95% confidence intervals) as calculated by means of logistic regression for the most sensitive functional and structural endpoints after a single or multiple application of a photosynthesis inhibitor. The values are calculated for 3 classifications, A: no versus slight and clear effect, B: no and slight versus clear effect, and C: recovery versus no recovery within 8 weeks. When the classification 'no versus slight and clear' effect is taken, a 50-percentile value of 0.17  $TU_{gsa}$  is calculated for the functional endpoints after a single application of a photosynthesis inhibitor. This means that it is predicted that the functional endpoints show a slight or clear response at a concentration of 0.17  $TU_{gsa}$  in 50% of the studies.



**Figure 5.** Classified effects of herbicides with a photosynthesis-inhibiting effect in (semi) field studies with stagnant or recirculating running test systems. The effects on the most sensitive endpoints in the separate studies are presented. The effects are also classified according to magnitude and duration. 1 = no significant effect, 2 = slight effect, 3 = clear short-term effect (< 8 weeks), 4 = clear effect in short-term study (recovery moment unknown), 5 = clear long-term effect (> 8 weeks). The closed circles (●) indicate the experiments with a single application.; the large open circles (○) indicate the experiments with multiple exposure.

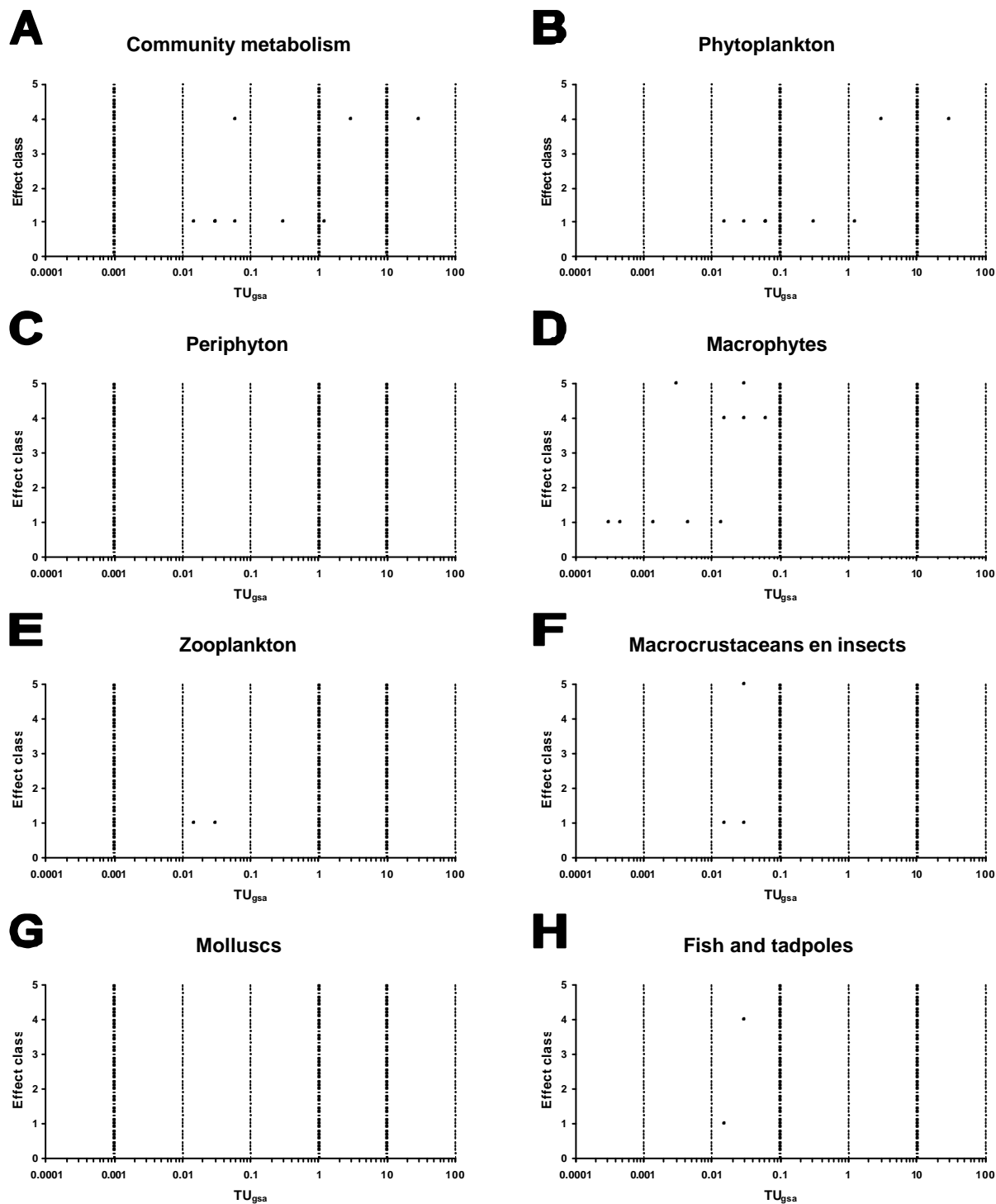
Figure 5 shows the classified effects on the most sensitive endpoints. Only slight effects on the most sensitive endpoints may be expected at a load around 0.1 TU<sub>gsa</sub> (Figure 4A, 5). Slight to clear effects may be expected at higher doses ((Figure 4B, 5). At doses of 1 TU<sub>gsa</sub> and higher it is to be expected that the recovery of sensitive endpoints takes more than 8 weeks (Figure 4C, Figure 5; see also Chapter 7).

There are probably only indirect effects at concentrations higher than 1 TU<sub>gsa</sub> on the other four structural groups (Figure 3E-H). These are discussed in Chapter 6. The only exception is a possible toxicity of linuron to rotifers at 15, 50 and 150 µg/L (Cuppen *et al.*, 1997) and to Cladocera and Copepoda at 1000 µg/L linuron (Stephenson & Kane, 1984).

Three substances with a photosynthesis-inhibiting effect were pulse-dosed in running systems. Pulses of 24 hours with a maximum concentration of 100 µg/L (1.5 TU<sub>gsa</sub>) atrazine had no effect on periphyton of a natural stream (Jurgensen & Hoagland, 1990). After an equally long exposure in a flow-through system terbutryn (maximum 50 µg/L; 18.5 TU<sub>gsa</sub>) neither had an effect on periphyton and the aquatic plant *Elodea canadensis*. Hexazinone in artificial streams, finally, hardly had negative effects on periphyton and on drift of invertebrates at 2700 to 82 000 µg/L (60-1800 TU<sub>gsa</sub>). In short, the effects of herbicides in running systems are much less serious than of the same concentrations in stagnant and recirculating ecosystems.

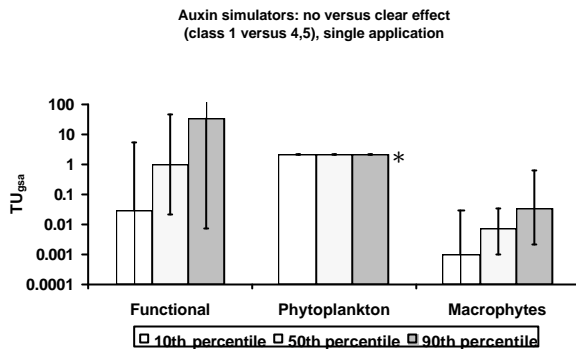
### 5.3 Auxin simulators

The number of suitable studies with substances with an auxin-simulating effect is much lower than those with the photosynthesis inhibitors (Figure 6). Studies with a constant exposure were not found and data about the effects on periphyton and molluscs are totally lacking (Figure 6 C and G). The picture of these compounds presented by the remaining endpoints contrasts sharply with the photosynthesis inhibitors. Macrophytes are the most sensitive organisms. The turning point for this group is between 0.003 and 0.01 TU<sub>gsa</sub> (Figure 6 D), whereas phytoplankton is less sensitive; here, the first population effects are only observed from 2 TU<sub>gsa</sub> (Figure 6 B). The turning point for community metabolism (primary production) lies between these two values, at about 0.05 TU<sub>gsa</sub> (Figure 6 A). The observed effects on macrocrustaceans, insects, fish and tadpoles are indirect effects (Chapter 6). All points in Figure 6, except Figure 6 D, only concern 2,4-D. The Figure for aquatic plants (6 D) also includes observations for picloram and clopyralid.

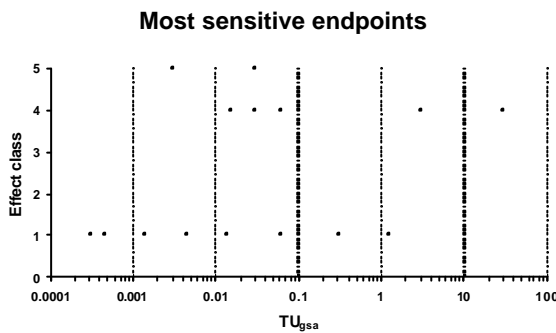


**Figure 6.** Classified effects of herbicides with an auxin-simulating effect in (semi) field studies with stagnant or recirculating test systems. The effects are classified into a functional category (community metabolism; A) and into several categories structural endpoints (B-H). The effects are also classified according to magnitude and duration. 1 = no significant effect, 2 = slight effect, 3 = clear short-term effect (< 8 weeks), 4 = clear effect in short-term study (recovery moment unknown), 5 = clear long-term effect (> 8 weeks). For an extensive description of the effect classes we refer to Section 2.4. The figure only includes studies with a single application. TU<sub>gsa</sub>: Toxic Unit on basis of the EC<sub>50</sub> of the most sensitive standard alga (see Section 2.5).





**Figure 7.** 10-, 50- and 90-percentile values (expressed in  $TU_{gsa}$  with 95% confidence intervals) as calculated by means of logistic regression for the functional and structural endpoints (split into phytoplankton and macrophytes) after a single application of an auxin simulator. The values could only be calculated for the classification (class 1 versus 4,5).  
\* confidence intervals could not be calculated.



**Figure 8.** Classified effects of herbicides with an auxin-simulating effect in (semi) field studies with stagnant or recirculating running test systems. The effects on the most sensitive endpoints are presented. The effects are also classified according to magnitude and duration. 1 = no significant effect, 2 = slight effect, 3 = clear short-term effect (< 8 weeks), 4 = clear effect in short-term study (recovery moment unknown), 5 = clear long-term effect (> 8 weeks). The figure only includes studies with a single application.

Due to the restricted number of data, logistic regression could only be performed on classification 1 versus classifications 4 and 5 as result of a single application (Figure 7). Because Figure 6 shows a large difference in response between aquatic plants and phytoplankton, the percentiles for these groups are calculated separately. As expected, the threshold values calculated for the macrophytes are much lower than those for phytoplankton and community metabolism. The 50-percentile for the effects on macrophytes after a single application of an auxin simulator is 0.007 (0.001-0.034)  $TU_{gsa}$ . The criterion given in the UP (0.1  $TU_{gsa}$ ) does therefore not guarantee protection of the macrophytes in case an auxin simulator is applied. Figure 8 presents the classified effects on the most sensitive endpoints. All effects reported for a dose below 0.1  $TU_{gsa}$  are effects on macrophytes (cf. Figure 7 and 6 D).

No suitable studies with running test systems were found for auxin-simulating herbicides.

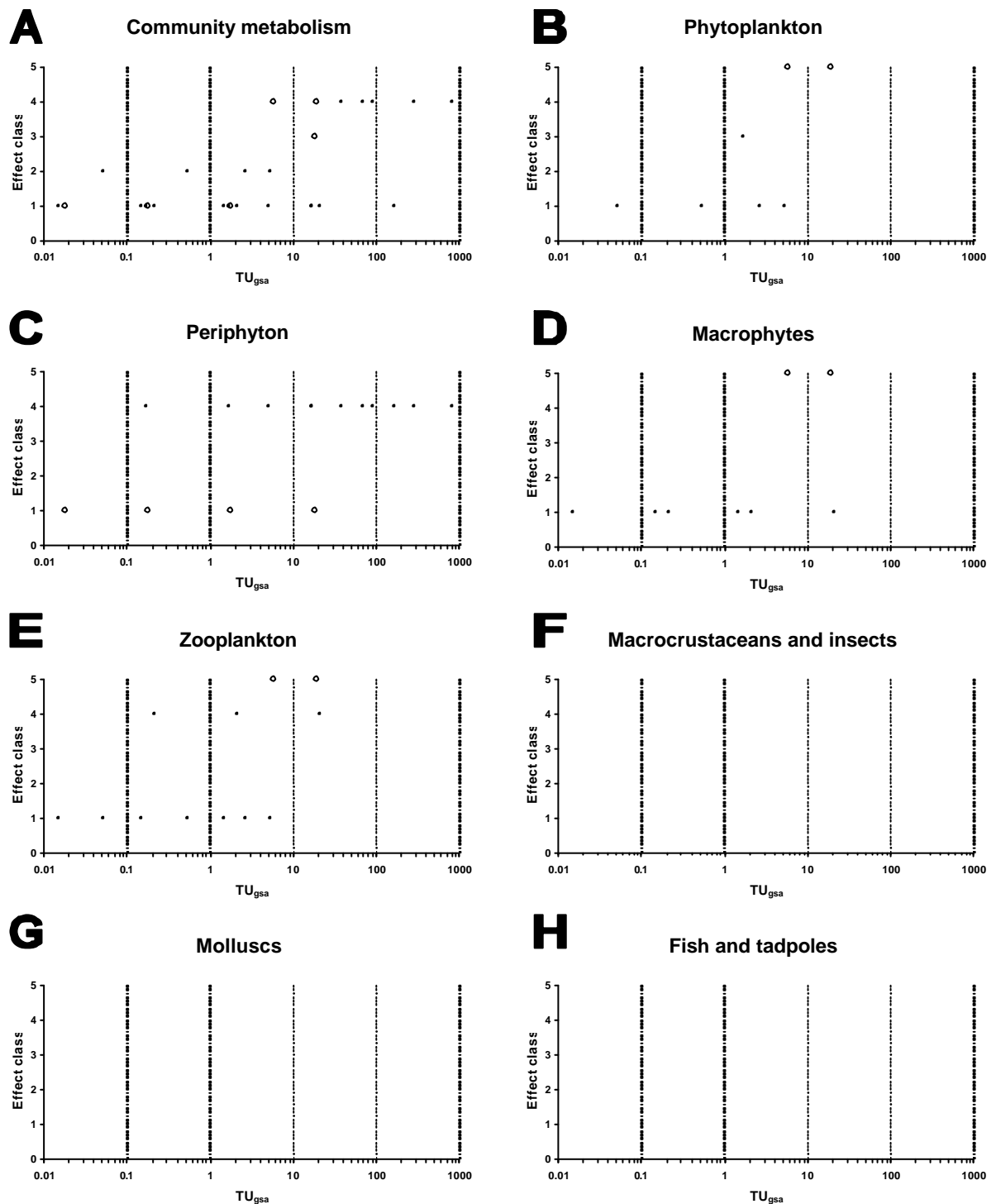
## 5.4 Other herbicides (growth inhibitors)

With one observation in Class 4 at a concentration of 0.17 TU<sub>gsa</sub>, periphyton seems to be the most sensitive to the group of other herbicides (Figure 9 C). The representative substances in this figure are alachlor, diquat and paraquat. This observation is based on the results of a single study into the effects of alachlor on the species composition of periphytic algae (Spawn *et al.*, 1997). Effects of alachlor on other parameters than species composition (such as cell density and chlorophyll concentration of the periphyton) were only found at 1.7 TU<sub>gsa</sub>. Negative effects on phytoplankton start to occur at 1 to 2 x EC<sub>50</sub> (Figure 9 B). The structure of the phytoplankton and periphyton communities seem to be the most sensitive endpoints for these growth-inhibiting herbicides. Aquatic plants are possibly somewhat less sensitive but this cannot be said with certainty due to the small number of studies (Figure 9 D). The effect on structural characteristics of primary producers probably only has measurable consequences for community metabolism at higher concentrations (first observed effects at 6 TU<sub>gsa</sub>, Figure 9 A). Nothing is known about the effects of this group of herbicides on macro-invertebrates, fish and tadpoles (Figure 9 F, G, H).

Figure 10 confirms the earlier presented picture; after a single application, the structural endpoints are more sensitive than the functional endpoints. The 50-percentile for these endpoints is 0.64 (0.07-6.14) TU<sub>gsa</sub> and 53 TU<sub>gsa</sub> (14-199), respectively. The same picture does not exist for a multiple application, but this evaluation is only based on one study.

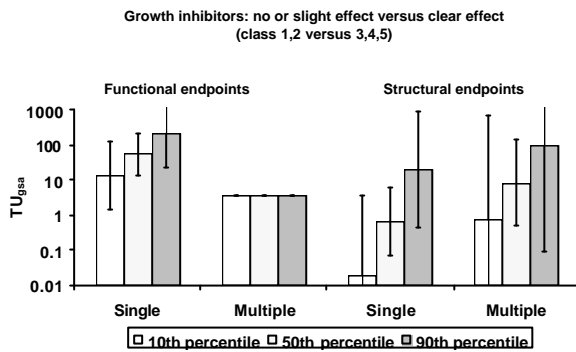
Figure 11 indicates that if effects would be expected at a dose of 0.1 TU<sub>gsa</sub>, these would probably only be slight. It is also clear that the number of studies is too small to draw a clear conclusion.

Figure 9 E shows that the first effects on zooplankton are observed at 0.3 TU<sub>gsa</sub> for standard algae. All effects shown in the figure in Class 4 concern the product triallate in the study of Johnson (1986). This substance probably has a direct effect on zooplankton. Johnson (1986) himself gives an EC<sub>50</sub> of 57 µg/L for *Daphnia magna*. This concentration corresponds with 1.2 TU<sub>gsa</sub>. This would explain a direct toxic effect of triallate in the study. When triallate is ignored, (indirect) effects on zooplankton are only observed around 6 TU.

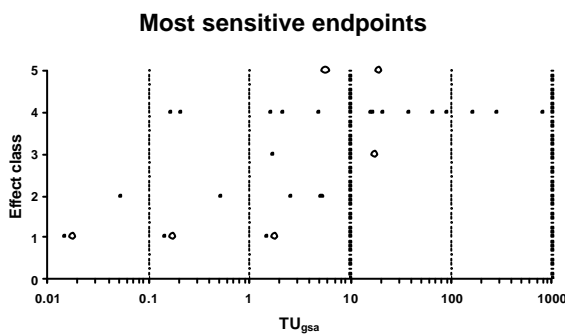


**Figure 9.** Classified effects of 'other group' herbicides (mainly with a growth-inhibiting effect) in (semi) field studies with stagnant or recirculating running test systems. The effects are classified into a functional category (community metabolism; A) and into several categories structural endpoints (B-H). The effects are also classified according to magnitude and duration. 1 = no significant effect, 2 = slight effect, 3 = clear short-term effect (< 8 weeks), 4 = clear effect in short-term study (recovery moment unknown), 5 = clear long-term effect (> 8 weeks). For a further description of the effect classes we refer to Section 2.4. The small closed circles (●) indicate the experiments with a single application; the large open circles (○) indicate the experiments with multiple or continuous exposure. TU<sub>gsa</sub>: Toxic Unit on basis of the EC<sub>50</sub> of the most sensitive standard alga (Section 2.5).

The only study with this group of herbicides in running systems concerns diquat. In a flow-through system, Paterson & Wright (1987) found no significant effect on the survival of *Elodea canadensis* in case of 24-hour exposure to pulses of 5 and 10 µg/L (0.1 and 0.2 TU<sub>gsa</sub>). Mortality was observed at 50 µg/L (1 TU<sub>gsa</sub>). All three concentrations showed a positive effect on the periphyton density which used *E. canadensis* as substrate. This should, however, probably be attributed to the leakage of nutrients from (slightly) affected plants.



**Figure 10.** 10-, 50- and 90-percentile values (expressed in TU<sub>gsa</sub> with 95% confidence intervals) as calculated by means of logistic regression for the functional and structural endpoints after single or multiple application of a herbicide belonging to the group of other herbicides (mainly with a growth-inhibiting effect). The values could be calculated for one classification, no and slight versus clear effect. Confidence intervals could not be calculated for functional endpoints at a multiple application.



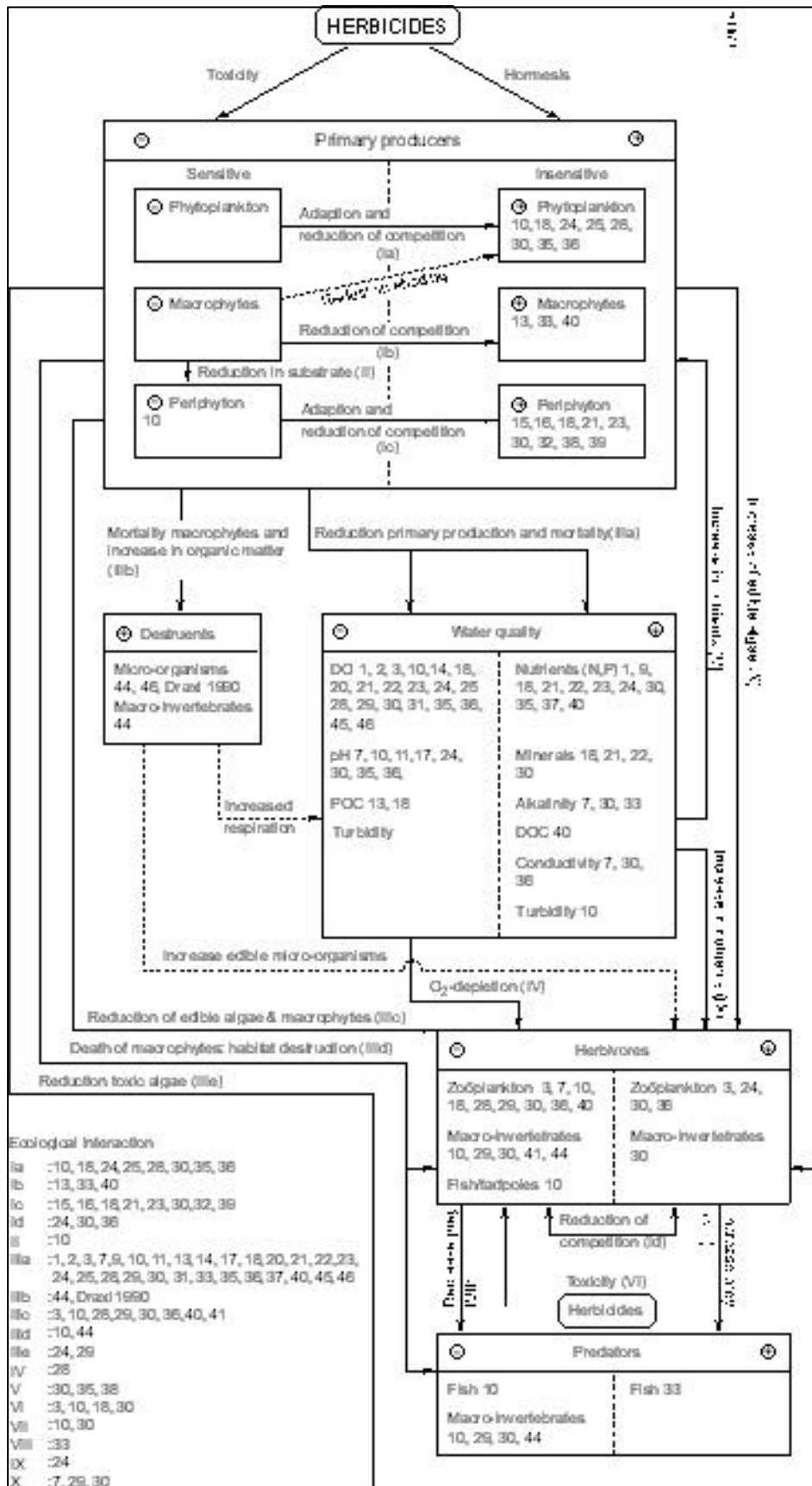
**Figure 11.** Classified effects of herbicides belonging to the group of other herbicides (mainly with a growth-inhibiting effect) in (semi) field studies with stagnant or recirculating running test systems. The effects on the most sensitive endpoints are presented. The effects are also classified according to magnitude and duration. 1 = no significant effect, 2 = slight effect, 3 = clear short-term effect (< 8 weeks), 4 = clear effect in short-term study (recovery moment unknown), 5 = clear long-term effect (> 8 weeks). The small closed circles (●) indicate the experiments with a single application; the large open circles (○) indicate the experiments with multiple exposure.

## 6 INDIRECT EFFECTS

Figure 12 summarises the indirect effects of herbicides as these were observed in the available studies. In this figure no distinction is made according to concentration level but the observed indirect effects usually originate from experiments in which relatively high concentrations have been studied ( $> 0.1 \text{ TU}_{\text{gsa}}$ ). Because the exact mechanism for indirect effects is difficult to establish in most cases, the shown routes are in fact based on appraisals of the best explanations, in which the discussions by the various authors of the papers as well as our own perceptions have played an important role. The figure should be interpreted as a presentation of the possible routes that follow from the observations. A number of effects, however, are occurring so frequently in combination with each other that the corresponding routes should be considered as most plausible explanation.

It has been observed for all groups of primary producers (phytoplankton, periphyton and macrophytes) that the disappearance of certain species may lead to an increase of other species within the same group. Primary producers mutually compete for nutrients, space,  $\text{CO}_2$  and light. Reduced competition of sensitive species may therefore shift the community towards less sensitive species. Shifts in species are in particular observed in phytoplankton and periphyton. Primary production often recovers after some time whereas the shift in species lasts much longer. This means that the ecosystem function often recovers as a result of other species taking over the role of the disappeared species (functional redundancy).

The disappearance of primary producers could in many cases be observed directly in the form of a decrease of the oxygen concentration and/or the pH of the water. In a single case oxygen depletion possibly caused a reduction of zooplankton. The most frequently suggested cause of negative effects on zooplankton and other herbivorous invertebrates, however, was the decrease of primary producers (algae and macrophytes) as source of food.



Death of primary producers and reduced primary production may also cause other changes in water quality. A significant increase in the amount of nutrients was the most frequently reported secondary effect besides reduced oxygen concentrations and pH. At the same time, a higher alkalinity, conductivity, turbidity and DOC (amount of dissolved organic carbon) were also found in some cases. This can easily be explained by the release of dissolved substances and particles that were until then part of the biomass of the primary producers. Next, an increase in nutrients may have a stimulating effect on less sensitive primary producers (especially algae), which may in turn result in a positive effect on the herbivores that feed on these algae, and finally even in an increase of predatory fish. Increases of certain herbivores may, however, also be caused by reduced competition with herbivores that disappear.

The most dramatic secondary effects were observed in the studies in which elimination of macrophytes occurred. Besides the above-mentioned effects on water quality and on herbivores this may lead to drastic shifts in the aquatic community by habitat destruction. The organisms for which aquatic plants play an important role include periphyton, crustaceans, aquatic insects (especially the larvae), molluscs, but also certain fish and tadpole species. All these groups may disappear or decrease in numbers as a result of mortality of macrophytes, where it makes no difference whether herbivorous or predatory species are involved. The disappearance of the vegetation may in some cases lead to a bloom of phytoplankton. This is possibly caused by decreased shading, as a result of which more light can penetrate the water, and the release of nutrients by the decomposition of dead plant material. There is thus a shift from a macrophyte- to a plankton-dominated ecosystem. Finally, as a result of the death of macrophytes there may be a (temporary) positive effect on the destruents in the community (microorganisms and macroinvertebrates), which may result in an increase in respiration (decrease in the amount of oxygen) and an increased amount of edible microorganisms for some zooplankton and macroinvertebrate species.

Figure 3 F and H show that in some studies with photosynthesis-inhibitors negative effects on macrocrustaceans, aquatic insects, fish and tadpoles already occur at  $0.2 \text{ TU}_{\text{gsa}}$ . In most cases this concerns habitat destruction by the disappearance of aquatic plants. The turning point for this effect is about the same as that for the macrophytes and is very close to the  $\text{NOEC}_{\text{ecor}}$ . Indirect effects on zooplankton (via phytoplankton) only arise at  $0.9 \text{ TU}_{\text{gsa}}$  and on molluscs (via periphyton) at  $9 \text{ TU}_{\text{gsa}}$  (Figure 3 E and G). The indirect effects of auxin simulators were hardly studied (Figure 6). Indirect effects of the other herbicides on zooplankton probably only occur at  $6 \text{ TU}_{\text{gsa}}$  (Figure 9 E).

**Figure 12. (page 38)** Schematic presentation of the indirect effects of herbicides in aquatic (semi) field experiments. The explanations given for the observations are partly based on the discussions of the various authors of publications that were consulted and partly on our own interpretation. The dotted lines, e.g., indicate the routes postulated by the authors of this report. The numbers refer to the study numbers given in Tables 1 - 3. The direct negative effects on primary producers are presented in Figures 3, 6, and 9.





## 7 RECOVERY

Figure 3 clearly shows for most endpoints that photosynthesis inhibitors have a more prolonged effect at higher concentrations. In case of single application, the functional endpoints do, however, generally recover faster (and after higher doses) than the structural endpoints (Figure 4 C). This difference is smaller in case of multiple applications or constant exposure (Figure 4 C). Short-term negative effects (< 8 weeks, class 3) on primary production are observed up to a maximum of 40 TU<sub>gsa</sub> (Figure 3A: there is one exception at 300 TU<sub>gsa</sub>). This maximum for phytoplankton is 10 TU<sub>gsa</sub> (Figure 3 B) and for zooplankton 5 TU<sub>gsa</sub> (Figure 3 E). The fact that primary production does in many cases recover earlier (and also after higher doses) than the structural endpoints for the primary producers themselves most probably indicates functional redundancy (less sensitive populations take over the role of sensitive populations).

Considering the most sensitive endpoints, recovery in case of photosynthesis-inhibitors usually occurs within 8 weeks after the last application at concentrations lower than 1 TU<sub>gsa</sub> (Figure 5). Observed effects of photosynthesis inhibitors on macrophytes at concentrations below 1 TU<sub>gsa</sub> do not always recover within 8 weeks (Figures 3 D and 4 C). The macrophyte-associated populations of macroinvertebrates, fish and tadpoles then neither recover within 8 weeks (Figures 3 F, G and H). This means that there is a clear difference in recovery between plankton- and macrophyte-dominated ecosystems. Below 1 TU<sub>gsa</sub> plankton communities usually recover within 8 weeks. The recovery of systems that strongly depend on macrophytes may sometimes take longer if the dominant macrophyte appears to be sensitive (2 of the 9 observations in the range 0.1-1 TU<sub>gsa</sub>).

For auxin simulators it is more difficult to come to general conclusions on the basis of the few available studies. Because data for class 2 and 3 are not available, conclusions on recovery can hardly be drawn (Figure 6). Most studies also had a short duration. Based on the findings for photosynthesis-inhibitors it seems, however, logical that in macrophyte-dominated systems this group of herbicides, which is in particular very toxic to aquatic plants, will also lead to far-reaching and prolonged changes at relatively low concentrations (Figure 7). The studies do not allow conclusions about the recovery of plankton-dominated communities after exposure to an auxin simulator. These types of systems are likely relatively insensitive to auxin simulators and may also be capable to recover from negative effects in a shorter period of time (Figure 7).

The recovery of the endpoints for the group 'other herbicides' – as for the photosynthesis inhibitors - decreases at a higher exposure (Figure 8). Most studies, however, did not last long enough to allow conclusions about the duration of recovery. It may be assumed that for these compounds there is also the same difference between the recovery of plankton- and macrophyte-dominated systems.

**Table 4.** NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values for studies with photosynthesis-inhibiting herbicides in (semi) field studies. A '-' indicates that no NOEC<sub>eco</sub> or LOEC<sub>eco</sub> could be derived from a series of experiments.

Active ingredient	Exposure	NOEC <sub>eco</sub> (µg/L)	LOEC <sub>eco</sub> (µg/L)	Reference(s)
<b>Stagnant and recirculating systems:</b>				
atrazine	single	≥ 5	-	Guessner & Watzin, 1996
	single	-	≤ 50	Fairchild <i>et al.</i> , 1994
	single	-	≤ 60	Stay <i>et al.</i> , 1985
	single	-	≤ 100	Moorhead & Kosinski, 1986
	single	20	100	Stay <i>et al.</i> , 1989
	single	50	100	Brockway <i>et al.</i> , 1984
	single	100	1000	Johnson, 1986
	constant	-	≤ 10	Kosinski, 1984
				Kosinski & Merkle, 1984
	constant	-	≤ 15	Detenbeck <i>et al.</i> , 1996
	multiple	-	≤ 20	DeNoyelles <i>et al.</i> , 1982
				Dewey, 1986
				Kettle <i>et al.</i> , 1987
				DeNoyelles <i>et al.</i> , 1989
				DeNoyelles <i>et al.</i> , 1994
	constant	-	≤ 24	Krieger <i>et al.</i> , 1988
	constant	5	50	Brockway <i>et al.</i> , 1984
	constant	≥ 5	-	Van den Brink <i>et al.</i> , 1995
	constant	10	32	Pratt <i>et al.</i> , 1988
constant	25	75	Jüttner <i>et al.</i> , 1995	
multiple, additive & single	-	≤ 80	Hamilton <i>et al.</i> , 1987	
constant	-	≤ 100	Hamala & Kollig, 1985	
multiple additive	-	≤ 155	Herman <i>et al.</i> , 1986	
			Hamilton <i>et al.</i> , 1988	
			Hamilton <i>et al.</i> , 1989	
simazine	single	-	≤ 100	Goldsborough & Robinson, 1983
				Goldsborough & Robinson, 1986
	single	100	500	Jenkins & Buikema, 1990
	single	100	1000	Goldsborough & Robinson, 1985
terbutryn	single	-	≤ 2000	Gumey & Robinson, 1989
	single	-	≤ 6	Struve <i>et al.</i> , 1991
hexazinone	single	-	≤ 10	Goldsborough & Robinson, 1983
	single	-	≤ 10	Goldsborough & Robinson, 1986
	single	10	100	Gumey & Robinson, 1989
linuron	single	-	≤ 1000	Thompson <i>et al.</i> , 1993 a
	constant	0.5	5	Thompson <i>et al.</i> , 1993 a
isoproturon	single	-	≤ 1000	Stephenson & Kane, 1984
	constant	0.5	5	Van den Brink <i>et al.</i> , 1997
diuron	single	-	≤ 5	Cuppen <i>et al.</i> , 1997
	single	2	9	Pérés <i>et al.</i> , 1996
	single	30	90	Feurtet-Mazel <i>et al.</i> , 1996
	single	2.9	28.5	Traunspurger <i>et al.</i> , 1996
	single	2.9	28.5	Flum & Shannon, 1987
<b>Running systems:</b>				
atrazine	two pulses	≥ 100	-	Jurgensen & Hoagland, 1990
terbutryn	pulse	≥ 50	-	Paterson & Wright, 1987
hexazinone	pulse	≥ 2700	-	Kreutzweiser <i>et al.</i> , 1995

## 8 EVALUATION OF THE SETTING OF CRITERIA

### 8.1 Photosynthesis inhibitors

Table 4 presents the NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values for photosynthesis-inhibiting herbicides which could be derived from the studies.

Next, a summarising NOEC<sub>eco</sub> and LOEC<sub>eco</sub> were derived for each substance; for the last parameter, the lowest LOEC<sub>eco</sub> of all relevant studies was taken. The summarising NOEC<sub>eco</sub> is the highest found NOEC<sub>eco</sub> that is lower or equal to the lowest found LOEC<sub>eco</sub>. In Table 5 these values are compared with the maximum permissible concentration (MPC) in accordance with the Dutch water quality criteria (Crommentuijn *et al.*, 1997), and the liberal and conservative criterion according to the Uniform Principles.

**Table 5.** Summarising NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values for studies with photosynthesis-inhibiting herbicides in (semi) field studies compared with different criteria.

Active ingredient	Exposure regime	NOEC <sub>eco</sub> (µg/L)	LOEC <sub>eco</sub> (µg/L)	MPC NW4 criterion (µg/L) <sup>1</sup>	Liberal UP criterion (µg/L)	Conservative UP criterion (µg/L)
<b>Stagnant and recirculating systems:</b>						
atrazine	single	20	50	2.9	6.7	0.4
	multiple	5	10	2.9	6.7	0.4
simazine	single	100	100	0.14	35.1	10
terbutryn	single	-	≤ 6	-	2.7	2.7
hexazinone	single	10	100	-	4.6	2.5
linuron	multiple	0.5	5	0.25	1.6	1.6
isoproturon	single	2	5	0.32	2.1	2.1
diuron	single	2.9	28.5	0.43	1.5	1.5
<b>Running systems:</b>						
atrazine	multiple	≥ 100	-	2.9	6.7	0.4
terbutryn	pulse	≥ 50	-	-	2.7	2.7
hexazinone	pulse	≥ 2700	-	-	4.6	2.5

<sup>1</sup> from Crommentuijn *et al.* (1997)

The criteria for the photosynthesis inhibitors are in all cases lower than the LOEC<sub>eco</sub> that could be derived from the (semi) field experiments. The criteria are also often lower or equal to the NOEC<sub>eco</sub>. The MPC values are in all cases lower than the liberal UP criterion. With exception of atrazine, this is also the case for the conservative UP criterion. The available suitable studies did not allow the derivation of summarising NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values for the substance terbutryn. The results of the studies, however, did not contradict the criteria. Recently, Beek & Knobens (1997) also calculated a MPC for atrazine with the Aldenberg & Slob (1993) method. Their result, 0.3 µg/L, is about 10 times as low as the MPC of Crommentuijn *et al.* (1997) and about 17 times as low as the NOEC<sub>eco</sub> that we derived from the (semi) field studies in this report.

## 8.2 Auxin simulators

The NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values for auxin-simulating substances are per study presented in Table 6.

**Table 6.** NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values for studies with auxin-simulating herbicides in (semi) field studies.

Active ingredient	Exposure	NOEC <sub>eco</sub> (µg/L)	LOEC <sub>eco</sub> (µg/L)	Reference(s)
<b>Stagnant and recirculating systems:</b>				
2,4-D	single	10	100	Forsyth <i>et al.</i> , 1997
	single	-	≤ 500	Boyle, 1980
	single	-	≤ 1000	Scott <i>et al.</i> , 1981; Stephenson & Mackie, 1986 Sherry, 1994
2,4,5-T	single	-	≤ 2000	Kobriae & White, 1996
	single	10000	100000	Sugiura, 1992
	single	≥ 100	-	Forsyth <i>et al.</i> , 1997
picloram	single	≥ 100	-	Forsyth <i>et al.</i> , 1997
clopyralid	single	≥ 100	-	Forsyth <i>et al.</i> , 1997
<b>Running systems:</b>				
trichlopyr	pulse (1 hour)	320	3200	Kreutzweiser <i>et al.</i> , 1992

Table 7 compares the summarising NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values from these studies with the criteria. The MPC value derived by Crommentuijn *et al.* (1997) for 2,4-D shows good agreement with the (semi) field studies. For 2,4,5-T, however, this criterion is a factor 1000 lower than the NOEC<sub>eco</sub>. This NOEC, however, is based on a single study (Sugiura, 1992) that has been conducted in a plankton-dominated system without aquatic plants. In view of the high sensitivity of macrophytes to these compounds it is to be expected that the NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values will be considerably lower in a macrophyte-dominated ecosystem. The UP criteria for 2,4-D, which are based on standard tests with algae, do not offer adequate protection because macrophytes are much more sensitive than algae. This is possibly also the case for picloram and clopyralid. This could, however, not be verified on the basis of the studies with these substances. The UP criteria neither seem to offer protection against trichlopyr in running systems. The measured effect in the particular study concerned the survival of some invertebrates. An explanation for this effect cannot be given. The LC<sub>50</sub> for *Daphnia magna*, e.g., is 133 000 µg/L. The MPC for 2,4-D estimated by Beek & Knobben (1997) is about 4 µg/L, comparable to that of Crommentuijn *et al.* (1997).

**Table 7.** Summarising NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values for studies with a single application of auxin-simulating herbicides in (semi) field studies compared with different criteria.

Active ingredient	NOEC <sub>eco</sub> (µg/L)	LOEC <sub>eco</sub> (µg/L)	MPC NW4 criterion (µg/L) <sup>1</sup>	Liberal UP criterion (µg/L)	Conservative UP criterion (µg/L)
<b>Stagnant and recirculating systems:</b>					
2,4-D	10	100	10	3289	2590
2,4,5-T	10000 <sup>2</sup>	100000	9	-	-
picloram	≥ 100	-	-	2170	2170
clopyralid	≥ 100	-	-	710	690
<b>Running systems:</b>					
trichlopyr	320	3200	-	4500	4500

<sup>1</sup> from Crommentuijn *et al.* (1997)

<sup>2</sup> based on phytoplankton-dominated test system without macrophytes

### 8.3 Other herbicides (growth inhibitors)

The NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values from the studies with these substances are presented in Table 8.

**Table 8.** NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values for studies with other herbicides in (semi) field studies.

Active ingredient	Exposure	NOEC <sub>eco</sub> (µg/L)	LOEC <sub>eco</sub> (µg/L)	Reference(s)
<b>Stagnant and recirculating running systems:</b>				
diquat	single	-	≤ 850	Pratt <i>et al.</i> , 1990
	single	-	≤ 3500	Barreiro Lozano & Pratt, 1994
	constant & single	-	≤ 300	Draxl <i>et al.</i> , 1991
paraquat	constant	1000 <sup>1</sup>	10000 <sup>1</sup>	Kosinski, 1984
fluridon	single	-	≤ 125	Struve <i>et al.</i> , 1991
metsulfuron-methyl	single	≥ 1000	-	Thompson <i>et al.</i> , 1993 a
				Thompson <i>et al.</i> , 1993 a
alachlor	single	-	≤ 1	Spawn <i>et al.</i> , 1997
trallate	single	-	≤ 10	Johnson, 1986
trifluralin	single	≥ 1000	-	Johnson, 1986
MSMA	constant	-	≤ 10	Kosinski, 1984
<b>Running systems:</b>				
diquat	pulse	10	50	Paterson & Wright, 1987

<sup>1</sup> based on an experiment where the substance was removed unnaturally fast

The number of suitable studies with these herbicides was rather scarce and they were often also of a lower quality than, e.g., those with the photosynthesis inhibitors. In most cases it was not possible to establish NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values per study so that 'higher or equal' or 'lower or equal' value will have to do. This also sets limits to the conclusions that can be drawn when these values are compared with the NW4 and UP criteria (Table 9). The criteria for diquat, paraquat, fluridon, alachlor, and triallate are in any case not in conflict with the experimental data that have been found. The UP criterion for metsulfuron-methyl, however, is very low in comparison with the results of the experiment of Thompson *et al.* (1993a, 1993b) and the same is true for trifluralin (study of Johnson, 1986). In the study of Kosinski (1984) and Merkle & Kosinski (1984), paraquat was removed in an artificial way. This may possibly explain why the UP criteria for paraquat are much lower than the NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values. A UP criterion could not be established for MSMA because algae data are lacking.

**Table 9.** Summarising NOEC<sub>eco</sub> and LOEC<sub>eco</sub> values for studies with single or multiple application of a herbicide of the group of 'other herbicides (growth inhibitors)' compared with different criteria. The experiments with the substances diquat, paraquat and MSMA were conducted with multiple exposure; experiments with all other substances with single exposure.

Active ingredient	NOEC <sub>eco</sub> (µg/L)	LOEC <sub>eco</sub> (µg/L)	MPC NW4 criterion (µg/L) <sup>1</sup>	Liberal UP criterion (µg/L)	Conservative UP criterion (µg/L)
<b>Stagnant and recirculating systems:</b>					
diquat	-	≤ 300	-	5.2	3.4
paraquat	1000 <sup>2</sup>	10000 <sup>2</sup>	-	56	56
fluridon	-	≤ 125	-	7.4	7.4
metsulfuron-methyl	≥ 1000	-	-	19	19
alachlor	-	≤ 1	-	0.6	0.6
triallate	-	≤ 10	1.9	4.7	4.7
trifluralin	≥ 1000	-	0.037	6.7	6.7
MSMA	-	≤ 10	-	-	-
<b>Running systems:</b>					
diquat	10	50	-	5.2	3.4

<sup>1</sup> from Crommentuijn *et al.* (1997)

<sup>2</sup> based on an experiment where the substance was removed unnaturally fast

## 9 GENERAL DISCUSSION

Only 11 of the 20 herbicides discussed in this report are registered in The Netherlands, and not all these substances are used on a large scale. Considering their use and presence in surface water, the data on atrazine, simazine, linuron, isoproturon, diuron, and 2,4-D are in particular relevant in the Dutch context. Unfortunately, no adequate studies were found for other compounds that are relevant in The Netherlands. Examples of substances for which it would, in the context of ecological criteria-setting, be meaningful to conduct studies in aquatic model ecosystems are mecoprop, metolachlor, propachlor, MCPA, dichlobenil, glyphosate and DNOC.

Atrazine is the only substance for which earlier literature reviews on ecological risks have been published. Eisler (1989) concluded that the first effects of atrazine on aquatic plants in single species tests occur at 1.5 µg/L. Huber (1993, 1994) put the turning point for ecological effects of atrazine at 20 µg/L. In her review of experiments in 'split ponds', Draxl (1994) also reports effects on physico-chemical parameters, macrophytes and zooplankton at this value. Lower concentrations, however, were not tested. Solomon *et al.* (1996) recently published a very extensive review of atrazine in North-American surface waters. These authors conclude that the disturbance of aquatic ecosystems starts at an exposure concentration above 20 µg/L but that ecologically important effects only start to occur at 50 µg/L and higher. Different studies in this report, however, clearly show that there may be considerable effects at 20 µg/L and lower (Table 4; see, e.g., also study no. 10). The NOEC<sub>eco</sub> of 5 µg/L that comes forward from our study is therefore a safer threshold value.

The criteria derived by Crommentuijn *et al.* (1997), which have also been included in the Fourth Memorandum Water Management (NW4), offer adequate protection against ecological effects in freshwater ecosystems for all herbicides in this report. This criterion could for some compounds (e.g. trifluralin) possibly be adjusted upwards on the basis of these studies. The conservative and liberal UP criteria, calculated in this report on the basis of algae tests, generally also seem to be adequate for photosynthesis- and growth-inhibiting herbicides. For substances with an auxin-simulating effect, such as 2,4-D, UP criteria based on algae data, however, underestimate the risk because the compounds have a specific effect on higher aquatic plants.

In case ecological threshold values for herbicides are exceeded, the very first effects occur in primary producers, for which community metabolism and the densities and biomass of algae and macrophytes are sensitive endpoints. There is an important difference between effects in plankton- and in macrophyte-dominated systems. Recovery and adaptation (recovery of functionality) in plankton-systems is more rapid and indirect effects on higher trophic levels (zooplankton) are only observed at higher concentrations. Recovery usually takes longer in ecosystems in which

macrophytes play an important role, while in some studies indirect effects by habitat destruction are already found at concentrations just above the threshold value.

It follows from the above that more attention should be paid to the effects of herbicides on higher aquatic plants in the context of ecological risk assessment and criteria-setting for herbicides. It has frequently been argued that algae tests cannot always act as substitute for higher aquatic plants (e.g., Sortkjaer, 1984; Benenati, 1990; Wang, 1991; Lewis, 1995a). Fletcher (1990) concluded on the basis of an analysis of available pesticide data that algae tests did not detect a response in 20% of the cases where vascular plants did show a response. The most frequently used aquatic plants in toxicity tests are various *Lemna* spp. (the organisation ASTM in the USA has recently published a protocol for a 7-day growth inhibition test for *L. gibba*; ASTM, 1997a). Besides the toxicity data on algae, Appendices 1-20 to this report present the data we found on *Lemna*. The results of these tests were of the same order of magnitude as those for standard algae for most of the photosynthesis- and growth-inhibiting herbicides. This was, however, not the case for sulfonylurea compounds such as metsulfuron-methyl and chlorosulfuron (Appendix 16; Fairchild *et al.*, 1997); the EC<sub>50</sub> for *Lemna* was up to a factor 400 lower for these substances. *Lemna* was less sensitive than algae to phenoxyacetic acids such as 2,4-D (Appendix 8) and clopyralid (Appendix 11) whereas field experiments show that rooting aquatic plants are very sensitive to this group of substances. For auxin-simulators this thus means that also tests with *Lemna* may underestimate the ecological risk. It is therefore essential for auxin-simulating herbicides that tests are conducted with relevant macrophytes, preferably rooting aquatic plants, besides the usual standard tests with aquatic organisms. Such tests have not often been performed in the past because they are awkward to conduct and last long (Lewis, 1995b). At the moment, however, especially in North America, quite some progress has been made with the development of standard tests with rooting aquatic plants (Freemark *et al.*, 1990; ASTM, 1997b).



## 10 CONCLUSIONS

- Provided that the experimental design meets a number of criteria (Section 2.2), the results of (semi) field experiments in freshwater ecosystems yield, after normalisation of the test concentrations, a well interpretable picture of the ecological effects of groups of herbicides with the same mode of action.
- Due to their effect on community metabolism, in particular on the oxygen regime, organic solvents that are used for the application of herbicides to experimental systems may, even at very low concentrations (ethanol possibly already from 2  $\mu\text{L}/\text{L}$ ), have a disturbing effect on the ecosystem.
- The most sensitive endpoints for the detection of negative effects of herbicides in aquatic ecosystems are community metabolism (decrease of oxygen and pH; increase of nutrients, alkalinity, conductivity, etc.), the structure of phytoplankton and periphyton communities (density, biomass, species composition, chlorophyll content) and the structure of the macrophyte community (cover, biomass).
- The functional endpoints are more sensitive to *photosynthesis inhibitors* than the structural endpoints. Macrophytes are most sensitive to *auxin simulators*. The few available studies show that structural endpoints, algae in particular, are possibly more sensitive to *growth inhibitors* than functional endpoints. The most sensitive endpoint generally determines the recovery rate of the ecosystem.
- Generally, too few data are available on ecological effects in aquatic (semi) field situations of herbicides other than photosynthesis inhibitors.
- Generally, effects due to multiple applications are already observed at lower (peak) concentrations than after a single application.
- Sometimes, a positive effect of low herbicide concentrations is observed on sensitive endpoints (hormesis). Usually, this phenomenon, is temporary. The ecological relevance of hormesis has insufficiently been studied.
- The ecological threshold levels for herbicides with a photosynthesis- or growth-inhibiting effect in stagnant and recirculating running test systems are generally equal to 0.1 times the geometric mean of the found  $\text{EC}_{50}$  values for the most sensitive standard alga. These levels are between 0.001 and 0.01 times the geometric mean of these  $\text{EC}_{50}$  values for substances that simulate the effect of the plant growth hormone auxin.
- For various reasons, less sensitive species often increase in number when sensitive primary producers disappear. This may lead to recovery of functional

endpoints while the effect on the structure of the ecosystem continues (functional redundancy).

- At concentrations higher than 0.1 times the geometric mean of the  $EC_{50}$  values of the most sensitive standard alga there is a considerable difference between the effects of herbicides in systems that are dominated by plankton or by macrophytes. Plankton-dominated communities adapt more rapidly at population and/or community level and, as a result, recover more rapidly than macrophyte-dominated systems. Below the geometric mean of the  $EC_{50}$  values of the most sensitive standard test alga, plankton-communities usually recover within 8 weeks from stress by photosynthesis inhibitors. The recovery of macrophyte-dominated systems may take longer if the dominant macrophyte appears to be sensitive.
- Indirect effects of photosynthesis inhibitors at higher trophic levels (consumers and predators) are only observed at concentrations around the  $EC_{50}$  for the standard alga. In systems dominated by aquatic plants, indirect effects on higher trophic levels as a result of habitat destruction are already observed as soon as the macrophytes disappear.
- Various indirect effects at ecosystem level may be observed at concentrations higher than 0.1-1 times the  $EC_{50}$  of the most sensitive standard alga. Some regularly reported indirect effects of herbicides are algal bloom (as a result of decreased competition for space, light and nutrients with sensitive primary producers such as macrophytes) and disappearance of macrophyte-associated animal populations by habitat destruction (as a result of the disappearance of macrophytes).
- The MPC values derived by Crommentuijn *et al.* (1997) appear to offer adequate protection of freshwater ecosystems against all herbicides in this study. A slight upward adjustment seems to be possible for some substances. Criteria based on algae tests in compliance with the Uniform Principles of the European Union are generally also adequate to prevent ecological effects of photosynthesis- and growth-inhibiting herbicides but these underestimate the threshold values for auxin-simulating compounds up to a factor 100. Because *Lemna* is insufficiently sensitive to these substances, toxicity tests with rooting aquatic plants will in this case have to bring a solution.

## **11 RECOMMENDATIONS FOR ECOSYSTEM EXPERIMENTS WITH HERBICIDES**

The studies evaluated in this report show that (semi) field experiments, provided that they are properly executed (see criteria in Section 2.2), present a uniform picture for groups of herbicides with a similar mode of action. The evaluation shows, however, that a number of lessons can be derived for future experiments.

- Several organic solvents that are used as 'carrier' for the application of pesticides may even at very low concentrations cause effects on the metabolism of model ecosystems. A minimum requirement is that the same concentrations of these solvents are added to the controls to allow establishment of the net effect of the pesticide. Controls with and without solvent may produce evidence whether or not solvents do affect the systems. It is even better, however, to use water as carrier. In case the water-solubility of the pesticides is insufficient, alternative application methods can be sought. Goldsborough & Robinson (1985) and Gurney & Robinson (1989), e.g., used micromesh sachets from which the herbicide dissolved in the water. Another elegant method is vapour-drying of the pesticide in the experimental system with highly volatile solvents. This can be done before the experimental system is filled with water (Sugiura, 1992).
- Further (semi) field studies into the ecological threshold values with other substances than photosynthesis inhibitors would be useful. Generally, too little information is available on the effects of these substances at ecosystem level.
- It is advisable that test systems for the ecological risk assessment of herbicides in shallow surface water contain higher aquatic plants. The reasons for this are already given in Chapter 9.
- More attention should be paid to effects on the heterotrophic component of aquatic ecosystems. Some studies clearly show that the death of primary producers, such as aquatic plants, causes indirect effects in destruents and bacteria.
- For studies with herbicides it is important that structural as well as functional endpoints are measured. Functions may recover by functional redundancy or adaptation before the structural endpoints are back to the original level. Here, it is thus important that the taxonomic level of the study is adequate to be able to detect changes in species composition of primary producers (e.g. algae).



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## **APPENDICES**





## Appendix 1

Substance: **atrazine**  
triazine

Available laboratory toxicity data for (standard) green algae (Chlorophyta).

Species	Exposure duration (h)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Scenedesmus subspicatus</i>	96	area below the curve	110	40	Geyer <i>et al.</i> 1985
	96	cell production	21	-	Kirby & Sheahan 1994
	72	growth rate	120	-	Tamerus 1996
	72	growth	72	22	Shäfer <i>et al.</i> 1994
<i>Scenedesmus parvus</i>	72	growth rate	27	7	Tamerus 1996
<i>Selenastrum capricornutum</i>	120	?	120 <sup>1)</sup>	-	USEPA 1994
	96	?	130 <sup>1)</sup>	-	Hoberg 1991
	96	?	50	-	Versteeg 1990
	120	?	55 <sup>1)</sup>	15 <sup>1)</sup>	Hoberg 1993
	96	cell count	4 <sup>1)</sup>	0.5 <sup>1)</sup>	Rodgers 1991
	120	growth rate	218	70	Abou-Waly <i>et al.</i> 1991
	96	growth	235	75	Fairchild <i>et al.</i> 1997
	72	growth rate	54	14	Tamerus 1996
	72	growth rate	110	-	Källqvist & Romstad 1994
	?	?	59 <sup>2)</sup>	-	Turbak <i>et al.</i> 1986
96	growth	147	-	Gaggi <i>et al.</i> 1995	
<i>Chlorella vulgaris</i>	144	growth rate	421	132	Véber <i>et al.</i> 1981
<i>Chlorella pyrenoidosa</i>	120	growth	175	-	Gramlich & Frans 1964
	120	?	282 <sup>1)</sup>	-	USEPA 1994
	96	growth	60	-	Maule & Wright 1984
<i>Chlamydomonas geitleri</i>	72	growth	481	326	François & Robinson 1990
<i>Chlamydomonas reinhardi</i>	72	growth	350	120	Schäfer <i>et al.</i> 1994

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna gibba</i>	14	density	50 <sup>1)</sup>	8.3 <sup>1)</sup>	Hoberg 1993a
	14	biomass	22 <sup>1)</sup>	8.3 <sup>1)</sup>	Hoberg 1993a
	7	Increase in density	180 <sup>1)</sup>	-	Hoberg 1991
	14	density	37 <sup>1)</sup>	-	Hoberg 1993b
	14	biomass	45 <sup>1)</sup>	7.7 <sup>1)</sup>	Hoberg 1993b
	5	?	170 <sup>1)</sup>	-	USEPA 1994
<i>Lemna minor</i>	4	?	-	10 <sup>1)</sup>	Rodgers 1991
	20	wetweight	±150	-	Beaumont <i>et al.</i> 1976
	4	density	56	-	Kirby & Sheahan 1994
	4	wetweight	60	-	Kirby & Sheahan 1994
	4	chlorophyll density	62	-	Kirby & Sheahan 1994
4	density	153	75	Fairchild <i>et al.</i> 1997	

1) quoted in Solomon *et al.* 1996

2) quoted in Källqvist & Romstad 1986

## Appendix 1 (atrazine cont.)

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Scenedesmus subspicatus</i>	67	30
<i>Scenedesmus parvus</i>	27	7
<i>Selenastrum capricornutum</i>	75	14
<i>Chlorella vulgaris</i>	421	132
<i>Chlorella pyrenoidosa</i>	144	-
<i>Lemna spp.</i>	72	13.2

Summary criteria.

UB conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
0.4	6.7	2.9

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
 liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
 MPC for freshwater from Crommentuijn *et al.* 1997

**Table Ia.** Studies with single and multiple peak loads (applications) and constant exposure with **atrazine** in stagnant and closed running ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L

Studied TU <sub>gsa</sub> [case number]	Concentration (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.007 [1]	0.5 single	12 d	lab stagnant microcosms	<i>No effect</i> Net O <sub>2</sub> production	-	Brockway <i>et al.</i> 1984 [1]I	1
0.007 [2]	0.5 constant	10 wks	lab running microcosms	<i>No effect</i> Net O <sub>2</sub> production, nitrate	-	Brockway <i>et al.</i> 1984 [1]II	1
0.05 [3]	3.2 constant	3 wks	lab running microcosms with artificial substrate	<i>No effect</i> DO, potassium, magnesium, calcium <i>Increase</i> Number of species, protein biomass, and Chl-a of Protozoa (slight, no clear dose-effect relationship, not used by authors for calculation of LOECs and NOECs)	-  > 3 wk	Pratt <i>et al.</i> 1988 [2]	1
0.075 [4]	5 constant	8 wks	enclosures in a pond	<i>No effect</i> DO, pH and conductivity <i>Increase</i> bloom of two algal species (but no dose-effect relationship), nauplii of Copepoda and egg production of <i>Daphnia</i> (slight, no clear dose-effect relationship)	-  2-6 wk  5-6 wk	Jüttner <i>et al.</i> 1995 [3]	1
0.075 [5]	5 constant	6 wk	lab running microcosms	<i>No effect</i> DO, pH, conductivity, alkalinity and species composition phytoplankton, zooplankton and macroinvertebrates	-	Van den Brink <i>et al.</i> 1995 [4]	1
0.075 [6]	5 single	12 d	lab stagnant microcosms	<i>No effect</i> Net O <sub>2</sub> production	-	Brockway <i>et al.</i> 1984 [1]I	1
0.075 [7]	5 single	10 wk	lab running microcosms	<i>No effect</i> Net O <sub>2</sub> production, nitrate	-	Brockway <i>et al.</i> 1984 [1]II	1
0.075 [8]	5 single	2 wk	recirculating artificial streams in lab	<i>No effect</i> Chl-a of periphyton on artificial substrate, insects in cages, leaching of macro-invertebrates <i>Increase</i> insects flying out (slight)	-  12 d	Guessner & Watzin 1996 [5]	2
0.15 [9]	10 constant	8 wk	enclosures in a pond	<i>No effect</i> one alga species <i>Decrease</i> DO, conductivity (slight) <i>Increase</i> pH (slight) bloom of an alga species (but no dose-effect relationship) nauplii of Copepoda, rotifers and egg production of <i>Daphnia</i> (slight, no clear dose-effect relationship)	n.a.  50 d  >63 d 25 d  18-39 d	Jüttner <i>et al.</i> 1995 [3]	2

**Table Ia. Continued** . Studies with single and multiple peak loads (applications) and constant exposure with **atrazine** in stagnant and closed running ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L

Studied TU <sub>gsa</sub> [case number]	Concentration (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.15 [10]	10 constant	3 wk	lab running microcosms with artificial substrates	<i>No effect</i> DO, potassium, magnesium, calcium <i>Increase</i> number of species, protein biomass and Chl-a Protozoa (slight, no clear dose-effect relationship, not used by authors for calculation of LOECs and NOECs)	-  >3 wk	Pratt <i>et al.</i> 1988 [2]	1
0.15 [11]	10 single	4 wk	lab stagnant microcosms	<i>No effect</i> pH, conductivity, alkalinity (day 30), microbial activity in the sediment, biomass of an alga species (day 30), biomass of macrophytes (day 30), survival of <i>Daphnia</i> <i>magna</i> <i>Decrease</i> gross primary production (slight)	-  7 d	Johnson 1986 [7]	2
0.15 [12]	10 constant	3 wk	recirculating artificial streams	<i>Decrease</i> gross primary production biovolume of periphyton on artificial substrate	>21 d >21 d	Kosinski 1984 Kosonski & Merkle 1984 [8]	4
0.22 [13]	15 constant	107 d	artificial flow- through swamp	<i>No effect</i> Chl-a and biomass of periphyton in bioassays, growth of two aquatic plants in bioassays, survival of <i>Daphnia</i> <i>magna</i> in bioassays, growth of fathead minnow and tadpoles <i>Decrease</i> DO, metabolism of periphyton in bioassays <i>Increase</i> nutrients	-  ?  ?	Detenbeck <i>et al.</i> 1996 [9]	4

**Table Ia. Continued** . Studies with single and multiple peak loads (applications) and constant exposure with **atrazine** in stagnant and closed running ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L.

Studied TU <sub>gsa</sub> [case number]	Concentration (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.30 [14]	20 multiple	1 yr	experimen- tal ponds	<i>No effect</i> isopods and dragonflies in bioassays, zooplankton community, biomass of four fish species after one year, snails in bioassays <i>Decrease</i> DO, pH <sup>14</sup> C-uptake and biomass phytoplankton cover by floating and submerged aquatic plants number of insects flying out biomass of tadpoles and number of young of a single fish species <i>Change</i> composition of phytoplankton species ( <i>Increase</i> in dinoflagellates)	-  7 d-<1 m 7-20 d > 1 yr ? >1yr  >136 d	DeNoyelles <i>et al.</i> 1982 Dewey 1986 Kettle <i>et al.</i> 1987 DeNoyelles <i>et al.</i> 1989 DeNoyelles <i>et al.</i> 1994 [10]	5
0.30 [15]	20 single	42 d	Lefler microcosms	<i>No effect</i> pH, primary production	-	Stay <i>et al.</i> 1989 [11]	1
0.36 [16]	24 constant	20 d	recirculating artificial streams	<i>No effect</i> uptake of phosphorous, silicium and nitrogen by periphyton <i>Decrease</i> Chl-a and biomass of periphyton	-  ?	Krieger <i>et al.</i> 1988 [12]	4
0.37 [17]	25 constant	8 wk	enclosures in a pond	<i>No effect</i> one alga species <i>Decrease</i> DO, conductivity (slight) <i>Increase</i> pH (slight) bloom of an alga species (but no dose-effect relationship) <i>nauplii of Copepoda, rotifers and egg production of</i> <i>Daphnia (slight, no clear dose-effect relationship)</i>	-  50->63 d  >63 d 21d  18-42 d	Jüttner <i>et al.</i> 1995 [3]	2

**Table Ia. Continued** . Studies with single and multiple peak loads (applications) and constant exposure with **atrazine** in stagnant and closed running ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L.

Studied TU <sub>gsa</sub> [case number]	Concentration (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.37 [18]	25 constant	107 d	artificial flow-through swamp	<i>No effect</i> Chl-a and biomass of periphyton in bioassays, growth of two aquatic plants in bioassays, survival of <i>Daphnia magna</i> in bioassays, growth of fathead minnow and tadpoles <i>Decrease</i> metabolism of periphyton in bioassays <i>Increase</i> nutrients	-  ? ?	Detenbeck <i>et al.</i> 1996 [9]	4
0.48 [19]	32 constant	3 wk	lab running microcosms with artificial substrates	<i>No effect</i> potassium, protein biomass, Chl-a of Protozoa <i>Decrease</i> DO magnesium, calcium (slight) <i>Increase</i> number of Protozoa species and (slight, no clear dose-effect relationship, not used by authors to calculate LOECs and NOECs)	-  ? ? ?	Pratt <i>et al.</i> 1988 [2]	4
0.75 [20]	50 single	4 m	experimen- tal ponds	<i>No effect</i> total biomass aquatic plants, zooplankton, survival and <i>Increase</i> of one fish species <i>Decrease</i> gross primary production and respiration Chl-a and POC phytoplankton (slight) <i>Change</i> <i>Chara</i> sp. replaces <i>Naja</i> sp.	-  2 wk 3 m  > 4 m	Fairchild <i>et al.</i> 1994 [13]	5
0.75 [21]	50 single	12 d	lab stagnant microcosms	<i>Decrease</i> Net O <sub>2</sub> -production (slight)	>12 d	Brockway <i>et al.</i> 1984 [1]	2
0.75 [22]	50 constant	10 wk	lab running microcosms	<i>Decrease</i> Net O <sub>2</sub> -production <i>Increase</i> Nitrate	1 d  2 d	Brockway <i>et al.</i> 1984 [1]	3

**Table Ia. Continued** . Studies with single and multiple peak loads (applications) and constant exposure with **atrazine** in stagnant and closed running ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L.

Studied TU <sub>gsa</sub> [case number]	Concentration (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.75 [23]	50 constant	107 d	artificial flow-through swamp	<i>No effect</i> Chl-a and biomass of periphyton in bioassays, growth of two aquatic plants in bioassays, survival of <i>Daphnia magna</i> in bioassays, growth of fathead minnow and tadpoles <i>Decrease</i> Metabolisms of periphyton in bioassays <i>Increase</i> nutrients	-  ? ?	Detenbeck <i>et al.</i> 1996 [9]	4
0.90 [24]	60 single	60 d	Taub microcosms	<i>Decrease</i> DO, <sup>14</sup> C-uptake, net primary production, respiration <sup>14</sup> C-uptake/Chl-a	20-27 d 53 d	Stay <i>et al.</i> 1985 [14]	3
1.1 [25]	75 constant	8 wk	enclosures in a pond	<i>No effect</i> One alga species, rotifers <i>Decrease</i> DO Conductivity (slight) Nauplii of Copedoda <i>Increase</i> pH (slight) bloom of an alga species (but no dose-effect relationship) egg production of <i>Daphnia</i> (slight, no clear dose-effect relationship)	-  >63 d 50 d 53 d  >63 d 46 d 35 d	Jüttner <i>et al.</i> 1995 [3]	5
1.1 [26]	75 constant	107 d	artificial flow-through swamp	<i>No effect</i> Chl-a and biomass of periphyton in bioassays, growth of two aquatic plants in bioassays, survival of <i>Daphnia magna</i> in bioassays, growth of fathead minnow and tadpoles <i>Decrease</i> Metabolism of periphyton in bioassays <i>Increase</i> nutrients	-  ? ?	Detenbeck <i>et al.</i> 1996 [9]	4

**Table Ia. Continued** . Studies with single and multiple peak loads (applications) and constant exposure with **atrazine** in stagnant and closed running ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L.

Studied TU <sub>gsa</sub> [case number]	Concentration (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
1.2 [27]	80 multiple	60 d	enclosures in a large lake	<i>Decrease</i> number, biomass and Chl-a of periphyton <i>Change</i> species composition of periphyton	49 d 223 d	Hamilton <i>et al.</i> 1987 [15]	5
1.5 [28]	100 multiple	1 yr	experimental ponds	<i>No effect</i> isopodes and dragonflies, zooplankton community, biomass of two fish species after one year, snails in bioassays <i>Decrease</i> DO, pH <sup>14</sup> C-uptake and biomass phytoplankton cover by emerged and submerged aquatic plants number of insects flying out biomass of tadpoles and two fish species	- ± 1 m 20 d >1 yr ? >1 yr	DeNoyelles <i>et al.</i> 1982 Dewey 1986 Kettle <i>et al.</i> 1987 DeNoyelles <i>et al.</i> 1989 DeNoyelles <i>et al.</i> 1994 [10]	5
1.5 [29]	100 single	60 d	Taub microcosms	<i>Decrease</i> DO, <sup>14</sup> C-uptake, net primary production, respiration <sup>14</sup> C-uptake/Chl-a	25-32 d 53 d	Stay <i>et al.</i> 1985 [14]	3
1.5 [30]	100 single	42 d	Lefler microcosms	<i>Decrease</i> pH, primary production	>42 d	Stay <i>et al.</i> 1989 [11]	4
1.5 [31]	100 constant	5 wk	running microcosms	<i>Decrease</i> primary production number of species, Chl-a and biomass of periphyton <i>Change</i> species composition of periphyton	20 d >20 d >20 d	Hamala & Kollig 1985 [16]	4
1.5 [32]	100 single	12 d	lab stagnant microcosms	<i>Decrease</i> net O <sub>2</sub> production	>7 d	Brockway <i>et al.</i> 1984 [1]	4
1.5 [33]	100 constant	10 wk	lab running microcosms	<i>Decrease</i> net O <sub>2</sub> production <i>Increase</i> nitrate	1 d 2 d	Brockway <i>et al.</i> 1984 [1]II	3



**Table Ia. Continued** . Studies with single and multiple peak loads (applications) and constant exposure with **atrazine** in stagnant and closed running ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L.

Studied TU <sub>gsa</sub> [case number]	Concentration (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
1.5 [34]	100 single	4 wk	lab stagnant microcosms	<i>No effect</i> pH, conductivity, alkalinity (day 30), microbial activity in sediment, biomass of an alga species (day 30), biomass of macrophytes (day 30), survival of <i>Daphnia magna</i> <i>Decrease</i> gross primary production (slight)	-  7 d	Johnson 1986 [7]	2
1.5 [35]	100 single	1 wk	recircula- ting artificial streams	<i>No effect</i> conductivity, alkalinity, soluble reactive phosphorous, respiration species composition of periphyton (study probably too short) <i>Decrease</i> pH, net primary production	-  7 d	Moorhead & Kosinski 1986 [17]	3
1.5 [36]	100 single	3 wk	recirculating artificial streams	<i>No effect</i> biovolume of periphyton on artificial substrate <i>Decrease</i> gross primary production	-  3 d	Kosinski 1984 Kosinski & Merkle 1984 [8]	3
1.6 [37]	110 constant	3 wk	lab running microcosms with artificial substrates	<i>No effect</i> potassium, calcium, number of species, protein biomass and Chl-a Protozoa <i>Decrease</i> DO magnesium (slight)	-  ? ?	Pratt <i>et al.</i> 1988 [2]	4
2.0 [38]	134 constant	20 d	recirculating artificial streams	<i>No effect</i> silicium uptake by periphyton <i>Decrease</i> uptake of phosphorous and nitrate by periphyton (slight) Chl-a and biomass of periphyton	-  ? ?	Krieger <i>et al.</i> 1988 [12]	4

**Table Ia. Continued** . Studies with single and multiple peak loads (applications) and constant exposure with **atrazine** in stagnant and closed running ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L.

Studied TU <sub>gsa</sub> [case number]	Concentration (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
2.1 [39]	140 multiple	60 d	enclosures in a large lake	<i>No effect</i> numbers, biomass, Chl-a and <sup>14</sup> C-uptake of periphyton <i>Change</i> species composition of periphyton	- >56 d	Hamilton <i>et al.</i> 1987 [15]	5
2.3 [40]	155 multiple	294 d	enclosures in a large lake	<i>No effect</i> sulphate, phosphorous, silicium, chloride, magnesium, potassium, rotifers <i>Decrease</i> DOC, POC (slight) DO, <sup>14</sup> C-uptake water <sup>14</sup> C-uptake periphyton Chl-a and biomass of periphyton numbers of one Cladocera, number of young of zooplankton (slight) <i>Increase</i> ammonium (light) nitrate, nitrite <i>Change</i> species composition phytoplankton, species composition periphyton	-  90 d 67-90 d 14 d >294 d 65-288 d  90 d >288 d  >294 d	Herman <i>et al.</i> 1986 Hamilton <i>et al.</i> 1988 Hamilton <i>et al.</i> 1989 [18]	5
3.0 [41]	200 multiple	1 yr	experimental ponds	<i>No effect</i> <sup>14</sup> C-uptake of phytoplankton, isopods and dragonflies in bioassays, zooplankton community, biomass of two fish species after one year, snails in bioassays <i>Decrease</i> DO, pH biomass phytoplankton cover by emerged and submerged aquatic plants number of insects flying out Biomass of tadpoles and two fish species	-  ± 1 m 20 d >1 yr ? > 1 yr	DeNoyelles <i>et al.</i> 1982 Dewey 1986 Kettle <i>et al.</i> 1987 DeNoyelles <i>et al.</i> 1989 DeNoyelles <i>et al.</i> 1994 [10]	5

**Table Ia. Continued** . Studies with single and multiple peak loads (applications) and constant exposure with **atrazine** in stagnant and closed running ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L.

Studied TU <sub>gsa</sub> [case number]	Concentration (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
3.0 [42]	200 constant	8 wk	enclosures in a pond	<i>No effect</i> rotifers <i>Decrease</i> DO conductivity (slight) alga species ( <i>Mallomonas</i> sp.) (slight) alga species ( <i>Cryptomonas</i> sp.) Nauplii of Copepoda <i>Increase</i> pH (slight) egg production of <i>Daphnia</i> (slight, no clear dose effect relationship)	-  >63 d 50 d 35 d >56 d 53 d  >63 d 35 d	Jüttner <i>et al.</i> 1995 [3]	5
3.0 [43]	200 single	60 d	Taub microcosms	<i>Decrease</i> DO, <sup>14</sup> C uptake net primary production, respiration, <sup>14</sup> C uptake/Chl-a	32-35 d >53 d	Stay <i>et al.</i> 1985 [14]	5
3.0 [44]	200 single	42 d	Lefler microcosms	<i>Decrease</i> pH, primary production	>42 d	Stay <i>et al.</i> 1989 [11]	4
5.0 [45]	337 constant	3 wk	lab running microcosms with artificial substrates	<i>Decrease</i> DO, K <sup>+</sup> magnesium, calcium (slight) number of species, protein biomass and Chl-a Protozoa	? ? ?	Pratt <i>et al.</i> 1988 [2]	4
5.4 [46]	360 constant	8 wk	enclosures in a pond	<i>Decrease</i> DO conductivity (slight) rotifers (slight) alga species ( <i>Mallomonas</i> sp.) (slight) alga species ( <i>Cryptomonas</i> sp.) Nauplii of Copepoda egg production of <i>Daphnia</i> (slight) <i>Increase</i> pH (slight)	>63 d 50 d 25d 35 d >56 d 53 d 39 d  >63 d	Jüttner <i>et al.</i> 1995 [3]	5

**Table Ia. Continued** . Studies with single and multiple peak loads (applications) and constant exposure with **atrazine** in stagnant and closed running ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L.

Studied TU <sub>gsa</sub> [case number]	Concentration (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference (study number)	Effect class sensitive endpoint
7.5 [47]	500 multiple	1 yr	experimental ponds	<i>No effect</i> isopods and dragonflies in bioassays, biomass of two fish species after one year, snails in bioassays <i>Decrease</i> DO, pH <sup>14</sup> C-uptake phytoplankton biomass phytoplankton number of one Copepoda species cover by emerged, floating and submerged aquatic plants number of insects flying out biomass of tadpoles and two fish species and number of young of one fish species <i>Change</i> species composition phytoplankton (and <i>Decrease</i> all important species)	-  ± 1 m 20-60 d 20-60 d ? >1 yr ? >1 yr  >63 d	DeNoyelles <i>et al.</i> 1982 Dewey 1986 Kettle <i>et al.</i> 1987 DeNoyelles <i>et al.</i> 1989 DeNoyelles <i>et al.</i> 1994 [10]	5
7.5 [48]	500 single	60 d	Taub microcosms	<i>Decrease</i> DO, <sup>14</sup> C-uptake, net primary production, respiration, <sup>14</sup> C- uptake/Chl-a	>53 d	Stay <i>et al.</i> 1985 [14]	5
7.5 [49]	500 single	42 d	Lefler microcosms	<i>Decrease</i> pH, primary production	>42 d	Stay <i>et al.</i> 1989 [11]	4
7.5 [50]	500 single	12 d	lab stagnant microcosms	<i>Decrease</i> net O <sub>2</sub> production	>12 d	Brockway <i>et al.</i> 1984 [1]	4
15 [51]	1000 single	60 d	Taub microcosms	<i>Decrease</i> DO, <sup>14</sup> C-uptake, net primary production, respiration, <sup>14</sup> C- uptake/Chl-a	>53 d	Stay <i>et al.</i> 1985 [14]	5
15 [52]	1000 single	42 d	Lefler microcosms	<i>Decrease</i> pH, primary production	>42 d	Stay <i>et al.</i> 1989 [11]	4
15 [53]	1000 single	4 w k	lab stagnant microcosms	<i>No effect</i> microbial activity in sediment, survival of <i>Daphnia magna</i> <i>Decrease</i> pH, gross primary production biomass of one alga species (day 30), biomass macrophytes (day 30) <i>Increase</i> pH, alkalinity (day 30)	-  >28 d >30 d  >28 d	Johnson 1986 [7]	4

**Table Ia. Continued** . Studies with single and multiple peak loads (applications) and constant exposure with **atrazine** in stagnant and closed running ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L.

Studied TU <sub>gsa</sub> [case number]	Concentration (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
15 [54]	1000 single	1 wk	recirculating artificial streams	<i>No effect</i> conductivity, alkalinity, soluble reactive phosphorous, respiration species composition periphyton (study probably too short) <i>Decrease</i> pH, net primary production	- - 7 d	Moorhead & Kosinski 1986 [17]	3
15 [55]	1000 single	3 wk	recirculating artificial streams	<i>Decrease</i> gross primary production biovolume periphyton on artificial substrate	> 21 d 14 d	Kosinski 1984 Kosinski & Merkle 1984 [8]	4
23 [56]	1560 multiple	60 d	enclosures in a large lake	<i>Decrease</i> number, biomass, Chl-a and <sup>14</sup> C-uptake of periphyton <i>Change</i> species composition periphyton	21-35 d >56 d	Hamilton <i>et al.</i> 1987 [15]	5
75 [57]	5000 single	60 d	Taub microcosms	<i>Decrease</i> DO, <sup>14</sup> C-uptake, net primary production, respiration, <sup>14</sup> C-uptake/Chl-a	>53 d	Stay <i>et al.</i> 1985 [14]	5
75 [58]	5000 single	42 d	Lefler microcosms	<i>Decrease</i> pH, primary production	>42 d	Stay <i>et al.</i> 1989 [11]	4
75 [59]	5000 single	12 d	lab stagnant microcosms	<i>Decrease</i> net O <sub>2</sub> production	>12 d	Brockway <i>et al.</i> 1984 [1]	4
149 [60]	10000 single	1 wk	recirculating artificial streams	<i>No effect</i> conductivity, alkalinity, soluble reactive phosphorous, respiration species composition periphyton (study probably too short) <i>Decrease</i> pH, net primary production	- - > 7 d	Moorhead & Kosinski 1986 [17]	4
149 [61]	10000 single	3 wk	recirculating artificial streams	<i>Decrease</i> gross primary production biovolume periphyton on artificial substrate	> 21 d > 21 d	Kosinski 1984 Kosinski & Merkle 1984 [8]	4

**Table Ib.** Multiple applications of **atrazine** in an open running ecosystem. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 67 µg/L. Exposure is two times 24 hours with an interval of 14 days. Observation duration concerns period after each treatment.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference (study number)	Effect class sensitive endpoint
<b>0.03</b> [1]	2	14 d	stream	<i>No effect</i> ash-free dry weight periphyton cell density algae	- -	Jurgensen & Hoagland 1990 [19]	1
<b>0.48</b> [2]	30	14 d	stream	<i>No effect</i> ash-free dry weight periphyton cell density algae	- -	Jurgensen & Hoagland 1990 [19]	1
<b>1.5</b> [3]	100	14 d	stream	<i>No effect</i> ash-free dry weight periphyton cell density algae	- -	Jurgensen & Hoagland 1990 [19]	1

## Appendix 2

Substance: **simazine**  
triazine

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	72	growth rate	350	-	Källqvist & Romstad 1994
	96	growth	1240	600	Fairchild <i>et al.</i> 1997
	96	?	100	-	Versteeg 1990
<i>Chlorella pyrenoidosa</i>	110	growth	-	52 <sup>1)</sup>	Foy & Hiranpradit 1977
<i>Chlamydomonas geitleri</i>	72	growth rate	863	-	François & Robinson 1990
	72	growth	1109	-	François & Robinson 1990

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (days)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
Lemna minor	4	density	166	75	Fairchild <i>et al.</i> 1997

1) quoted in Crommentuijn *et al.* 1997

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	351	600
<i>Chlorella pyrenoidosa</i>	-	52
<i>Chlamydomonas geitleri</i>	878	-
<i>Lemna spp.</i>	166	75

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
10	35.1	0.14

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UB criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 2.** Single peak loads **simazine** in stagnant ecosystems. UB criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 351 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.28 [1]	100	6 wk	enclosures in a swamp	<i>No effect</i> nitrate, silicium Chl-a and biovolume periphyton <sup>14</sup> C uptake periphyton <i>Decrease</i> DO <i>Change</i> species composition periphyton (slight)	- - - < 1 wk ± 2 wk	Goldsborough & Robinson 1983 Goldsborough & Robinson 1986 [21]	3
0.28 [2]	100	24 d	enclosures in a swamp	<i>No effect</i> DO silicium <i>Increase</i> ammonium (slight) phosphorous (slight)	- - ? ?	Goldsborough & Robinson 1985 [22]	2
0.28 [3]	100	21 d	bottles of natural water in a pond	<i>No effect</i> DO, pH, nitrate, ammonium cell density and species composition phytoplankton juvenile Copepoda numbers and species composition rotifers numbers of bacteria	- - - - -	Jenkins & Buikema 1990 [24]	1
1.4 [4]	500	21 d	bottles of natural water in a pond	<i>No effect</i> nitrate juvenile Copepoda numbers of bacteria <i>Decrease</i> DO, pH cell density phytoplankton <i>Increase</i> ammonium numbers and species composition rotifers <i>Change</i> species composition phytoplankton and rotifers	- - - ? ? ? ? ? ?	Jenkins & Buikema 1990 [24]	4



**Table 2. Continued – 1.** Single peak loads **simazine** in stagnant ecosystems. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 351 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
2.8 [5]	1000	6 wk	enclosures in a swamp	<i>Decrease</i> Chl-a and biovolume periphyton <sup>14</sup> C uptake periphyton DO <i>Increase</i> nitrate and silicium <i>Change</i> species composition periphyton	> 3 wk > 3 wk < 1 wk  < 1 wk  > 3 wk	Goldsborough & Robinson 1983 Goldsborough & Robinson 1986 [21]	4
2.8 [6]	1000	24 d	enclosures in a swamp	<i>Decrease</i> DO <i>Increase</i> silicium (slight) ammonium phosphorous (slight)	n.a.  n.a. ? ?	Goldsborough & Robinson 1985 [22]	4
2.8 [7]	1000	21 d	bottles of natural water in a pond	<i>No effect</i> juvenile Copepoda numbers of bacteria <i>Decrease</i> DO, pH, nitrate cell density phytoplankton <i>Increase</i> ammonium numbers and species composition of rotifers <i>Change</i> species composition periphyton	- -  ? ?  ? ?  ?	Jenkins & Buikema 1990 [24]	4
5.7 [8]	2000	86 d	enclosures in a swamp	<i>No effect</i> silicium cell density periphyton <i>Decrease</i> DO, <sup>14</sup> C uptake periphyton Chl-a (slight) biovolume periphyton, <sup>14</sup> C uptake benthic algae <i>Increase</i> ammonium and phosphorous <i>Change</i> species composition periphyton	- -  14 d 14 d > 80 d  14 d  > 80 d	Gurney & Robinson 1989 [23]	5

**Table 2. Continued .** Single peak loads **simazin** in stagnant ecosystems. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 351 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference (study number)	Effect class sensitive endpoint
14 [9]	5000	6 wk	enclosures in a swamp	<i>Decrease</i> Chl-a and biovolume periphyton <sup>14</sup> C uptake periphyton DO <i>Increase</i> nitrate and silicium <i>Change</i> species composition periphyton	>3 wk >3 wk <1 wk  <1 wk  >3 wk	Goldsborough & Robinson 1983 Goldborough & Robinson 1986 [21]	4
14 [10]	5000	24 d	enclosures in a swamp	<i>Decrease</i> DO <i>Increase</i> silicium (slight) ammonium phosphorous	n.a.  n.a. ? ?	Goldsborough & Robinson 1985 [22]	4

### Appendix 3

Substance: **terbutryn**  
triazine herbicide

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	96	growth	2.7	-	Gaggi <i>et al.</i> 1995
<i>Chlorella pyrenoidosa</i>	48	growth	6	-	Lefebvre-Drouet&Calvet 1978
<i>Chlamydomonas geitleri</i>	72	growth rate	4.8	-	François & Robinson 1990
	72	growth	7.2	-	François & Robinson 1990

Available laboratory toxicity data for duckweed (Lemnaceae): **none**.

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	2.7	-
<i>Chlorella pyrenoidosa</i>	6	-
<i>Chlamydomonas geitleri</i>	5.9	-

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
0.27	0.27	-

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UB criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 3a.** Single peak loads of **terbutryn** in stagnant ecosystems. UP criterion based on *Selenastrum capricornutum*: gg-EC<sub>50</sub> 2.7 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
2.2 [1]	6	2 m	enclosures in a fish pond	<i>No effect</i> turbidity, ammonia, nitrite, COD numbers and species composition of phytoplankton <i>Decrease</i> DO (slight) Chl-a	- - 8 wk 4 wk	Struve <i>et al.</i> 1991 [25]	3
3.7 [2]	10	6 wk	enclosures in a swamp	<i>Decrease</i> Chl-a and biovolume periphyton <sup>14</sup> C uptake periphyton DO <i>Increase</i> nitrate and silicium <i>Change</i> species composition periphyton	>3 wk 3 wk <1 wk  <1 wk  >3 wk	Goldsborough & Robinson 1983 Goldsborough & Robinson 1986 [21]	4
3.7 [3]	10	86 d	enclosures in a swamp	<i>No effect</i> silicium cell density periphyton <i>Decrease</i> DO, <sup>14</sup> C uptake periphyton Chl-a (slight) biovolume periphyton, <sup>14</sup> C uptake benthic algae <i>Increase</i> ammonium and phosphorous <i>Change</i> species composition periphyton	- - 21 d 21 d >80 d  21 d  >80 d	Gurney & Robinson 1989 [23]	5
4.4 [4]	12	2 m	enclosures in a fish pond	<i>No effect</i> ammonia, nitrite, COD numbers of phytoplankton <i>Decrease</i> DO turbidity Chl-a <i>Change</i> species composition phytoplankton	- - 8 wk ? 6 wk  ?	Struve <i>et al.</i> 1991 [25]	4

**Table 3a. Continued** . Single peak loads of **terbutryn** in stagnant ecosystems. UP criterion based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 2.7 µg/L

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
8.9 [5]	24	2 m	enclosures in a fish pond	<i>No effect</i> ammonia, nitrite, COD numbers of phytoplankton <i>Decrease</i> DO turbidity Chl-a <i>Change</i> species composition phytoplankton	- - 8 wk ? 6 wk ?	Struve <i>et al.</i> 1991 [25]	4
37 [6]	100	6 wk	enclosures in a swamp	<i>Decrease</i> Chl-a and biovolume periphyton <sup>14</sup> C uptake periphyton DO <i>Increase</i> nitrate and silicium <i>Change</i> species composition periphyton	>3 wk >3 wk <1 wk <1 wk >3 wk	Goldborough & Robinson 1983 Goldborough & Robinson 1986 [21]	4
370 [7]	1000	6 wk	enclosures in a swamp	<i>Decrease</i> Chl-a and biovolume periphyton <sup>14</sup> C uptake periphyton DO <i>Increase</i> nitrate and silicium <i>Change</i> species composition periphyton	> 3 wk > 3 wk <1 wk <1 wk >3 wk	Goldborough & Robinson 1983 Goldborough & Robinson 1986 [21]	4

**Table 3b.** Single peak loads of **terbutryn** in an open running ecosystem. UB criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 2.7 µg/L. Exposure duration is 24 hours.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
1.9 [1]	5	21 d	running system with aquatic plants	No effect periphyton on <i>Elodea</i> survival <i>Elodea canadensis</i>	- -	Paterson & Wright 1987 [38]	1
3.7 [2]	10	21 d	running system with aquatic plants	No effect periphyton on <i>Elodea</i> survival <i>Elodea canadensis</i>	- -	Paterson & Wright 1987 [38]	1
19 [3]	50	21 d	running system with aquatic plants	No effect periphyton on <i>Elodea</i> survival <i>Elodea canadensis</i>	- -	Paterson & Wright 1987 [38]	1

## Appendix 4

Substance: **hexazinone**  
triazinone

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	96	growth	24.5	-	St. Laurent <i>et al.</i> 1992
	120	growth rate	85	-	Abou-Waly <i>et al.</i> 1991

Available laboratory toxicity data for duckweed (Lemnaceae): **none**.

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	46	-

Summary criteria.

UB conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
2.5	4.6	-

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 4a.** Single peak loads of **hexazinone** in a stagnant ecosystem. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 46 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.22 [1]	10	77 d	enclosures in a lake	No effect numbers of zooplankton Decrease DO (slight) biomass phytoplankton (slight)	- 42 d 14 d	Thompson <i>et al.</i> 1993a Thompson <i>et al.</i> 1993b [28]	2
2.2 [2]	100	77 d	enclosures in a lake	Decrease DO biomass phytoplankton numbers of zooplankton	49 d >77 d <77 d	Thompson <i>et al.</i> 1993a Thompson <i>et al.</i> 1993b [28]	5
22 [3]	1000	77 d	enclosures in a lake	Decrease DO biomass phytoplankton numbers of zooplankton	49 d >77 d <77 d	Thompson <i>et al.</i> 1993a Thompson <i>et al.</i> 1993b [28]	5
217 [4]	10000	77 d	enclosures in a lake	Decrease DO biomass phytoplankton numbers of zooplankton	49 d >77 d <77 d	Thompson <i>et al.</i> 1993a Thompson <i>et al.</i> 1993b [28]	5



**Table 4b.** Single peak loads of **hexazinone** in an open running ecosystem. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 46 µg/L. Exposure was 12 hours or 1 hour.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
59 [1]	2700	3 d	lab running system	<i>No effect</i> Chl-a periphyton <i>Decrease</i> oxygen production periphyton (slight)	- 3 h	Kreutzweiser <i>et al.</i> 1995 [26]	2
59 [2]	2700	14 d	artificial streams outdoors	<i>No effect</i> Chl-a periphyton drift and density macroinvertebrates	- -	Kreutzweiser <i>et al.</i> 1995 [26]	1
200 [3]	9200	48 h	open artificial streams	<i>No effect</i> drift of some benthic insect larvae survival of some benthic insect larvae	- -	Kreutzweiser <i>et al.</i> 1992 [27]	1
1783 [4]	82000	48 h	open artificial streams	<i>No effect</i> survival of some benthic insect larvae <i>Increase</i> drift of some benthic insect larvae	? ?	Kreutzweiser <i>et al.</i> 1992 [27]	4

## Appendix 5

Substance: **linuron**  
urea

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (h)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Scenedesmus subspicatus</i>	?	?	16 <sup>1)</sup>	5.6 <sup>1)</sup>	IRC 1997
<i>Scenedesmus actus</i>	72	growth	6	1.2	Snel <i>et al.</i> 1998
<i>Selenastrum capricornutum</i>	?	?	70 <sup>1)</sup>	-	IRC 1997
<i>Chlorella vulgaris</i>	72-148	growth rate	50	-	Stephenson & Kane 1984
<i>Chlorella sp.</i>	96	growth	100 <sup>2)</sup>	50	Knauf & Schultze 1972
<i>Ankistrodesmus falcatus</i>	?	?	4.9 <sup>1)</sup>	2.5 <sup>1)</sup>	IRC 1997

1) from Crommentuijn *et al.* 1997

2) geometric average of NOEC and LOEC

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna minor</i>	5	growth rate	70	-	Stephenson & Kane 1984
	21	growth	10	-	Van den Brink pers. comm.

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Scenedesmus subspicatus</i>	16	5.6
<i>Scenedesmus acutus</i>	6	1.2
<i>Selenastrum capricornutum</i>	70	-
<i>Chlorella spp.</i>	71	50
<i>Ankistrodesmus falcatus</i>	4.9	2.5
<i>Lemna spp.</i>	26	-

Summary criteria.

UB conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
1.6	1.6	0.25

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UB criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997



**Table 5. Continued.** Studies conducted with single peak loads and constant exposure of **linuron** in stagnant ecosystems. UP criterion based on *S. subspicatum*: gm-EC<sub>50</sub> 16 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
3.1 [4]	50 constant	5 wk	lab stagnant microcosms	<i>No effect</i> potassium, calcium, nitrate, sodium, ammonium, phosphorous; Chl-a phytoplankton; numbers of Copepoda; numbers of arthropods and molluscs decomposition organic matter <i>Decrease</i> DO, pH growth and final weight <i>Elodea</i> in bioassays numbers of rotifers, Ostracoda <i>Increase</i> conductivity, alkalinity Chl-a periphyton numbers of Cladocera <i>Change</i> species composition phytoplankton species composition periphyton	- - - - >5 wk >5 wk >5 wk >5 wk ? 5 wk ? >5 wk	Van den Brink <i>et al.</i> 1997 Cuppen <i>et al.</i> 1997 [30]	4
9.4 [5]	150 constant	5 wk	lab stagnant microcosms	<i>No effect</i> sodium, ammonium, phosphorous decomposition organic substance <i>Decrease</i> DO, pH growth and final weight <i>Elodea</i> in bioassays numbers of rotifers, Ostracoda numbers of arthropods and molluscs <i>Increase</i> conductivity, alkalinity, potassium, calcium, nitrate Chl-a phytoplankton Chl-a periphyton numbers of Cladocera and Copepoda <i>Change</i> species composition phytoplankton species composition periphyton	- - >5 wk >5 wk >5 wk >5 wk >5 wk <5 wk ? 5 wk >5 wk >5 wk	Van den Brink <i>et al.</i> 1997 Cuppen <i>et al.</i> 1997 [30]	4
63 [6]	1000 single	42 d	enclosures in a pond	<i>No effect</i> Chl-a phytoplankton numbers of molluscs and oligochates <i>Decrease</i> DO, pH quantity of macrophytes numbers of Cladocera and Copepoda numbers of arthropods <i>Increase</i> Alkalinity	- - >42 d >28 d >42 d >49 d >42 d	Stephenson & Kane 1984 [29]	4

## Appendix 6

Substance: **isoproturon**  
urea

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Scenedesmus subspicatus</i>	96	growth	21	-	Kirby & Sheahan 1994
<i>Chlorella pyrenoidosa</i>	48	growth	40	-	Lefebvre-Drouet&Calvet 1978
<i>Chlamydomonas reinhardi</i>	72	?	40	-	Traunspurger <i>et al.</i> 1996

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna minor</i>	10	growth	33	-	Kirby & Sheahan 1994

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Scenedesmus subspicatus</i>	21	-
<i>Chlorella pyrenoidosa</i>	40	-
<i>Chlamydomonas reinhardi</i>	40	-
<i>Lemna spp.</i>	33	-

Summary criteria.

UB conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
2.1	2.1	0.32

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UB criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 6.** Studies conducted with single peak loads and constant exposure to **isoproturon** in stagnant ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 21 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.10 [1]	2 single	3 wk	lab stagnant microcosms	No effect biomass <i>Elodea densa</i> biomass <i>Ludwigia natans</i> Decrease DO (slight)	- - >21 d	Feurtet-Mazel <i>et al.</i> 1996 [31]	2
0.29 [2]	6 single	71 d	lab stagnant microcosms	Decrease density and species composition Bacillariophyta within periphyton	71 d	Pèrès <i>et al.</i> 1996 [32]	3
0.38 [3]	8 constant via sediment	71 d	lab stagnant microcosms	Decrease density and species composition Bacillariophyta within periphyton	71 d	Pèrès <i>et al.</i> 1996 [32]	3
0.43 [4]	9 single	3 wk	lab stagnant microcosms	No effect biomass <i>Ludwigia natans</i> Decrease DO biomass <i>Elodea densa</i>	- >21 d >21 d	Feurtet-Mazel <i>et al.</i> 1996 [31]	4
0.48 [5]	10 single	56 d	lab stagnant microcosms	No effect production of carbon dioxide & methane sediment density and species composition phytoplankton numbers and species composition zooplankton numbers of nematodes, Phyllopora numbers of snails and <i>Puntius</i> (fish) in cages	- - - - -	Traunsprunger <i>et al.</i> 1996 [42]	1
1.0 [6]	21 constant via sediment	71 d	lab stagnant microcosms	Decrease density and species composition Bacillariophyta within periphyton	>71 d	Pèrès <i>et al.</i> 1996 [31]	5
1.4 [7]	30 single	56 d	lab stagnant microcosms	No effect production of carbon dioxide & methane sediment density and species composition phytoplankton numbers and species composition zooplankton numbers of nematodes, Phyllopora numbers of snails and <i>Puntius</i> (fish) in cages	- - - - -	Traunsprunger <i>et al.</i> 1996 [42]	1
1.5 [8]	31 single	3 wk	lab stagnant microcosms	No effect biomass <i>Ludwigia natans</i> Decrease DO biomass <i>Elodea densa</i>	- >21 d >21 d	Feurtet-Mazel <i>et al.</i> 1996 [31]	4

**Table 6. Continued.** Studies conducted with single peak loads and constant exposure to **isoproturon** in stagnant ecosystems. UP criterion is based on *Scenedesmus subspicatus*: gm-EC<sub>50</sub> 21 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
1.7 [9]	35 constant via sediment	71 d	lab stagnant microcosms	<i>Decrease</i> density and species composition Bacillariophyta within periphyton	>71 d	Péres <i>et al.</i> 1996 [32]	5
3.0 [10]	62	3 wk	lab stagnant microcosms	<i>No effect</i> biomass <i>Ludwigia natans</i> <i>Decrease</i> DO biomass <i>Elodea densa</i>	- >9 d >21 d	Feurtet-Mazel <i>et al.</i> 1996 [31]	4
4.3 [11]	90	56 d	lab stagnant microcosms	<i>No effect</i> density and species composition phytoplankton numbers and species composition zooplankton numbers of nematodes, Phyllopoda numbers of snails and <i>Puntius</i> (fish) in cages <i>Decrease</i> production of carbon dioxide & methane sediment	- - - - >56 d	Traunsprunger <i>et al.</i> 1996 [42]	5
4.8 [12]	100	3 wk	lab stagnant microcosms	<i>No effect</i> biomass <i>Ludwigia natans</i> <i>Decrease</i> DO biomass <i>Elodea densa</i>	- >21 d >21 d	Feurtet-Mazel <i>et al.</i> 1996 [31]	4
6.0 [13]	125	3 wk	lab stagnant microcosms	<i>Decrease</i> DO biomass <i>Ludwigia natans</i> biomass <i>Elodea densa</i>	>21 d >21 d >21 d	Feurtet-Mazel <i>et al.</i> 1996 [31]	4
12 [14]	250	3 wk	lab stagnant microcosms	<i>Decrease</i> DO biomass <i>Ludwigia natans</i> biomass <i>Elodea densa</i>	>21 d >21 d >21 d	Feurtet-Mazel <i>et al.</i> 1996 [31]	4
24 [15]	500	3 wk	lab stagnant microcosms	<i>Decrease</i> DO biomass <i>Ludwigia natans</i> biomass <i>Elodea densa</i>	>21 d >21 d >21 d	Feurtet-Mazel <i>et al.</i> 1996 [31]	4
48 [16]	1000	3 wk	lab stagnant microcosms	<i>Decrease</i> DO biomass <i>Ludwigia natans</i> biomass <i>Elodea densa</i>	>21 d >21 d >21 d	Feurtet-Mazel <i>et al.</i> 1996 [31]	4

## Appendix 7

Substance: **diuron**  
urea

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Scenedesmus subspicatus</i>	72	growth	72	10	Schäfer <i>et al.</i> 1994
	72	growth rate	36	5	Tamerus 1996
<i>Scenedesmus parvus</i>	72	growth rate	27	7	Tamerus 1996
<i>Selenastrum capricornutum</i>	72	growth rate	15	3	Tamerus 1996
<i>Chlorella pyrenoidosa</i>	72	growth	-	2.3 <sup>1)</sup>	Davis <i>et al.</i> 1976
	96	growth	25	-	Maule & Wright 1984

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna major</i>	7	number	41	2.3 <sup>1)</sup>	Liu & Cedeño-Maldonado 1974
<i>Lemna gibba</i>	14	biomass	±23	-	Wejnar <i>et al.</i> 1992
<i>Lemna perpusila</i>	7	number	15	2.3 <sup>1)</sup>	Liu & Cedeño-Maldonado 1974

1) derived from data

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Scenedesmus subspicatus</i>	51	7
<i>Scenedesmus parvus</i>	27	7
<i>Selenastrum capricornutum</i>	15	3
<i>Chlorella pyrenoidosa</i>	25	2.3
<i>Lemna spp.</i>	24	2.3

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
1.5	1.5	0.43

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UP criterion based on gg-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997



**Table 7.** Single peak loads of **diuron** in a stagnant ecosystem. UP criterion is based on *Selenestrum capricornutum*:: gm-EC<sub>50</sub> 15 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
<b>0.02</b> [1]	0.29	42 d	lab stagnant microcosms	<i>No effect</i> DO	-	Flum & Shannon 1987 [20]	1
<b>0.19</b> [2]	2.9	42 d	lab stagnant microcosms	<i>No effect</i> DO	-	Flum & Shannon 1987 [20]	1
<b>1.9</b> [3]	28.5	42 d	lab stagnant microcosms	<i>Decrease</i> DO	?	Flum & Shannon 1987 [20]	4
<b>19</b> [4]	285	42 d	lab stagnant microcosms	<i>Decrease</i> DO	?	Flum & Shannon 1987 [20]	4
<b>190</b> [5]	2850	42 d	lab stagnant microcosms	<i>Decrease</i> DO	>42 d	Flum & Shannon 1987 [20]	4

## Appendix 8

Substance: **2,4-D**  
phenoxy acetic acid

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	96	growth	25 900	-	St. Laurent <i>et al.</i> 1992 Fairchild <i>et al.</i> 1997
		growth	41 772	25 000	
<i>Chlorella pyrenoidosa</i>	120	growth	28 288	-	Gramlich & Frans 1964
<i>Chlamydomonas reinhardi</i>	192	growth rate	36 286	-	Wong & Chang 1988

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna minor</i>	4	density	>100 000 - 200 000 <sup>1)</sup> -	-	Fairchild <i>et al.</i> 1997 Taraldsen & Norberg-King 1990
	4	density			

1) 0.5 times ChV (geometric average of NOEC and LOEC)

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	32 892	-
<i>Chlorella pyrenoidosa</i>	28 288	-
<i>Chlamydomonas reinhardi</i>	36 286	-
<i>Lemna spp.</i>	200 000	-

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
2590	3289	10

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997



**Table 8. Continued.** Single peak loads of **2,4-D** in stagnant ecosystems. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 32 892 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.030 [5]	1000	338 d	experimental ponds	<i>No effect</i> turbidity, DO, alkalinity, POC ammonia, nitrogen, phosphorous, silicium <i>Decrease</i> carbon uptake <i>Myriophyllum</i> stems density <i>Elodea</i> , <i>Potamogeton</i> , <i>Typha</i> , <i>Myriophyllum</i> diversity macroinvertebrates <i>Increase</i> DOC, nitrate density <i>Chara</i> <i>Change</i> species composition micro-organisms	- - >20 d >57 d >338 d ? ? 114 d	Scott <i>et al.</i> 1981 Stephenson & Mackie 1986 Sherry 1994 [44]	5
0.061 [6]	2000	8 d	lake, field	<i>No effect</i> pH, alkalinity Chl-a phytoplankton <i>Decrease</i> DO (slight) gross primary production and respiration, density of macrophytes <i>Increase</i> nitrate, phosphorous density and biovolume phytoplankton	- - 5 d >7 d  >8 d ?	Kobriæ & White 1996 [35]	4
0.061 [7]	2000	8 d	bottles of lake water	<i>No effect</i> DO, pH, alkalinity nitrate, phosphorous respiration Chl-a phytoplankton density and biovolume phytoplankton <i>Increase</i> gross primary production	- - - - - ?	Kobriæ & White 1996 [35]	1
0.30 [8]	10 000	8 d	bottles of lake water	<i>No effect</i> DO, pH, alkalinity nitrate, phosphorous gross primary production and respiration Chl-a phytoplankton density and biovolume phytoplankton	- - - - -	Kobriæ & White 1996 [35]	1

**Table 8. Continued .** Single peak loads of 2,-D in stagnant ecosystems. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 32 892 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
1.2 [9]	40 000	8 d	bottles of lake water	<i>No effect</i> pH, alkalinity nitrate, phosphorous gross primary production and respiration Chl-a phytoplankton density and biovolume phytoplankton <i>Decrease</i> DO (slight)	- - - - - ?	Kobriæ & White 1996 [35]	2
3.0 [10]	100 000	8 d	bottles of lake water	<i>No effect</i> alkalinity nitrate, phosphorous respiration <i>Decrease</i> DO, pH gross primary production Chl-a phytoplankton density and biovolume phytoplankton	- - - - >8 d >8 d >8 d	Kobriæ & White 1996 [35]	4
30 [11]	100 000	8 d	bottles of lake water	<i>No effect</i> alkalinity nitrate, phosphorous <i>Decrease</i> DO, pH gross primary production and respiration Chl-a phytoplankton density and biovolume phytoplankton	- - - >8 d >7 d >8 d >8 d	Kobriæ & White 1996 [35]	4

## Appendix 9

Substance: **2,4,5-T**  
phenoxy acetic acid

Available laboratory toxicity data for standard algae (Chlorophyta and Cyanophyta)

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Scenedesmus quadricauda</i>	192	growth	-	>220 000	Bringman & Kühn 1978
<i>Microcystis aeruginosa</i> (Cyanophyta)	192	growth	-	52 000	Bringman & Kühn 1978

Available laboratory toxicity data for duckweed (Lemnaceae): **none**.

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC50 (µg/L)	gm-NOEC (µg/L)
<i>Scenedesmus quadricauda</i>	-	>220 000
<i>Microcystis aeruginosa</i> (Cyanophyta)	-	52 000

Summary criteria.

Up conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
-	-	9

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 9.** Single peak loads of **2,4,5-T** in a stagnant ecosystem. No toxicity data on algae found.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
- [1]	100	25 d	lab stagnant microcosms	<i>No effect</i> Primary production and respiration density phytoplankton density rotifers density oligochaetes density micro-organisms	- - - - -	Sugiura 1992 [46]	1
- [2]	1000	25 d	lab stagnant microcosms	<i>No effect</i> Primary production and respiration density phytoplankton density rotifers density oligochaetes density micro-organisms	- - - - -	Sugiura 1992 [46]	1
- [3]	10000	25 d	lab stagnant microcosms	<i>No effect</i> Primary production and respiration density phytoplankton density rotifers density oligochaetes density micro-organisms	- - - - -	Sugiura 1992 [46]	1
- [4]	100000	25 d	lab stagnant microcosms	<i>No effect</i> respiration density phytoplankton density rotifers density oligochaetes <i>Decrease</i> primary production <i>Chlorella</i> density micro-organisms (afterwards <i>Increase</i> )	- - - - - 14 d 21 d >25 d	Sugiura 1992 [46]	4

## Appendix 10

Substance: **picloram**  
pyridine

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	96	growth	21 700	-	St. Laurent <i>et al.</i> 1992

Available laboratory toxicity data for duckweed (Lemnaceae): **none**.

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	21 700	-

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
2170	2170	-

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997



**Table 10.** Single peak loads of **picloram** in a stagnant ecosystem. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 21 700 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
<b>0.00046</b> [1]	10	60 d	enclosures in a pond	<i>No effect</i> survival and weight <i>Potamogeton pectinatus</i> survival and weight <i>Myriophyllum sibiricum</i>	- -	Forsyth <i>et al.</i> 1997 [34]	1
<b>0.0046</b> [2]	100	60 d	enclosures in a pond	<i>No effect</i> survival and weight <i>Potamogeton pectinatus</i> survival and weight <i>Myriophyllum sibiricum</i>	- -	Forsyth <i>et al.</i> 1997 [34]	1

## Appendix 11

Substance: **clopyralid**  
pyridine

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	96	cell density	6 900	-	Tomlin 1994
		cell volume	7 300	-	Tomlin 1994

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna gibba</i>	4	?	89 000	-	Tomlin 1994

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	7 097	-
<i>Lemna spp.</i>	89 000	-

Summary criteria.

UB conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
690	710	-

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UB criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 11.** Single peak loads of **clopyralid** in a stagnant ecosystem. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 7 097 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
<b>0.0014</b> [1]	10	60 d	enclosures in a pond	<i>No effect</i> survival and weight <i>Potamogeton pectinatus</i> survival <i>Myriophyllum sibiricum</i> <i>Increase</i> weight <i>Myriophyllum sibiricum</i>	- - >60 d	Forsyth <i>et al.</i> 1997 [34]	1
<b>0.014</b> [2]	100	60 d	enclosures in a pond	<i>No effect</i> survival and weight <i>Potamogeton pectinatus</i> survival and weight <i>Myriophyllum sibiricum</i>	- -	Forsyth <i>et al.</i> 1997 [34]	1

## Appendix 12

Substance: **trichlopyr**  
pyridiloxy acetic acid

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	120	?	45 000	-	Tomlin 1994

Available laboratory toxicity data for duckweed (Lemnaceae): **none**.

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	45 000	-

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
4 500	4 500	-

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 12.** Single peak loads of **trichlopyr** in an open running ecosystem. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 45 000 µg/L. Exposure duration was one hour.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.0071 [1]	320	48 h	open artificial streams	<i>No effect</i> drift of (some) benthic insect larvae survival of (some) benthic insect larvae	- -	Kreutzweiser <i>et al.</i> 1992 [27]	1
0.071 [2]	3 200	48 h	open artificial streams	<i>Decrease</i> survival of (some) benthic insect larvae <i>Increase</i> drift of (some) benthic insect larvae	? ?	Kreutzweiser <i>et al.</i> 1992 [27]	4
0.71 [3]	32 000	48 h	open artificial streams	<i>Decrease</i> survival of (some) benthic insect larvae <i>Increase</i> drift of (some) benthic insect larvae	? ?	Kreutzweiser <i>et al.</i> 1992 [27]	4
7.1 [4]	320 000	48 h	open artificial streams	<i>Decrease</i> survival of (some) benthic insect larvae <i>Increase</i> drift of (some) benthic insect larvae	? ?	Kreutzweiser <i>et al.</i> 1992 [27]	4

## Appendix 13

Substance: **diquat (dibromide)**  
bipyridilium

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	96	growth	34.2	-	St. Laurent <i>et al.</i> 1992
	96	growth	80	44	Fairchild <i>et al.</i> 1997

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna minor</i>	4	density	18	<11	Fairchild <i>et al.</i> 1997

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	52	44
<i>Lemna spp.</i>	18	<11

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
3.4	5.2	-

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 13a.** Chronic studies and single peak loads of **diquat** in stagnant ecosystems. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 52 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
5.8 [1]	300 single + constant	4 m	lab stagnant microcosms	<i>Decrease</i> DO, pH numbers of Cryptophyta survival <i>Elodea canadensis</i> numbers of <i>Alona costata</i> total density Cladocera numbers of nauplii, copepodits and fully grown Copepoda <i>Increase</i> conductivity numbers of Conjugophyta numbers of <i>Simocephalus vetulus</i> total density algae	? >2½ m >4 m ? ? >2½ m  2½ m >2½ m ? ?	Draxl <i>et al.</i> 1991 [36]	5
16 [2]	850 single	3 wk	lab microcosms with artificial substrate	<i>No effect</i> DO biomass protein periphyton <i>Decrease</i> number of species of Protozoa periphyton	- - >3 wk	Pratt <i>et al.</i> 1990 [45]	4
19 [3]	1000 single + constant	4 m	lab stagnant microcosms	<i>Decrease</i> DO, pH numbers of Cryptophyta survival <i>Elodea canadensis</i> numbers of <i>Alona costata</i> total density Cladocera numbers of nauplii, copepodits and fully grown Copepoda <i>Increase</i> conductivity numbers of Conjugophyta numbers of <i>Simocephalus vetulus</i> total density algae	? >4 m >4 m ? ? >4 m  4 m 4 m ? ?	Draxl <i>et al.</i> 1991 [36]	5
38 [4]	2000 single	3 wk	lab microcosms with artificial substrate	<i>No effect</i> biomass protein periphyton <i>Decrease</i> DO number of species of Protozoa periphyton	- >3 wk >3 wk	Pratt <i>et al.</i> 1990 [45]	4

**Table 13a. Continued .** Chronic studies and single peak loads of **diquat** in stagnant ecosystems. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 52 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
67 [5]	3500 single	23 d	lab microcosms with artificial substrate	<i>Decrease</i> gross productivity biomass protein periphyton (at low nutrient levels) <i>Increase</i> nitrate phosphorous	>23 d >23 d ?	Barreiro Lozano & Pratt 1994 [37]	4
90 [6]	4700 single	3 wk	Lab microcosms with artificial substrate	<i>Decrease</i> DO biomass protein periphyton number of species Protozoa periphyton	>3 wk 2 wk >3 wk	Pratt <i>et al.</i> 1990 [45]	4
288 [7]	15 000 single	3 wk	Lab microcosms with artificial substrate	<i>Decrease</i> DO biomass protein periphyton number of species Protozoa periphyton	>3 wk 2 wk >3 wk	Pratt <i>et al.</i> 1990 [45]	4
827 [8]	43 000 single	3 wk	Lab microcosms with artificial substrate	<i>Decrease</i> DO biomass protein periphyton number of species Protozoa periphyton	>3 wk >3 wk >3 wk	Pratt <i>et al.</i> 1990 [45]	4



**Table 13b.** Single peak loads of **diquat** in an open running ecosystem. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 52 µg/L. Exposure duration 24 hours.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference (study number)	Effect class sensitive endpoint
<b>0.10</b> [1]	5	21 d	running system with aquatic plants	<i>No effect</i> survival <i>Elodea canadensis</i> <i>Increase</i> periphyton on <i>Elodea</i>	-  >14 d	Paterson & Wright 1987 [38]	1
<b>0.19</b> [2]	10	21 d	running system with aquatic plants	<i>No effect</i> survival <i>Elodea canadensis</i> <i>Increase</i> periphyton on <i>Elodea</i>	-  >21 d	Paterson & Wright 1987 [38]	1
<b>0.96</b> [3]	50	21 d	running system with aquatic plants	<i>No effect</i> survival <i>Elodea canadensis</i> <i>Increase</i> periphyton on <i>Elodea</i>	>21 d  >14 d	Paterson & Wright 1987 [38]	4

## Appendix 14

Substance: **paraquat (dichloride)**  
bipyridilium

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	96	growth	559	114	Fairchild <i>et al.</i> 1997
<i>Chlamydomonas eugametos</i>	48	growth	116	-	Hess 1980

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna minor</i>	4	density	51	14	Fairchild <i>et al.</i> 1997
<i>Lemna gibba</i>	20	biomass	-	2.6	Wejnar <i>et al.</i> 1992

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	559	114
<i>Chlamydomonas eugametos</i>	116	-
<i>Lemna spp.</i>	51	6

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
56	56	-

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 14.** Single peak loads of **paraquat** in a closed running ecosystem. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 559 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
<b>0.018</b> [1]	10 constant	3 wk	recirculating artificial streams	<i>No effect</i> gross primary production biovolume periphyton on artificial substrate	- -	Kosinski 1984 Kosinski & Merkle 1984 [8]	1
<b>0.18</b> [2]	100 single	3 wk	recirculating artificial streams	<i>No effect</i> gross primary production biovolume periphyton on artificial substrate	- -	Kosinski 1984 Kosinski & Merkle 1984 [8]	1
<b>1.8</b> [3]	1000 single	3 wk	recirculating artificial streams	<i>No effect</i> gross primary production biovolume periphyton on artificial substrate	- -	Kosinski 1984 Kosinski & Merkle 1984 [8]	1
<b>18</b> [4]	10 000 single	3 wk	recirculating artificial streams	<i>No effect</i> biovolume periphyton on artificial substrate <i>Decrease</i> gross primary production	- 7 d	Kosinski 1984 Kosinski & Merkle 1984 [8]	3

## Appendix 15

Substance: **fluridon**  
4-pyridon

Available laboratory toxicity data for algae.

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Chlamidomonas eugametos</i> (Chlorophyta)	48	growth	1482 <sup>1)</sup>	329 <sup>1)</sup>	Hess 1980
<i>Oscillatoria agardhii</i> (Cyanophyta)	96	growth	74 <sup>2)</sup>	9.1 <sup>2)</sup>	Millie <i>et al.</i> 1990

1) deduced from raw data

2) deduced by means of DEBtox

Available laboratory toxicity data for duckweed (Lemnaceae): **none**.

Summary of available laboratory toxicity data for algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Chlamidomonas eugametos</i>	1482	-
<i>Oscillatoria agardhii</i>	74	9.1

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
7.4	7.4	-

conservative UP criterion based on lowest EC<sub>50</sub> for algae (due to the absence of toxicity data for standard algae)

liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive algae (due to the absence of toxicity data for standard algae)

MPC for freshwater from Crommentuijn *et al.* 1997

**Table 15.** Single peak loads of fluridon in a stagnant ecosystem. UP criterion is based on *Oscillatoria agardhii*: gm-EC<sub>50</sub> 74 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
1.7  [1]	125	2 m	enclosures in a fish pond	<i>No effect</i> DO, turbidity, COD ammonia, nitrate, nitrite, phosphorous, species composition phytoplankton <i>Decrease</i> density phytoplankton Chl-a phytoplankton	- - - 5 wk 5 wk	Struve <i>et al.</i> 1991 [25]	3

## Appendix 16

Substance: **metsulphuron-methyl**  
sulphonylurea

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	96	growth	190	<19	Fairchild <i>et al.</i> 1997

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna minor</i>	4	density	0.4	<0.2	Fairchild <i>et al.</i> 1997

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	190	<19
<i>Lemna spp.</i>	0.4	<0.2

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
19	19	-

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UP criterion based on gg-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 16.** Single peak loads of **metsulfuron-methyl** in a stagnant ecosystem. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 190 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
<b>0.053</b>	10	77 d	enclosures in a lake	<i>No effect</i> biomass phytoplankton numbers of zooplankton <i>Decrease</i> DO (slight)	- - >77 d	Thompson <i>et al.</i> 1993a Thompson <i>et al.</i> 1993b [28]	2
<b>0.53</b>	100	77 d	enclosures in a lake	<i>No effect</i> biomass phytoplankton numbers of zooplankton <i>Decrease</i> DO (slight)	- - >77 d	Thompson <i>et al.</i> 1993a Thompson <i>et al.</i> 1993b [28]	2
<b>2.6</b>	500	77 d	enclosures in a lake	<i>No effect</i> biomass phytoplankton numbers of zooplankton <i>Decrease</i> DO (slight)	- - >77 d	Thompson <i>et al.</i> 1993a Thompson <i>et al.</i> 1993b [28]	2
<b>5.3</b>	1000	77 d	enclosures in a lake	<i>No effect</i> biomass phytoplankton numbers of zooplankton <i>Decrease</i> DO (slight)	- - >35 d	Thompson <i>et al.</i> 1993a Thompson <i>et al.</i> 1993b [28]	2

## Appendix 17

Substance: **alachlor**  
anilid

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	96	growth	6	4	Fairchild <i>et al.</i> 1997
<i>Chlorella pyreniodosa</i>	24	growth	1430	-	Hawxby <i>et al.</i> 1977
<i>Chlamydomonas euglametos</i>	48	growth	110 <sup>1)</sup>	30 <sup>1)</sup>	Hess 1980

1) derived from raw data

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna minor</i>	4	density	198	32	Fairchild <i>et al.</i> 1997

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	6	4
<i>Chlorella pyreniodosa</i>	1430	-
<i>Chlamydomonas euglametos</i>	121	-
<i>Lemna spp.</i>	198	32

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
0.6	0.6	-

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997



**Table 17.** Single peak loads of **alachlor** in a closed running ecosystem. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 6 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.17 [1]	1	21 d	recirculating artificial streams	<i>No effect</i> DO nitrogen, phosphorous Chl-a periphyton on artificial substrate density periphyton on artificial substrate <i>Change</i> species composition periphyton on artificial substrate	- - - - >21 d	Spawn <i>et al.</i> 1997 [39]	4
1.7 [2]	10	21 d	recirculating artificial streams	<i>No effect</i> DO nitrogen, phosphorous <i>Decrease</i> Chl-a periphyton on artificial substrate density periphyton on artificial substrate <i>Change</i> species composition periphyton on artificial substrate	- - >21 d 14 d >21 d	Spawn <i>et al.</i> 1997 [39]	4
5.0 [3]	30	21 d	recirculating artificial streams	<i>No effect</i> DO nitrogen, phosphorous <i>Decrease</i> Chl-a periphyton on artificial substrate density periphyton on artificial substrate <i>Change</i> species composition periphyton on artificial substrate	- - >21 d 14 d >21 d	Spawn <i>et al.</i> 1997 [39]	4
17 [4]	100	21 d	recirculating artificial streams	<i>No effect</i> DO nitrogen, phosphorous <i>Decrease</i> Chl-a periphyton on artificial substrate density periphyton on artificial substrate <i>Change</i> species composition periphyton on artificial substrate	- - >21 d >21 d >21 d	Spawn <i>et al.</i> 1997 [39]	4
167 [5]	1000	21 d	recirculating artificial streams	<i>No effect</i> DO nitrogen, phosphorous <i>Decrease</i> Chl-a periphyton on artificial substrate density periphyton on artificial substrate <i>Change</i> species composition periphyton on artificial substrate	- - >21 d >21 d >21 d	Spawn <i>et al.</i> 1997 [39]	4

## Appendix 18

Substance: **triallate**  
thiocarbamate

Available laboratory toxicity data for test organisms.

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	96	growth	47	12.5	Fairchild <i>et al.</i> 1997
<i>Daphnia magna</i>	?	?	57	-	Johnson 1986

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna minor</i>	4	density	>10 000	-	Fairchild <i>et al.</i> 1997

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	47	12.5
<i>Lemna spp.</i>	>10 000	-

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
0.57	0.57	1.9

conservative UP criterion based on lowest EC<sub>50</sub> for *Daphnia*

liberal UP criterion based on gm-EC<sub>50</sub> for *Daphnia*

MPC for freshwater from Crommentuijn *et al.* 1997

**Table 18.** Single peak loads of **trilialate** in a stagnant ecosystem. UP criterion is based on *Daphnia magna*: gm-EC<sub>50</sub> 57 µg/L. For comparison with other studies the EC<sub>50</sub> of *Selenastrum capricornutum* was used: gm-EC<sub>50</sub> 47 µg/L

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.21 [1]	10	28 d	lab stagnant microcosms	<i>No effect</i> pH, alkalinity, conductivity gross primary production microbial activity in sediment biomass <i>Selenastrum capricornutum</i> biomass macrophytes <i>Decrease</i> survival <i>Daphnia magna</i>	- - - - - - >3 d	Johnson 1986 [7]	4
2.1 [2]	100	28 d	lab stagnant microcosms	<i>No effect</i> pH, alkalinity, conductivity microbial activity in sediment biomass macrophytes <i>Decrease</i> biomass <i>Selenastrum capricornutum</i> survival <i>Daphnia magna</i> <i>Increase</i> gross primary production	- - - ? >3 d 21 d	Johnson 1986 [7]	4
21 [3]	1000	28 d	lab stagnant microcosms	<i>No effect</i> pH, alkalinity, conductivity microbial activity in sediment biomass macrophytes <i>Decrease</i> biomass <i>Selenastrum capricornutum</i> survival <i>Daphnia magna</i> <i>Increase</i> gross primary production	- - - ? >3 d 28 d	Johnson 1986 [7]	4

## Appendix 19

Substance: **trifluralin**  
dinitroanilin

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Selenastrum capricornutum</i>	96	growth	673	150	Fairchild <i>et al.</i> 1997
<i>Chlamydomonas euglametos</i>	48	growth	151 <sup>1)</sup>	36 <sup>1)</sup>	Hess 1980

Available laboratory toxicity data for duckweed (Lemnaceae).

Species	Exposure duration (d)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Lemna minor</i>	4	density	170	75	Fairchild <i>et al.</i> 1997

1) deduced from raw data

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Selenastrum capricornutum</i>	673	150
<i>Chlamydomonas euglametos</i>	151	-
<i>Lemna minor</i>	170	75

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
67	67	0.037

conservative UP criterion based on lowest EC<sub>50</sub> for standard alga  
liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive standard alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 19.** Single peak loads of **trifluralin** in a stagnant ecosystem. UP criterion is based on *Selenastrum capricornutum*: gm-EC<sub>50</sub> 673 µg/L.

Studied Tu <sub>gsa</sub> [case number]	Studied conc. (µg/L)	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
0.015 [1]	10	28 d	lab stagnant microcosms	<i>No effect</i> pH, alkalinity, conductivity gross primary production microbial activity in sediment biomass <i>Selenastrum capricornutum</i> biomass macrophytes survival <i>Daphnia magna</i>	- - - - -	Johnson 1986 [7]	1
0.15 [2]	100	28 d	lab stagnant microcosms	<i>No effect</i> pH, alkalinity, conductivity gross primary production microbial activity in sediment biomass <i>Selenastrum capricornutum</i> biomass macrophytes survival <i>Daphnia magna</i>	- - - - -	Johnson 1986 [7]	1
1.5 [3]	1000	28 d	lab stagnant microcosms	<i>No effect</i> pH, alkalinity, conductivity microbial activity in sediment biomass <i>Selenastrum capricornutum</i> biomass macrophytes survival <i>Daphnia magna</i> <i>Increase</i> gross primary production	- - - - - 28 d	Johnson 1986 [7]	1

## Appendix 20

Substance: **MSMA (monosodium methylarsonate)**  
organoarsenic

Available laboratory toxicity data for standard green algae (Chlorophyta).

Species	Exposure duration (hour)	Endpoint	EC <sub>50</sub> (µg/L)	NOEC (µg/L)	Reference
<i>Chlorella pyreniodosa</i>	96	density etc. growth	-	>3.10 <sup>6</sup>	Blythe <i>et al.</i> 1979
	72?		-	>810	Davis <i>et al.</i> 1976

Available laboratory toxicity data for duckweed (Lemnaceae): **none**.

Summary of available laboratory toxicity data for algae, green algae and duckweed.

Species	gm-EC <sub>50</sub> (µg/L)	gm-NOEC (µg/L)
<i>Chlorella pyreniodosa</i>	-	-

Summary criteria.

UP conservative (µg/L)	UP liberal (µg/L)	MPC freshwater (µg/L)
-	-	-

conservative UP criterion based on lowest EC<sub>50</sub> for alga  
liberal UP criterion based on gm-EC<sub>50</sub> for most sensitive alga  
MPC for freshwater from Crommentuijn *et al.* 1997

**Table 20.** Single peak loads and constant exposure to **MSMA** in a closed running ecosystem. No data on *Selenastrum capricornutum* available.

Studied $Tu_{gsa}$ [case number]	Studied conc. ( $\mu\text{g/L}$ )	Observation duration	Ecosystem	Results	Recovery	Reference [study number]	Effect class sensitive endpoint
- [1]	10 constant	3 wk	recirculating artificial streams	<i>No effect</i> gross primary production <i>Decrease</i> biovolume periphyton on artificial substrate	-  >21 d	Kosinski 1984 Kosinski & Merkle 1984 [8]	4
- [2]	100 single	3 wk	recirculating artificial streams	<i>No effect</i> gross primary production biovolume periphyton on artificial substrate	- -	Kosinski 1984 Kosinski & Merkle 1984 [8]	1
- [3]	1000 single	3 wk	recirculating artificial streams	<i>No effect</i> gross primary production biovolume periphyton on artificial substrate	- -	Kosinski 1984 Kosinski & Merkle 1984 [8]	1
- [4]	10 000 single	3 wk	recirculating artificial streams	<i>No effect</i> gross primary production <i>Decrease</i> biovolume periphyton on artificial substrate	-  >21 d	Kosinski 1984 Kosinski & Merkle 1984 [8]	4

