National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

Environmental risk limits for metazachlor in water

A proposal for water quality standards in accordance with the Water Framework Directive

RIVM Letter Report 601714024/2013
J.W. Vonk | C.E. Smit | F.M.W. de Jong

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This investigation has been performed by order and for the account of Ministry of Infrastructure and the Environment, Sustainability Directorate, within the framework of the project "Chemical aspects of WFD and RPS".

Rapport in het kort

Milieurisicogrenzen voor metazachloor in water
Een voorstel voor waterkwaliteitsnormen volgens de Kaderrichtlijn Water

Het RIVM heeft in opdracht van het ministerie van Infrastructuur en Milieu (I&M) de milieurisicogrenzen voor metazachloor in water aangepast. Metazachloor is een onkruidbestrijdingsmiddel. De stof is opgenomen in de Regeling Monitoring Kaderrichtlijn Water (KRW), waarin staat aan welke eisen oppervlaktewater in Nederland moet voldoen. Nieuwe waterkwaliteitsnormen zijn nodig omdat de huidige norm voor metazachloor niet is afgeleid volgens de meest recente methodiek. Het ministerie stelt deze nieuwe normen vast op basis van de wetenschappelijke advieswaarden van het RIVM.

Twee waterkwaliteitsnormen
De Kaderrichtlijn Water hanteert twee typen waterkwaliteitsnormen: de Jaargemiddelde Milieukwaliteitsnorm (JG-MKN) en de Maximaal Aanvaardbare Concentratie (MAC-MKN). De JG-MKN is de concentratie in water waarbij geen schadelijke effecten te verwachten zijn nadat waterorganismen en mensen er langdurig aan zijn blootgesteld. De MAC-MKN is de norm die het ecosysteem beschermt tegen kortdurende concentratiepieken. Het gemiddelde van de gemeten concentraties over een jaar moet lager zijn dan de JG-MKN. Individuele meetwaarden kunnen hoger zijn dan het jaargemiddelde, maar de hoogst gemeten concentratie mag niet boven de MAC-MKN uitkomen.

Voor de JG-MKN zijn twee ‘routes’ onderzocht: directe effecten op waterorganismen en indirecte effecten op mensen via het eten van vis. Dit levert twee veilige concentraties op; de laagste bepaalt de voorgestelde JG-MKN (0,08 microgram per liter). Het voorstel voor de MAC-MKN is 0,48 microgram per liter. Op basis van meetgegevens over de afgelopen jaren wordt verwacht dat de voorgestelde normen op een aantal locaties worden overschreden. Als dit inderdaad zo blijkt te zijn, zal dit worden meegewogen bij de toekomstige beoordeling van deze stof als gewasbeschermingsmiddel.

Trefwoorden:
milieurisicogrenzen; JG-MKN; MAC-MKN; Kaderrichtlijn water

Abstract

Environmental risk limits for metazachlor in water
A proposal for water quality standards in accordance with the Water Framework Directive

RIVM has revised the environmental risk limits (ERLs) for metazachlor in water by order of the Ministry of Infrastructure and the Environment. Metazachlor is used as a herbicide. The compound is included in the Dutch decree on water quality objectives in the context of the Water Framework Directive (WFD). The current standard for metazachlor has to be updated according to the new WFD-methodology. The ERLs in this report are advisory values that serve as a scientific background for the ministry, which is responsible for setting those standards.

Two types of quality standards
There are two types of water quality standards under the WFD: the Annual Average Environmental Quality Standard (AA-EQS) and the Maximum Acceptable Concentration EQS (MAC-EQS). The AA-EQS is the concentration in water at which no harmful effects are to be expected for ecosystem and human health after long-term exposure. The MAC-EQS protects the ecosystem against short-term concentration peaks. The average measured concentration over a year should not exceed the AA-EQS. Individual monitoring data may be higher than the annual average, but concentrations should not exceed the MAC-EQS. In the Dutch derivation procedure, the risk limits corresponding to AA- and MAC-EQS are denoted as Maximum Permissible Concentration (MPC) and Maximum Acceptable Concentration (MAC_{eco}), respectively.

Two routes were considered for derivation of the MPC: direct ecotoxicity and consumption of fish by humans. Direct ecotoxicity is the most critical of these two routes and determines the proposed MPC (0.08 microgram per litre). The proposed MAC is 0.48 microgram per litre. Monitoring data from recent years indicate that these levels will probably be exceeded at several locations. If this is indeed the case, this will be taken into account in the future authorisation procedure for the use of this compound as plant protection product.

Key words:
water quality standard; AA-EQS; MAC-EQS; WFD

Contents

Summary

In this report, RIVM presents environmental risk limits (ERL) for metazachlor in water. Metazachlor is used as a broad spectrum herbicide. The compound is listed as a *specific pollutant* in the context of the Water Framework Directive (WFD). The current water quality standard for chronic exposure is 34 µg/L. This value dates back to 1997. A maximum acceptable level for peak exposure, which is also required according to the WFD, is not available.
Based on the data from the national and European authorisation dossiers and additional information obtained from the open literature, environmental risk limits for metazachlor in water have been derived that can be used to set updated water quality standards. The methods used are in accordance with the methodology of the WFD and national frameworks for risk limit derivation.

Five types of ERL are considered, each representing a different protection aim.

- The Maximum Permissible Concentration in water (MPC). The MPC represents the concentration that protects man and environment from adverse effects due to chronic exposure. According to the WFD-methodology, three routes have been taken into account for derivation of the MPC: direct exposure of aquatic organisms, secondary poisoning of predatory birds and mammals, and exposure of humans via fish consumption.

- The Maximum Acceptable Concentration for aquatic ecosystems (MAC_{eco}). The MAC_{eco} is the concentration that protects aquatic ecosystems from adverse effects of short-term concentration peaks. The MPC and MAC_{eco} are equivalent to the long-term and short-term water quality standards that are indicated as AA-EQS and MAC-EQS in the WFD-guidance. They are derived for both the freshwater and saltwater compartment.

- The Negligible Concentration in water (NC). The NC is calculated by applying an additional factor of 100 to the MPC. The NC represents the concentration at which effects to ecosystems are expected to be negligible and functional properties of ecosystems are fully safeguarded. In the Dutch policy on substances, the NC is used to define a safety margin that takes combination toxicity into account.

- The Serious Risk Concentration for ecosystems (SRC_{eco}). This is the concentration at which serious ecotoxicological risks might occur in aquatic ecosystems.

- The Maximum Permissible Concentration in water for drinking water abstraction ($MPC_{dw, hh}$). The $MPC_{dw, hh}$ represents the concentration at which surface water can be used for production of drinking water without further treatment.

Where applicable, ERLs are derived for freshwater and saltwater. An overview of the newly derived ERLs is presented in Table 1. ERLs that are equivalent to water quality standards required under the WFD are indicated in bold. Monitoring data from 2006 to 2010 indicate that these levels will probably be exceeded at several locations. Saltwater monitoring data are not available.

Table 1 Environmental risk limits for metazachlor in water

Environmental risk limit	**Value [µg/L]**
Freshwater	
MPC_{fw}	**0.08**
$MAC_{eco, fw}$	**0.48**
NC_{fw}	0.0008
$SRC_{eco, fw}$	60
Surface water for drinking water production	
$MPC_{dw, hh}$	**0.1**
Saltwater	
MPC_{sw}	**0.008**
$MAC_{eco, sw}$	**0.048**
NC_{sw}	0.00008
$SRC_{eco, sw}$	60

1 Introduction

1.1 Project framework

In this report, environmental risk limits (ERLs) for surface water are derived for metazachlor. Metazachlor is a herbicide that is considered as a specific pollutant for the Netherlands in the context of the Water Framework Directive (WFD). The compound is listed in the Dutch decree on WFD-monitoring (*Regeling monitoring Kaderrichtlijn water*). The aim of this report is to present updated risk limits that can be used to set water quality standards in accordance with the WFD. The derivation of the ERLs is performed in the context of the project Chemical aspects of the Water Framework Directive. The following ERLs are considered:

- Maximum Permissible Concentration (MPC) – defined in VROM (1999, 2004) as the standard based on scientific data which indicates the concentration in an environmental compartment for which:
  1 no effect to be rated as negative is to be expected for ecosystems;
  2a no effect to be rated as negative is to be expected for humans (for non-carcinogenic substances);
  2b for humans no more than a probability of 10^{-6} per year of death can be calculated (for carcinogenic substances). Within the scope of the Water Framework Directive (WFD), a probability of 10^{-6} on a life-time basis is used.

  The MPC for water should not result in risks due to secondary poisoning and/or risks for human health aspects. These aspects are therefore also addressed in the MPC derivation. Separate MPC-values are derived for the freshwater and saltwater environment.

- Negligible Concentration (NC) – the concentration in fresh- and saltwater at which effects to ecosystems are expected to be negligible and functional properties of ecosystems are safeguarded fully. It defines a safety margin which should exclude combination toxicity. The NC is derived by dividing the MPC by a factor of 100.

- Maximum Acceptable Concentration (MAC_{eco}) for aquatic ecosystems – the concentration protecting aquatic ecosystems from effects due to short-term exposure or concentration peaks. The MAC_{eco} is derived for freshwater and saltwater ecosystems.

- Serious Risk Concentration for ecosystems (SRC_{eco}) – the concentration in water at which possibly serious ecotoxicological effects are to be expected. The SRC_{eco} is valid for the freshwater and saltwater compartment.

- Maximum Permissible Concentration for surface water that is used for drinking water abstraction ($MPC_{dw, hh}$). This is the concentration in surface water that meets the requirements for use of surface water for drinking water production. The $MPC_{dw, hh}$ specifically refers to locations that are used for drinking water abstraction.

The quality standards in the context of the WFD refer to the absence of any impact on community structure of aquatic ecosystems. Hence, not the potential to recover after transient exposure, but long-term undisturbed function is the protection objective under the WFD. Recovery in a test situation, after a limited exposure time, is therefore not included in the derivation of the MPC and MAC.

1.2 Current standards for metazachlor

The current MPC_{water} for metazachlor is 34 μg/L. The scientific background of this value dates back to 1997 (Crommentuijn et al., 1997), when the WFD-methodology was not yet adopted.

1.3 Methodology

The methodology is in accordance with the European guidance document for derivation of environmental quality standards under the WFD (EC, 2011). This document is further referred to as the WFD-guidance. Additional guidance for derivation of ERLs that are specific for the Netherlands, such as the NC and SRC, can be found in Van Vlaardingen and Verbruggen (2007). This guidance document was prepared for derivation of ERLs in the context of the project "International and national environmental quality standards for substances in the Netherlands (INS)", and is further referred to as the INS-guidance. Similar to the WFD-guidance, the INS-guidance is based on the Technical Guidance Document (TGD), issued by the European Commission and developed in support of the risk assessment of new notified chemical substances, existing substances and biocides (EC, 2003) and on the Manual for the derivation of Environmental Quality Standards in accordance with the Water Framework Directive (Lepper, 2005). The WFD-guidance also takes into account the most recent guidance developed under REACH (ECHA, 2008).
It should be noted that the recent WFD-guidance deviates from the INS-guidance for some aspects. This specifically applies to the treatment of data for freshwater and marine species (see section 4.1) and the derivation of the MAC (see section 4.4). This also holds for the MPC for surface waters intended for the abstraction of drinking water ($MPC_{dw, hh}$, see section 4.3). In the INS-guidance, this is one of the MPCs from which the lowest value should be selected as the general MPC_{water} (see section 3.1.6 and 3.1.7 of the INS-Guidance). According to the new guidance, the $MPC_{dw, hh}$ is not taken into account for the derivation of the general MPC_{water}, but specifically refers to locations that are used for drinking water abstraction. In addition, the terminology that is used in the present report is harmonised as much as possible with WFD-guidance.

1.3.1 Data sources

The derivation of the ERLs for metazachlor is based on the data available in the EU-dossier. Data from the Draft Assessment Report (DAR; EC, 2005) were re-assessed for their reliability in view of the specific use for ERL derivation. In addition, an on-line literature search was performed via SCOPUS, available via http://www.scopus.com/. For information on coverage, see http://info.scopus.com/detail/what/. For search profile see Annex 3. This search did result in some additional references from which an endpoint could be derived. The registrants for metazachlor-based plant protection products in the Netherlands gave permission to consult the dossiers available at the Dutch authorisation board (Ctgb) and relevant studies were included in this report.

1.3.2 Data evaluation

Ecotoxicity studies were screened for relevant endpoints (i.e. those endpoints that have consequences at the population level of the test species) and thoroughly evaluated with respect to the validity (scientific reliability) of the study. A detailed description of the evaluation procedure is given in section 2.2.2

and 2.3.2 of the INS-Guidance and in the Annex to the draft EQS-guidance under the WFD. In short, the following reliability indices were assigned, based on Klimisch et al. (1997):

Ri 1: Reliable without restriction
'Studies or data … generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline … or in which all parameters described are closely related/comparable to a guideline method.'

Ri 2: Reliable with restrictions
'Studies or data … (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.'

Ri 3: Not reliable
'Studies or data … in which there are interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g., unphysiologic pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which is not convincing for an expert judgment.'

Ri 4: Not assignable
'Studies or data … which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.).'

Citations
In case of (self-)citations, the original (or first cited) value is considered for further assessment, and an asterisk is added to the Ri of the endpoint that is cited.

All available studies are summarised in data-tables, that are included as Annexes to this report. These tables contain information on species characteristics, test conditions and endpoints. Explanatory notes are included with respect to the assignment of the reliability indices.

1.4 Status of the results

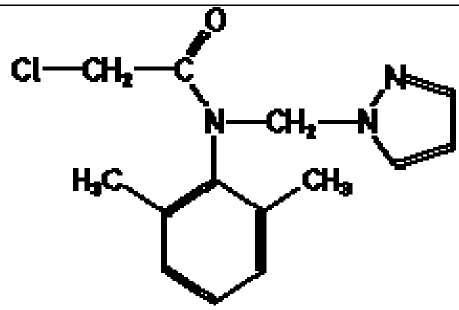
The results presented in this report have been discussed by the members of the scientific advisory group for standard setting in the Netherlands (WK-INS). It should be noted that the ERLs in this report are scientifically derived values, based on (eco)toxicological, fate and physico-chemical data. They serve as advisory values for the Dutch Ministry of Infrastructure and Environment, that is responsible for setting Environmental Quality Standards (EQSs). ERLs should thus be considered as advisory values that do not have an official status.

2 Information on the substance

2.1 Identity

Metazachlor is a herbicide. The compound is approved for use in the European Union under Regulation (EC) No 1107/2009 (repealing Directive 91/414/EEC). Products based on metazachlor are authorised in 25 Member States, including the Netherlands (EU Pesticides Database, accessed November 2012).

Table 2 Substance identification

Name	Metazachlor
Chemical name	2-chloro-N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-ylmethyl)-acetamide
CAS number	67129-08-2
Molecular formula	$C_{14}H_{16}ClN_3O$
Molar mass	277.8 g/mol
EC number	266-583-0
Structural formula	
SMILES code	O=C(N(c1c(cccc1C)C)Cn2nccc2)CCl
Use class	Herbicide
Mode of action	Pre-emergence herbicidal action to grasses and some dicotyledonous weeds. The mode of action is inhibition of the synthesis of very long chain fatty acids, possibly by reaction of the CH2-Cl moiety with certain enzymes involved in ergosterol or fatty acid incorporation (Böger, 2003; EC, 2005). No fungicidal action is known from this substance.

2.2 Physico-chemical properties

Table 3 Physico-chemical properties

Parameter	Unit	Value	Remark	Reference
Water solubility	[mg/L]	630	pH 7, 25 °C	EC, 2005
pK_a		-	No dissociation in water	EC, 2005
log K_{ow}		2.5	pH 7, 21-22 °C	EC, 2005
Vapour pressure	[Pa]	9×10^{-5}	20 °C	EC, 2005
Henry's law constant	[Pa.m^3/mol]	5.9×10^{-5}	20 °C	EC, 2005
Melting point	[°C]	78-81		EC, 2005
Boiling point	[°C]	-		EC, 2005

2.3 Fate and behaviour

Selected environmental properties of metazachlor are given in Table 4.

Table 4 Selected environmental properties of metazachlor

Parameter	Name/Unit	Value	Remark	Reference
log K_{oc}	log [L/kg]	2.04	Median of 25 values	EC, 2005
Hydrolysis half-life	DT_{50} [d]	-	Stable at pH 4 – 9 , 20-25 °C	EC, 2005
Photolysis half-life	DT_{50} [d]	-	Stable to direct aqueous photolysis	EC, 2005
Biodegradation in water/sediment systems	DT_{50} [d]	19.3	Whole system; geometric mean of 4 systems	EC, 2005

2.4 Bioconcentration and biomagnification

There are no experimental data available for bioconcentration in fish. Since the trigger for the log K_{ow} is not exceeded (log K_{ow} < 3), there is no need to derive an MPC for secondary poisoning. Using the log K_{ow}, the BCF for fish was calculated to be 26.6 L/kg according to EC (2011) and Van Vlaardingen and Verbruggen (2007).

3 Human toxicology and ecotoxicological effect data

3.1 Human toxicological threshold limits and carcinogenicity

The proposed harmonised classification of metazachlor under CLP Regulation 1272/2008/EC (Committee for Risk Assessment, 2011) is as follows: H317 (Skin sensitation category 1), H351 (carcinogenic category 2). This is equivalent to R43 and R40, respectively, under Directive 67/548/EEC. According to the triggers as given in WFD-Guidance, the $MPC_{water,\ hh\ food}$ for human exposure via fish should be derived. The ADI is 0.08 mg/kg bw/d, based on the NOAEL of 8.5 mg/kg bw/d from a chronic study with rat and an assessment factor of 100 (List of Endpoints of the DAR; EC, 2005). There are no indications that metazachlor has a potential for endocrine disruption (KEMI, 2008; Spruijt et al., 2008).

3.2 Ecotoxicological effect data

3.2.1 Laboratory data

Detailed aquatic toxicity data for metazachlor are tabulated in Annex 1. Unbound values are not used in ERL derivation, unless they indicate that the derived value is not protective and consequently the assessment factor should be adapted. The selected valid acute and chronic ecotoxicity data for freshwater organisms are summarised in Tables 5 and 6. No data for marine species are available.

For cyanobacteria and algae, endpoints based on growth rate are preferred over biomass, in accordance with OECD 201 (OECD, 2006a). The selection of the endpoint for *Lemna* sp. is briefly explained here. For *Lemna*, the relevant endpoints according to OECD 221 (OECD, 2006b) are growth rate or yield, which can be based on frond number, frond area, dry weight or wet weight. However, it is explicitly stated in the guideline that similar to algae, growth rate is preferred over yield from a scientific point of view. Recommended test duration is 7 days, but older tests have been performed with a test duration of 14 days. Therefore, for the aggregated data table, the geometric mean of growth rate values, based on the same parameter (frond number, area or weight) was calculated from the 7-days tests, and the lowest of these was chosen. Details can be found in the aggregated data tables. However, if for a species only a single endpoint is reported, this is used if no other preferred endpoints are available for that particular species. For sediment-rooted macrophytes, the AMRAP workshop recommended to use biomass and growth as regulatory endpoints (Maltby et al., 2010). In the DAR, a study with several macrophyte species is included, and for each of these the lowest relevant endpoint was included in the toxicity tables in Annex 1.

3.2.2 Field data

In Annex 2 field studies are described and a conclusion is drawn on the usability of the studies for ERL derivation. From the results it is concluded that 2 µg/L can be used as the NOEC from the field studies. The lowest laboratory NOEC was generated for *L. gibba.* Following the recommendations of Brock et al. (2011), the test duration of 7 days is used to calculate the time weighted average exposure concentration in the mesocosm study. Given the DT50 of 33 d from the relevant field study, the NOEC expressed as Time Weighted Average concentration (TWA) is rounded to 1.9 µg/L.

Table 5 Selected acute ecotoxicity data of metazachlor for freshwater organisms. The lowest available endpoint is indicated in bold.

Endpoints	L(E)C50 [μg/L]	Remark
Cyanobacteria[a]		
Anabaena flos-aquae	> 32000	Growth rate
Algae[a]		
Chlorella fusca	1630	Growth rate
Navicula pelliculosa[d]	72500	Growth rate
Pseudokirchneriella subcapitata	19.3	Geometric mean of 31.8 and 11.7 μg/L (preferred endpoint growth rate)
Scenedesmus subspicatus	39.4	Geometric mean of 50 and 31 μg/L (preferred endpoint growth rate)
Macrophyta		
Callitriche palustris	24	Wet weight
Ceratophyllum demersum	24	Length
Elodea canadensis	73	Wet weight
Lemna gibba	11.3	Geometric mean of 10.7, 7.1, 14.3 and 14.9 μg/L; growth rate based on frond numbers, exposure 7 d[b].
Lemna minor	**2.9**	Lowest relevant endpoint; growth rate based on frond area[c].
Lemna paucicostata	106	Frond area
Myriophyllum heterophyllum	80	Length
Potamogeton natans	> 200	Wet weight
Stratiotes aloides	49	Wet weight
Crustacea		
Daphnia magna	29369	Geometric mean of 33700, 33000, 30000 and 22300 μg/L (immobilisation)
Pisces		
Cyprinus carpio	13447	Geometric mean of 12300 and 14700 μg/L (mortality)
Lepomis macrochirus	10900	Mortality
Lepomis gibbosus	6800	Mortality
Oncorhynchus mykiss	5988	Geometric mean of 8500, 8900, 4900, 4420 and 4700 μg/L (mortality)

a: For studies with cyanobacteria and algae, endpoints based on growth rate are preferred over endpoints based on biomass.
b: This value is lower than the 7-days EC50 for growth rate based on dry weight (37.6 μg/L).
c: This value is lower than the geometric mean EC50 for growth rate based on frond numbers of 7.3 μg/L (individual values 3.8 and 14 μg/L).
d: See comment on taxonomic position of diatoms in section 4.2.1.2

Table 6 Selected chronic ecotoxicity data of metazachlor for freshwater organisms. The lowest available endpoint is indicated in bold.

Endpoints	NOEC/EC10 [µg/L]	Remark
Cyanobacteria[a]		
Anabaena flos-aquae	13900	Growth rate
Algae and diatomeae[a]		
Chlorella fusca	340	Growth rate
Navicula pelliculosa[d]	3200	Growth rate
Pseudokirchneriella subcapitata	5.7	Geometric mean of 6.1 and 5.4 µg/L; preferred endpoint growth rate
Scenedesmus subspicatus	5.2	Geometric mean of 1.8 and 15 µg/L; preferred endpoint growth rate
Macrophyta		
Callitriche palustris	5	Wet weight
Ceratophyllum demersum	10	Wet weight, length
Elodea canadensis	31.5	Wet weight, length
Lemna gibba	**0.245**	Lowest relevant endpoint, growth rate based on dry weight, exposure 7 d[b]
Lemna minor	0.8	Lowest relevant endpoint, growth rate based on frond area[c]
Myriophyllum heterophyllum	20	Wet weight
Potamogeton natans	≥ 200	Wet weight
Stratiotes aloides	5	Wet weight
Crustacea		
Daphnia magna	2396	Geometric mean of 6250, 100 and 22000 µg/L; endpoint reproduction
Insecta		
Chironomus riparius	5700	Emergence
Pisces		
Oncorhynchus mykiss	2318	Geometric mean of 2500 and 2150 µg/L; endpoint growth

a: For studies with cyanobacteria and algae, endpoints based on growth rate are preferred over endpoints based on biomass.
b: This value is lower than the geometric mean NOEC/EC10-value for growth rate based on frond numbers of 0.57 µg/L (individual data 0.6, 0.36, 2.48, 1.93 µg/L), the NOEC for yield based on frond numbers of 2.48 µg/L and the NOEC for yield based on dry weight of 0.772 µg/L.
c: This value is lower than the geometric mean NOEC/EC10-value for growth rate based on frond numbers of 0.92 µg/L (individual data 0.6 and 1.4 µg/L).
d: See comment on taxonomic position of diatoms in section 4.2.1.2

4 Derivation of ERLs for water

4.1 Treatment of data for freshwater and marine species

The dataset for fresh water species is used for risk limit derivation of marine species, since no marine dataset is available for metazachlor.

4.2 MPC_{fw} and MPC_{sw}

4.2.1 *$MPC_{fw, eco}$– ecotoxicity data*

NOECs are available for 15 species from six taxonomic groups covering at least three trophic levels[1]. In addition, an unbound value is available for the macrophyte *Potamogeton natans*. Although the NOECs for fish are derived from a 28 days test and not from an ELS- or FLC test, the base set is considered complete, because metazachlor is a herbicide primarily acting on algae and plants. The variation in sensitivity between individual macrophyte species is quite large and hard to explain. Both monocots (*Elodea canadensis*, *Lemna* sp., *Potamogeton natans*, *Stratoides aloides*) and dicots (*Callitriche palustris*, *Ceratophyllum demersum*, *Myriophyllum heterophyllum*) include sensitive as well as insensitive species. A possible factor might be whether or not a species is sediment-rooting and was tested as such in the ecotoxicity tests. There seems to be a tendency towards lower toxicity (higher effect values) for species that were tested as potted plants (*E. canadensis*, *P. natans* and *M. heterophyllum*). This cannot be explained by dissipation of the compounds from the test system, since (no) effect values are based on concentrations in the water phase. Metazachlor affects plant growth by inhibiting the synthesis of very long chain fatty acids (VLCFA), thereby disturbing cell metabolism and division (Böger, 2003; Couderchet et al., 1996; Mohr et al., 2007; Schmalfuss et al., 1998). VLCFA are precursors of epicuticular waxes in the epidermis cells of plants and components of cell and plasma membranes. According to Mohr et al. (2007) both monocots and dicots generally have high amounts of VLCFA, but still it could be possible that differences in sensitivity relate to species-specific differences in the presence of VLCFA. It is also possible that the normal growth rate of plants plays a role, i.e. that there is a difference in sensitivity between slowly growing plants and plants that grow relatively fast.

4.2.1.1 Assessment factor approach

The $MPC_{eco, water}$ is derived in the first instance by putting an assessment factor of 10 to the lowest NOEC of 0.245 μg/L for the aquatic macrophyte *L. gibba*, resulting in an $MPC_{fw, eco}$ of 0.02 μg/L.

4.2.1.2 SSD approach

The dataset does not fully meet the criteria for construction of a Species Sensitivity Distribution (SSD) as listed in the WFD-guidance. According to the guidance, the output from an SSD-based quality standard is considered reliable if the database contains preferably more than 15, but at least 10 datapoints, from different species covering at least eight taxonomic groups. Below, the

[1] For a decision on the height of the assessment factor, the WFD-guidance often refers to the number of trophic levels represented in the dataset. Especially for compounds with a specific mode of action, the use of this term may be complicating because species belonging to the same trophic level may differ greatly in sensitivity because of their different taxonomic position (e.g. crustaceans and insects in case of certain insecticides). When deciding on the AF, the question whether or not the potentially most sensitive taxonomic group is represented in the dataset is more relevant than the trophic level.

criteria are copied, together with the representative species from the present dataset:

- Fish (species frequently tested include salmonids, minnows, bluegill sunfish, channel catfish, etc.): *Oncorhynchus mykiss*, family Salmonidae
- A second family in the phylum Chordata (e.g. fish, amphibian, etc.): no data
- A crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.): *Daphnia magna*
- An insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.): *Chironomus riparius*
- A family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.): *Anabaena flos-aquae*, phylum Cyanophycota, family Nostocaceae
- A family in any order of insect or any phylum not already represented: no data
- Algae: *Scenedesmus subspicatus*
- Higher plants: *Lemna gibba*

From this list it can be seen that data are missing for two of the required taxa. However, in view of the fact that metazachlor is a herbicide and non-target taxa are clearly not sensitive, derivation of ERLs by means of Species Sensitivity Distributions (SSDs) is considered justified. First, the HC5 value is estimated using ETX 2.0 (Van Vlaardingen et al., 2004) with all NOEC/EC10 data. The result is presented in Figure 1. The goodness-of-fit is accepted at all levels, except for 0.1 (Anderson-Darling test).
The HC5 is 0.16 µg/L, with upper and lower limit of 0.007 and 1.2 µg/L, respectively. Applying the default safety factor of 5, the resulting $MPC_{fw, eco}$ would be 0.032 µg/L.

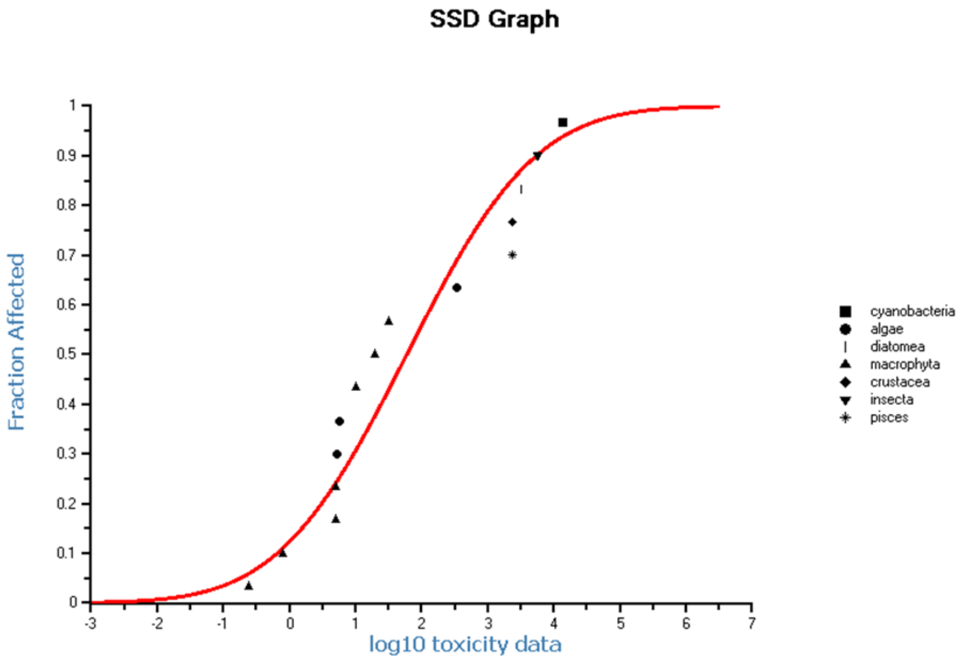


Figure 1. SSD graph of the NOEC/EC10 values of all species listed in Table 6. HC5 = 0.16 µg/L.

As can be seen from Figure 1, the SSD shows a separation between the algae and macrophytes at one side and the less sensitive groups at the other side. The diatom *Navicula pelliculosa* is clearly insensitive. In the INS-guidance, algae and diatoms are considered as belonging to the same taxonomic group. However, recent information from taxonomic databases shows that this is not the case (ITIS, 2012; AlgaeBase, 2012), and this might be the reason that *N. pelliculosa* is at the upper end of the distribution.

Because metazachlor is a herbicide, and 10 NOEC/EC10 values are available for the potentially sensitive species groups of algae and macrophytes, an SSD was also performed using the data for these species (Fig. 2). *N. pelliculosa* is not included in view of the above.
The goodness-of-fit is accepted at all levels. The HC5 is 0.25 µg/L, with lower and upper level of 0.02 and 0.95 µg/L, respectively. The HC5 is equal to the lowest NOEC of 0.245 µg/L for *L. gibba*. Applying the standard maximum assessment factor of 5 would result in an $MPC_{fw,\ eco}$ of 0.05 µg/L. Brock et al. (2011) suggest that the default assessment factor of 5 may be lowered to 3 when the chronic SSD is generated using NOEC/EC10 values for the sensitive species. This would result in an $MPC_{fw,\ eco}$ of 0.08 µg/L.
The original dataset does not meet the criteria that are set in the WFD-guidance, and with 10 values, the number of data used for the specific SSD is at the minimum of what is considered reliable. It is also not clear whether the tested species cover the range of sensitive species related to the mode of action. This might be a reason for not lowering the default assessment factor. However, according to the WFD-guidance (EC, 2011), a comparison with mesocosm studies can also be used to decide on the assessment factor and in this case the results of the mesocosms (see 3.2.2, and further below) favour the use of the lower assessment factor. It is decided to use the HC5 based on algae and macrophytes with an assessment factor of 3, and the SSD-based $MPC_{fw,\ eco}$ is set to 0.08 µg/L.

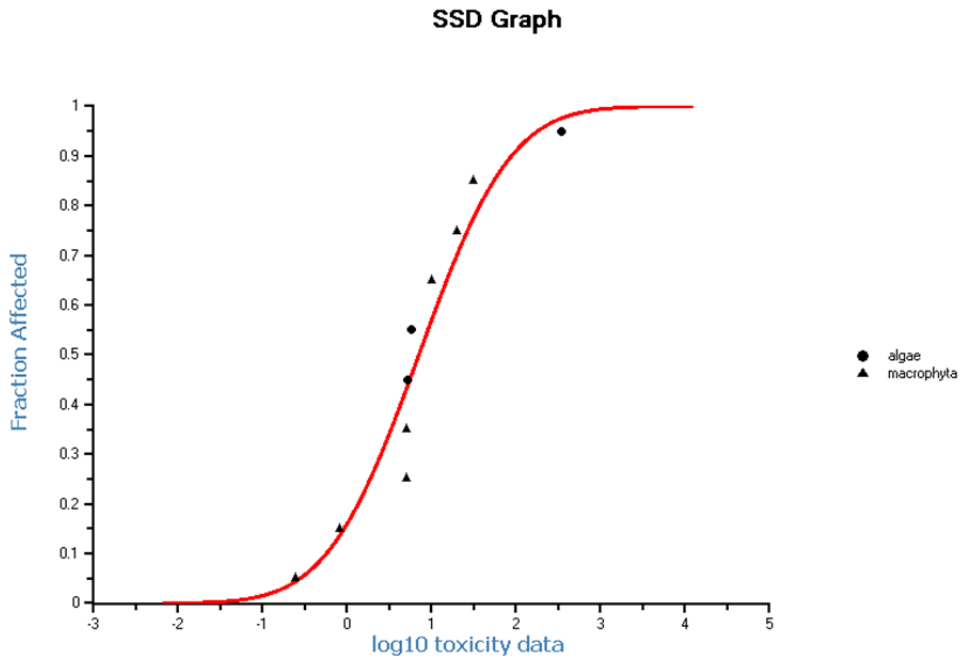


Figure 2. SSD graph of the NOEC/EC10 values of algae and macrophyta in Table 6. Navicula pelliculosa *excluded. HC5 = 0.25 µg/L.*

4.2.1.3 Mesocosm

As indicated in section 3.2.2, the NOEC based on mesocosm experiments is 2.0 μg/L, which expressed as TWA concentration is rounded to 1.9 μg/L. For pesticide registration purposes, EFSA proposed to use 5 μg/L as NOEAEC with an assessment factor of 3 (EC, 2005). However, ERL-derivation according to the WFD-guidance does not take account of recovery and the NOEC rather than the NOEAEC should be used. For derivation of water quality standards, Brock et al. (2011) propose an assessment factor of 2-4 on the NOEC of a reliable mesocosm study. The choice of the factor depends on the additional information that is available. One other reliable mesocosm is available (Mohr et al., 2007; 2008). However, according to the description *Lemna*, which is by far the most sensitive macrophyte species, was not present in that study. Therefore, it is decided to use the highest assessment factor of 4 on the NOEC of 1.9 μg/L, resulting in mesocosm-based $MPC_{fw,\ eco}$ of 0.48 μg/L.

4.2.1.4 Choice of the $MPC_{fw,\ eco}$

The $MPC_{fw,\ eco}$ derived by the assessment factor approach is 0.02 μg/L, the SSD approach results in 0.08 μg/L and the mesocosm approach in 0.48 μg/L. The results of the assessment factor approach and SSD approach differ by a factor of 4, while the difference between the SSD and mesocosm approach is almost a factor of 10. There is no clear reason for the relatively high NOEC of 1.9 μg/L observed in the mesocosm as compared to the lowest laboratory NOEC of 0.245 μg/L. A possible explanation might be the fact that growth rate of *Lemna* sp. in the mesocosms was very low. As indicated above, it is not known how the mode of action of metazachlor relates to the observed differences in sensitivity among plant species. It may be possible that effects of the herbicide become more prominent under optimal growth conditions. Since the mesocosm-based $MPC_{fw,\ eco}$ might therefore be under protective for *Lemna* sp. and other potentially sensitive species, it is decided to use the SSD-based value of 0.08 μg/L as the $MPC_{fw,\ eco.}$

4.2.2 $MPC_{sw,\ eco}$– ecotoxicity data

The $MPC_{sw,\ eco}$ is derived on the basis of the same dataset that was used for the $MPC_{fw,\ eco}$. A NOEC from a specific marine taxonomic group is not available. In this case an additional assessment factor of 10 is applied to the MPC derived for freshwater, resulting in an $MPC_{sw,\ eco}$ of 0.008 μg/L.

4.2.3 $MPC_{water,\ hh\ food}$ – human exposure

Metazachlor is assigned H351. According to the triggers in the WFD-Guidance, the $MPC_{water,\ hh\ food}$ should be derived. The $MPC_{hh,\ food}$ is calculated from the ADI (0.08 mg/kg bw/d), a body weight of 70 kg and a daily fish consumption of 115 g and a maximum contribution of fish consumption to the ADI of 10%. The resulting $MPC_{hh,\ food}$ is 0.1 x 0.08 x 70 / 0.115 = 4.87 mg/kg fd. Subsequently the $MPC_{water,\ hh\ food}$ is calculated using the (estimated) BCF of 26.6 L/kg and BMF of 1 as 4.87 / (26.6 x 1) = 0.183 mg/L = 183 μg/L. The $MPC_{water,\ hh\ food}$ is valid for both the freshwater and the marine compartment.

4.2.4 Selection of the MPC_{fw} and MPC_{sw}

The lowest of the two routes – direct ecotoxicity and human exposure via fish – is selected as the final MPC. For freshwater as well as the marine environment, direct ecotoxicity is the most critical route. The MPC_{fw} is 0.08 μg/L, the MPC_{sw} is 0.008 μg/L.

4.3 $MPC_{dw, hh}$ – surface water for abstraction of drinking water

Metazachlor is an organic pesticide. The drinking water standard according to Directive 98/83/EC is 0.1 μg/L. According to the WFD-guidance, a substance specific removal rate should be considered to derive the $MPC_{dw, hh}$. At present, such information is not available and water treatment is not taken into account. The $MPC_{dw, hh}$ is 0.1 μg/L.

4.4 $MAC_{fw, eco}$ and $MAC_{sw, eco}$

L(E)C50 values are available for 17 species from six taxonomic groups including three trophic levels. In addition, unbound values for *A. flos-aquae* and *P. natans* are available (Table 5). As for the chronic dataset, the range of sensitivity within the group of macrophytes is relatively large.

4.4.1.1 Assessment factor approach

The $MAC_{fw, eco}$ is derived in the first instance from the lowest acute toxicity value available: the EC50 of 2.9 μg/L for *Lemna minor*. An assessment factor of 10 can be applied, because representatives of the potentially most sensitive taxonomic groups are included in the dataset. The $MAC_{fw, eco}$ is 0.29 μg/L.

4.4.1.2 SSD-approach

Following the same reasoning as for the $MPC_{fw, eco}$, the derivation of a $MAC_{fw, eco}$ via the SSD-approach was also investigated. As for the chronic data, the acute dataset does not fully meet the criteria for construction of an SSD. Below, the criteria are copied with the representative species from the present dataset:

- Fish (species frequently tested include salmonids, minnows, bluegill sunfish, channel catfish, etc.): *Oncorhynchus mykiss*, family Salmonidae
- A second family in the phylum Chordata (e.g. fish, amphibian, etc.): *Cyprinus carpio*, family Cyprinidae
- A crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.): *Daphnia magna*
- An insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.): no data
- A family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.): *Anabaena flos-aquae*, phylum Cyanophycota, family Nostocaceae (unbound value)
- A family in any order of insect or any phylum not already represented: no data
- Algae: *Scenedesmus subspicatus*
- Higher plants: *Lemna minor*

Again, two taxa are missing and for one taxon only an unbound value is available. The SSD with ETX 2.0 using all species is presented in Figure 3. Using all data, the goodness-of-fit is rejected at two levels (Anderson-Darling test). There is a clear distinction in the relatively sensitive algae and macrophytes on the left hand side, and the other species on the right hand side. The specific SSD with the algae and macrophytes (*Navicula pelliculosa* excluded) is presented in Figure 4. The goodness-of-fit is accepted at all levels (Anderson-Darling test). The resulting HC5 is 2.86 μg/L, with lower and upper limit of 0.49 and 8.1 μg/L, respectively. The default assessment factor for derivation of the $MAC_{fw, eco}$ by the SSD approach is 10, resulting in a $MAC_{fw, eco}$ of 0.29 μg/L. Brock et al. (2011) propose to lower this assessment to 6 in case the SSD is constructed for the sensitive taxa. This would result in a $MAC_{fw, eco}$ of 0.48 μg/L.

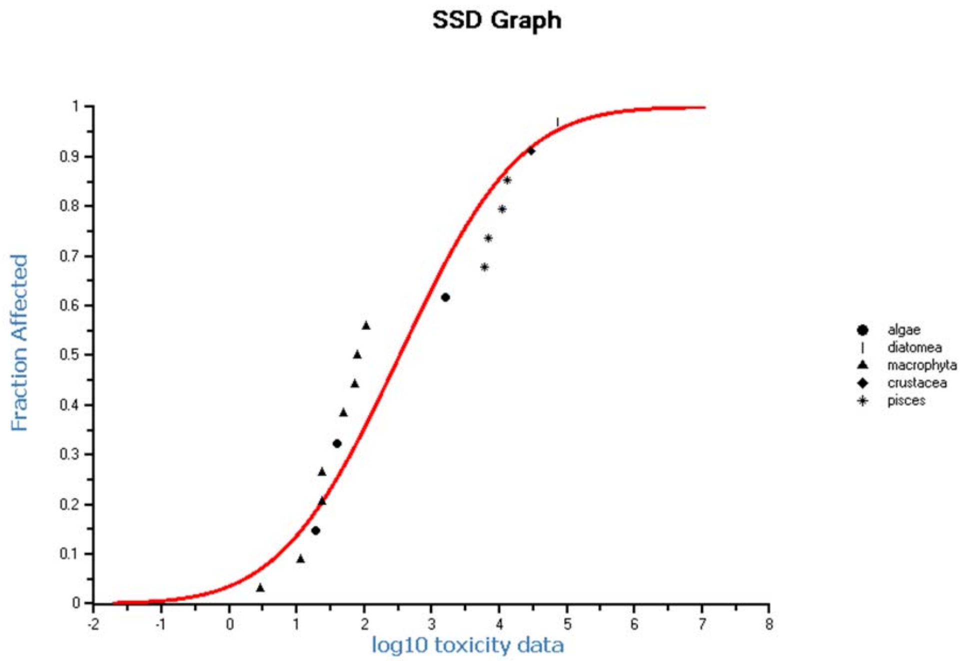


Figure 3. SSD graph of the EC50 values in Table 5, unbound values not included.

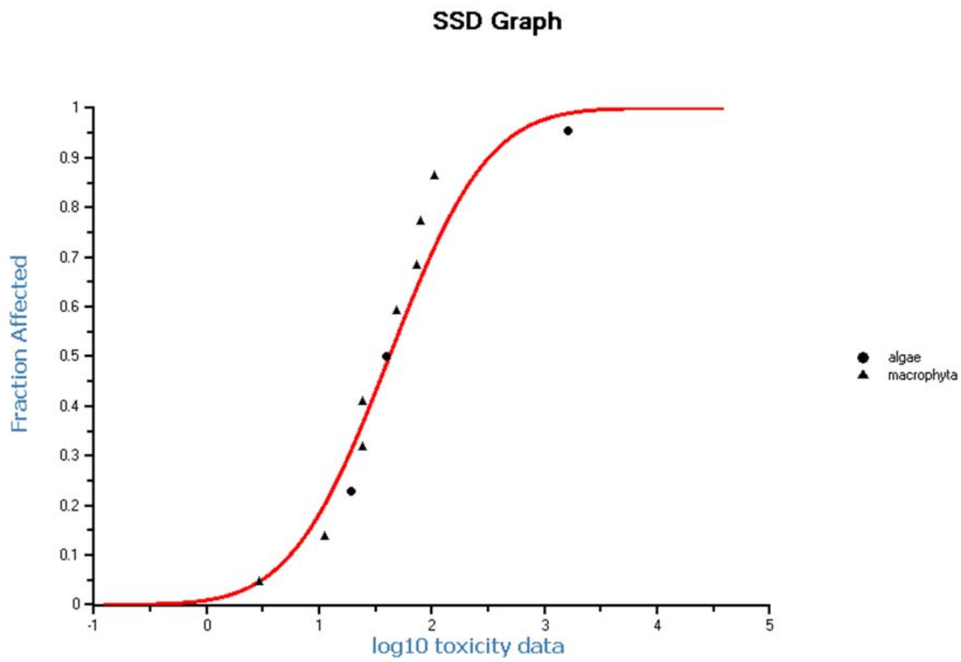


Figure 4. SSD graph of the EC50 values for algae and macrophytes in Table 5, Navicula pelliculosa *and unbound values not included. HC5 = 2.86 µg/L.*

The generic dataset does not meet the criteria that are set in the WFD-guidance, and with 11 values, the number of data used for the specific SSD is at the minimum of what is considered reliable. This would not favour the use of a lower assessment factor.

Another consideration to lower the assessment factor can be the ratio between the acute EC50 and the NOEC/EC10. The $MAC_{fw, eco}$ represents an acute no-effect level. Because for algae and macrophytes the EC50 and NOEC/EC10 values originate from the same studies and mostly refer to the same endpoint, the $MAC_{fw, eco}$ should be set at a level that is protective for chronic effects too. If the ratio between the EC50 and NOEC/EC10 is larger than 10, the default assessment factor is needed as a minimum to account for the fact that the SSD is based on 50%-effect values, while the $MAC_{fw, eco}$ represents a no-effect level. For the 10 species for which both EC50 and NOEC/EC10 values are available, the ratio between the two values is between 2.3 and 9.8, the exception is *L. minor*, for which the ratio is 46. This is probably due to the fact that the basis for the acute endpoint (frond numbers) differs from that for the chronic endpoint (frond weight). For seven out of 10 species the ratio is below 6, which would justify the use of a factor lower than 10. The $MAC_{fw, eco}$ of 0.48 µg/L that is derived using a factor of 6 is similar to the lower limit of the EC50-based SSD (0.49 µg/L). This indicates that at this $MAC_{fw, eco}$, exposure of species above their EC50 is not likely. However, when using the default factor of 10, the resulting $MAC_{fw, eco}$ is at the level of the chronic HC5 of 0.25 µg/L and effects cannot be excluded when a lower assessment is used.
On the other hand, it is noted that the standard test duration for *Lemna* of 7 days probably exceeds the duration of "short-term concentration peaks" for which the $MAC_{fw, eco}$ was introduced. While the endpoints from the agreed study duration should be used as input for the SSD, the information from intermediate time-points may be considered to underpin the choice of the assessment factor. In this case, additional studies indicate that effects of metazachlor on *L. gibba* (the most sensitive species from the chronic dataset) are reduced when exposure time is limited to 48 hours. In the study from Wenzel (2011a) no effects were seen when *L. gibba* was initially exposed for 24 hours to 10 µg/L, followed by another 24 hours at 1.4 µg/L and clean medium thereafter until day 7 (see Annex 1 Table A1.1). The time-dependent effect of metazachlor is confirmed by a study in the DAR in which *L. gibba* showed no effects after exposure to 3 µg/L for 72 hours, but displayed 25.8% growth inhibition after 5 days (Junker & Kubitza, 2003 in EC, 2005). In another study, the NOEC was 0.572 µg/L after exposure for 72 hours (LOEC 2.8 µg/L), but was 0.193 µg/L after exposure for 7 days (Scheerbaum, 2000 in EC, 2005). In a study with *L. minor*, the NOEC was 4.4 µg/L after two days and dropped to 1.4 µg/L after 4 and 7 days (Scheerbaum, 2004 in EC, 2005). Taking all available information into account, an SSD-based $MAC_{fw, eco}$ of 0.48 µg/L derived using an assessment factor of 6 seems to be protective taking the time-frame of the MAC into consideration.

4.4.1.3 Mesocosm

Usable field studies are available, from which a NOEC of 2 µg/L was derived. Brock et al. (2011) propose to use an assessment factor of 2-3 on the 48/72 hours TWA concentration for derivation of the $MAC_{fw, eco}$ in case a NOEC from a reliable mesocosm study is available. With the DT50 of 33 days, the rounded NOEC is 2.0 µg/L. As for derivation of the $MPC_{fw, eco}$, the higher assessment factor of 3 is used because it is not known whether *Lemna* sp. was present in the other mesocosm studies. The resulting mesocosm-based $MAC_{fw, eco}$ is 0.67 µg/L.

4.4.2 *Selection of the $MAC_{fw, eco}$ and $MAC_{sw, eco}$*

The $MAC_{fw, eco}$ derived by the assessment factor approach is 0.29 µg/L, the SSD approach results in 0.48 µg/L and the mesocosm approach in 0.67 µg/L. The difference between lowest and highest value is a factor of 2.3. As for the $MPC_{fw, eco}$, the SSD-based value is considered most appropriate. The $MAC_{fw, eco}$ is set to 0.48 µg/L.

The $MAC_{sw, eco}$ is derived on the basis of the freshwater dataset. Since there are no acute data from specific marine taxa, an additional assessment factor of 10 is applied to the $MAC_{fw, eco}$. This results in an $MAC_{sw, eco}$ of 0.048 µg/L.

4.5 NC_{fw} and NC_{sw}

The NC_{fw} is calculated by dividing the MPC_{fw} by a factor of 100. The NC_{fw} is 0.0008 µg/L.
The NC_{sw} is calculated by dividing the MPC_{sw} by a factor of 100. The NC_{sw} is 0.00008 µg/L.

4.6 $SRC_{fw, eco}$ and $SRC_{sw, eco}$

Since more than three long-term NOECs of all required trophic levels are available, the $SRC_{fw, eco}$ is derived from the geometric mean of all available NOECs with an assessment factor of 1. The geometric mean is 60 µg/L. Therefore, the $SRC_{fw, eco}$ is 60 µg/L. This value is also valid as $SRC_{sw, eco}$.

5 Conclusions

Based on the available information, environmental risk limits (ERLs) for metazachlor in freshwater and saltwater are derived according to the methodology of the WFD and INS. An overview of the derived ERLs is presented in the table below. ERLs that are equivalent to water quality standards required under the WFD are indicated in bold.

Table 7 Environmental risk limits for metazachlor in water

Environmental risk limit	**Value [µg/L]**
Freshwater	
$MPC_{fw, eco}$	0.08
$MPC_{water, hh food}$	183
MPC_{fw}	**0.08**
$MAC_{fw, eco}$	**0.48**
NC_{fw}	0.0008
$SRC_{fw, eco}$	60
Surface water for drinking water production	
$MPC_{dw, hh}$	**0.1**
Saltwater	
$MPC_{sw, eco}$	0.008
$MPC_{water, hh food}$	183
MPC_{sw}	**0.008**
$MAC_{sw, eco}$	**0.048**
NC_{sw}	0.00008
$SRC_{sw eco}$	60

According to the Bestrijdingsmiddelenatlas (www.bestrijdingsmiddelenatlas.nl) concentrations in 2010 were <0.34 µg/L (1/100 of the current quality standard of 34 µg/L) for the majority of monitoring locations, eight locations had concentrations between 0.34 and 34 µg/L.
In 2009, metazachlor was detected at 26 WFD-monitoring locations with 90^{th} percentile yearly concentrations ranging from 0.01 to 1.34 µg/L (Van Duijnhoven, 2011). The frequency of detection ranged from 15% (1 out of 7 samples) to 100% (detected in all samples). Because the comparison with the new MPC_{fw} will be based on annual average concentrations, these data cannot be used directly but they indicate that the MPC_{fw} will probably be exceeded. This also holds for the $MAC_{fw ,eco}$.

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List of abbreviations

ADI	Acceptable Daily Intake
BCF	Bioconcentration Factor
BMF	Biomagnification Factor
EC_x	Concentration at which x% effect is observed
ERL	Environmental Risk Limit
INS	International and National Environmental Quality Standards for Substances in the Netherlands
LC_{50}	Concentration at which 50% mortality is observed
MAC_{eco}	Maximum Acceptable Concentration for ecosystems
$MAC_{fw, eco}$	Maximum Acceptable Concentration for ecosystems in freshwater
$MAC_{sw, eco}$	Maximum Acceptable Concentration for ecosystems in the saltwater compartment
Marine species	Species that are representative for marine and brackish water environments and that are tested in water with salinity > 0.5 ‰.
MPC	Maximum Permissible Concentration
MPC_{fw}	Maximum Permissible Concentration in freshwater
MPC_{sw}	Maximum Permissible Concentration in the saltwater compartment
$MPC_{fw, eco}$	Maximum Permissible Concentration in freshwater based on ecotoxicological data
$MPC_{sw, eco}$	Maximum Permissible Concentration in the saltwater compartment based on ecotoxicological data
$MPC_{fw, secpois}$	Maximum Permissible Concentration in freshwater based on secondary poisoning
$MPC_{sw, secpois}$	Maximum Permissible Concentration in the saltwater compartment based on secondary poisoning
$MPC_{water, hh food}$	Maximum Permissible Concentration in freshwater and saltwater based on consumption of fish and shellfish by humans
$MPC_{dw, hh}$	Maximum Permissible Concentration in water used for abstraction of drinking water
NC	Negligible Concentration
NC_{fw}	Negligible Concentration in freshwater
NC_{sw}	Negligible Concentration in saltwater
NOEC	No Observed Effect Concentration
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NOEAEC	No Observed Ecosystem Adverse Effect Level
SRC_{eco}	Serious Risk Concentration for ecosystems
$SRC_{water, eco}$	Serious risk concentration for freshwater and saltwater ecosystems
TGD	Technical Guidance Document
TWA	Time Weighted Average
WFD	Water Framework Directive (2000/60/EC)

Annex 1 Detailed ecotoxicity data

Legend to column headings	
A	test water analysed Y(es)/N(o)
Test type	S = static; Sc = static closed; R = renewal; F = flow through; CF = continuous flow; IF = intermittent flow system
Purity	refers to purity of active substance or content of active substance in formulation; ag = analytical grade
Test water	am = artificial medium; dtw = dechlorinated tap water; dw = deionised/dechlorinated/distilled water; nw = natural water; rw = reconstituted water; rtw = reconstituted tap water; tw = tap water
T	temperature
Ri	Reliability index according to Klimisch et al. (1997); asterisk indicates citation

Table A1.1. Acute toxicity of metazachlor to freshwater organisms

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [µg a.s./L]	Ri	Notes	Ref.
Cyanobacteria																
Anabaena flos-aquae	3 x 10^4 cells/mL	Y	S	a.s.	98.7	am	7.5-7.7	23-25		96 h	EC50	growth rate	>32000	2	10	EC, 2005 (Kubitza, 1998b)
Anabaena flos-aquae	3 x 10^4 cells/mL	Y	S	a.s.	98.7	am	7.5-7.7	23-25		96 h	EC50	biomass (AUGC)*	25200	2	10	EC, 2005 (Kubitza, 1998b)
Algae																
Chlorella fusca	2 x 10^4 cells/mL	N	S	a.s	90-95	am	7.1	20		96 h	EC50	growth rate	16300	2	16	Panman and Linders, 1990
Navicula pelliculosa	1 x 10^4 cells/mL	Y	S	a.s.	97.7	am	7.9-8.0	23±2		72 h	EC50	growth rate	72500	2	10	EC, 2005 (Scheerbaum, 2000)
Navicula pelliculosa	1 x 10^4 cells/mL	Y	S	a.s.	97.7	am	7.9-8.0	23±2		72 h	EC50	biomass (AUGC)*	13700	2	10	EC, 2005 (Scheerbaum, 2000)
Pseudokirchneriella subcapitata	1 x 10^4 cells/mL	Y	S	a.s.	98.7	am	8.0	21-23		72 h	EC50	growth rate	31.8	2	10	EC, 2005 (Kubitza, 1998a)
Pseudokirchneriella subcapitata	1 x 10^4 cells/mL	Y	S	a.s.	98.7	am	8.0	21-23		72 h	EC50	biomass (AUGC)*	16.2	2	10	EC, 2005 (Kubitza, 1998a)
Pseudokirchneriella subcapitata	1 x 10^4 cells/mL	Y	S	BAS 479 22 H	43.5 (m/m)	am	8.0	22±1		72 h	EC50	growth rate	11.7	2	15	EC, 2005 (Kubitza, 2000)

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [µg a.s./L]	Ri	Notes	Ref.
Pseudokirchneriella subcapitata	1 x 10^4 cells/mL	Y	S	BAS 479 22 H	43.5 (m/m)	am	8.0	22±1		72 h	EC50	biomass (AUGC)*	7.5	2	15	EC, 2005 (Kubitza, 2000)
Pseudokirchneriella subcapitata	3 x 10^4 cells/mL	N	S	BAS 479 14 H	43.9 (m/m)	am	7.9-8.4	24±1		72 h	EC50	biomass (AUGC)*	9	2	16	EC, 2005 (Dohmen, 1990)
Scenedesmus subspicatus	1 x 10^4 cells/mL	Y	S	FSG 02094 H	45.4 (m/m)	am	7.3-8.3	22.0-23.0		72 h	EC50	growth rate	50	2	10	EC, 2005 (Scheerbaum, 1997)
Scenedesmus subspicatus	1 x 10^4 cells/mL	Y	S	FSG 02094 H	45.4 (m/m)	am	7.3-8.3	22.0-23.0		72 h	EC50	biomass (AUGC)*	29	2	10	EC, 2005 (Scheerbaum, 1997
Scenedesmus subspicatus	1 x 10^4 cells/mL	Y	S	a.s.	97.7	am	7.9-8.1	23±2		72 h	EC50	growth rate	31	2	11	EC, 2005 (Scheerbaum, 2000)
Scenedesmus subspicatus	1 x 10^4 cells/mL	Y	S	a.s.	97.7	am	7.9-8.1	23±2		72 h	EC50	biomass (AUGC)*	8.8	2	11	EC, 2005 (Scheerbaum, 2000)
Scenedesmus acutus		N	S	a.s.	ag	am		23		24 h	EC50	biomass	500	3	3	Grossmann et al. 1992
Scenedesmus acutus	50 µg chorophyll/mL	N	S	a.s.	ag	am		25		3h	EC50	biomass	28	3	2	Couderchet et al., 1998
Scenedesmus acutus	7-15 µg chlorophyll/mL	N	S	a.s.	ag	am		25		50 h	EC50	biomass	1400	3	1	Couderchet et al., 1998
Scenedesmus vacuolatus		Y	S	a.s.	98	am		28		24 h	EC50	biomass	46.6	3	5	Junghans et al., 2003
Macrophyta																
Callitriche palustris	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	EC50	biomass (wwt)	24	2	13	EC 2005 (Kubitza and Dohmen, 2002)
Ceratophyllum demersum	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	EC50	length	24	2	13	EC 2005 (Kubitza and Dohmen, 2002)
Elodea canadensis	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	EC50	biomass (wwt)	73	2	13	EC 2005 (Kubitza and Dohmen, 2002)
Lemna gibba	14 fronds/vessel	Y	S	a.s.	98.7	am	6.6-9.1	25-26		7 d	EC50	growth rate (frond #)	10.7	2	12	EC, 2005 (Dohmen, 1998b)
Lemna gibba	14 fronds/vessel	Y	S	a.s.	98.7	am	6.6-9.1	25-26		7 d	EC50	frond #	4.7	2	12	EC, 2005 (Dohmen, 1998b)
Lemna gibba	3 fronds/plant, 4 plants	Y	R	a.s.	97.7	am	7.5±0.1	25±2		7d	EC50	growth rate (frond #)	7.1	2	14	EC, 2005 (Scheerbaum, 2000)
Lemna gibba	3 fronds/plant, 4 plants	Y	R	a.s.	97.7	am	7.5±0.1	25±2		7d	EC50	biomass (AUGC)*	2.3	2	14	EC, 2005 (Scheerbaum, 2000)

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [µg a.s./L]	Ri	Notes	Ref.
Lemna gibba	4 fronds/plant, 3 plants	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.5-8.9	24-25		7 d	EC50	growth rate (frond #)	14.3	2	12	EC, 2005 (Junker, 2003)
Lemna gibba	4 fronds/plant, 3 plants	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.5-8.9	24-25		7 d	EC50	frond #	2.2	2	12	EC, 2005 (Junker, 2003)
Lemna gibba	9 fronds/vessel	Y	Sc	metazachlor 50 SC	500 g/L	am	7.5-8.5	22-24		7 d	EC50	growth rate (frond #)	14.9	1	24	Juckeland, 2011
Lemna gibba	9 fronds/vessel	Y	Sc	metazachlor 50 SC	500 g/L	am	7.5-8.5	22-24		7 d	EC50	growth rate (dwt)	37.6	1	24	Juckeland, 2011
Lemna gibba	9 fronds/vessel	Y	Sc	metazachlor 50 SC	500 g/L	am	7.5-8.5	22-24		7 d	EC50	yield (frond #)	7.52	1	24	Juckeland, 2011
Lemna gibba	9 fronds/vessel	Y	Sc	metazachlor 50 SC	500 g/L	am	7.5-8.5	22-24		7 d	EC50	yield (dwt)	7.61	1	24	Juckeland, 2011
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	EC50	growth rate (frond #)	> 80	2	25	Wenzel, 2011a
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	NOEC	growth rate (frond #)	20	2	25	Wenzel, 2011a
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	EC50	growth rate (dwt)	> 80	2	25	Wenzel, 2011a
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	NOEC	growth rate (dwt)	10	2	25	Wenzel, 2011a
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	EC50	growth rate (frond area)	> 80	2	25	Wenzel, 2011a
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	NOEC	growth rate (frond area)	10	2	25	Wenzel, 2011a
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	EC50	yield (frond #)	> 80	2	25	Wenzel, 2011a

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [µg a.s./L]	Ri	Notes	Ref.
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	NOEC	yield (frond #)	20	2	25	Wenzel, 2011a
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	EC50	yield (dwt)	> 80	2	25	Wenzel, 2011a
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	NOEC	yield (dwt)	40	2	25	Wenzel, 2011a
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	EC50	yield (frond area)	93.4	2	25	Wenzel, 2011a
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		48 h	NOEC	yield (frond area)	10	2	25	Wenzel, 2011a
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	EC50	growth rate (frond #)	> 400	2	26	Wenzel, 2011b
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	NOEC	growth rate (frond #)	≥ 400	2	26	Wenzel, 2011b
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	EC50	growth rate (dwt)	> 400	2	26	Wenzel, 2011b
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	NOEC	growth rate (dwt)	50	2	26	Wenzel, 2011b
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	EC50	growth rate (frond area)	> 400	2	26	Wenzel, 2011b
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	NOEC	growth rate (frond area)	50	2	26	Wenzel, 2011b
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	EC50	yield (frond #)	> 400	2	26	Wenzel, 2011b
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	NOEC	yield (frond #)	25	2	26	Wenzel, 2011b
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	EC50	yield (dwt)	> 400	2	26	Wenzel, 2011b

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [µg a.s./L]	Ri	Notes	Ref.
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	NOEC	yield (dwt)	<25	2	26	Wenzel, 2011b
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	EC50	yield (frond area)	> 400	2	26	Wenzel, 2011b
Lemna gibba	12 fronds/vessel	Y	R	a.s.	96.7	am	7.6-9.1	23-26		24 h	NOEC	yield (frond area)	<25	2	26	Wenzel, 2011b
Lemna minor	3 fronds/plant, 4 plants	N	S	a.s.	ag	am	5.5	24±2		7 d	EC50	growth rate (frond area)	2.9	2	6	Müller et al., 2010
Lemna minor	3 fronds/plant, 4 plants	N	S	a.s.	ag	am	5.5	24±2		7 d	EC50	growth rate (frond #)	3.8	2	6	Müller et al., 2010
Lemna minor	3 fronds/plant, 4 plants	N	S	a.s.	ag	am				7 d	EC50	growth rate (frond area)	4.7	3	18	Müller et al., 2010
Lemna minor	3 fronds/plant, 4 plants	N	S	a.s.	ag	am				7 d	EC50	growth rate (frond #)	52.9	3	18	Müller et al., 2010
Lemna minor	4 fronds/plant, 3 plants	Y	R	FUEGO SC	45.3 (m/m)	am	6.5±0.2	24±2		7 d	EC50	growth rate (frond #)	14	2	17	EC, 2005 (Scheerbaum, 2004)
Lemna minor	4 fronds/plant, 3 plants	Y	R	FUEGO SC	45.3 (m/m)	am	6.5±0.2	24±2		7 d	EC50	biomass (AUGC)*	22	2	17	EC, 2005 (Scheerbaum, 2004)
Lemna minor	4 fronds/plant, 3 plants	Y	R	FUEGO SC	45.3 (m/m)	am	6.5±0.2	24±2		7 d	EC50	biomass (dwt)	> 45	2	17	EC, 2005 (Scheerbaum, 2004)
Lemna paucicostata	4 fronds/vessel	N	S	a.s.	ag	am		25		8 d	EC50	frond area	106	2	4	Grossmann et al. 1992
Myriophyllum heterophyllum	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	EC50	length	80	2	13	EC 2005 (Kubitza and Dohmen, 2002)
Potamogeton natans	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	EC50	biomass (wwt)	> 174	2	13	EC 2005 (Kubitza and Dohmen, 2002)
Stratiotes aloides	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	EC50	biomass (wwt)	49	2	13	EC 2005 (Kubitza and Dohmen, 2002)

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [µg a.s./L]	Ri	Notes	Ref.
Crustacea																
Daphnia magna	<20 h	N	S	a.s.	90-95	rw	8.3-8.6	21	204	48 h	EC50	mobility	22300	2	20	Panman and Linders, 1990
Daphnia magna	<24 h	Y	S	a.s.	98.7	rw	8.0-8.1	19.2-21	254	48 h	EC50	mobility	33700	2	9	EC, 2005 (Dohmen,2001)
Daphnia magna	<24 h	Y	S	a.s.	97.7	rw	7.5	20.9	255	48 h	EC50	mobility	33000	2	9	EC, 2005 (Noack, 2000)
Daphnia magna	<24 h	Y	S	BAS 479 14 H	43.5 (m/m)	rw	8.0	20.3-21.0	270	48 h	EC50	mobility	>43000	2	9	EC, 2005 (Elendt-Schneider, 1991)
Daphnia magna	<24 h	Y	S	FSG 02094 H	45.4 (m/m)	rw	7.4-7.9	21±1		48 h	EC50	mobility	30000	2	9	EC, 2005 (Noack and Geffke, 1997)
Pisces																
Cyprinus carpio	3.3 cm, 0.68 g	Y	S	a.s.	97.7	dtw	7.2-7.7	20-21.5	69	96 h	LC50	mortality	12300	2	8	EC, 2005 (Scheerbaum, 2000b)
Cyprinus carpio	8.4 cm, 10.0 cm	N	S	a.s.		dtw	7.8	16-20	260	96 h	LC50	mortality	14700	2	23	Panman and Linders. 1990
Lepomis gibbosus	5.8 cm, 2.8 g	N	S	a.s			6.7	22±1	130-260	96 h	LC50	mortality	6800	2	22	Panman and Linders. 1990
Lepomis macrochirus	4.8 cm, 1.4 g	Y	S	a.s.	96.6		8.2-8.5	22	250	96 h	LC50	mortality	10900	2	7	EC, 2005 (Zok, 2001g)
Oncorhynchus mykiss	8.7 cm, 8.8 g	N	S	a.s.		dtw	7.8	16-20	260	96 h	LC50	mortality	4420	2	21	Panman and Linders, 1990
Oncorhynchus mykiss	6.4 cm, 2.4 g	Y	S	a.s.	96.6		8.1-8.5	12	250	96 h	LC50	mortality	8500	2	7	EC, 2005 (Zok, 2001f)
Oncorhynchus mykiss	5 cm, 1.4 g	Y	S	a.s.	97.7	dtw	7.3-7.6	13-14	115	96 h	LC50	mortality	8900	2	8	EC, 2005 (Scheerbaum, 2000a)
Oncorhynchus mykiss	6.2 cm, 3.0 g	Y	S	BAS 479 14 H	43.5 (m/m)		7.0-8.1	12±1	250	96 h	LC50	mortality	4900	2	7	EC, 2005 (Munk and Kitsch, 1990b)
Oncorhynchus mykiss	5.9 cm, 2.8 g	Y	S	FSG 02094 H	43.7 (m/m)	tw	7.0-7.3	15±2	67	96 h	LC50	mortality	4700	2	7	EC, 2005 (Scheerbaum and Geffke, 1997)

* AUGC = Area Under the Growth Curve

Notes

1 Results based on nominal concentrations. Approximate endpoint value. Algae were incubated with one concentration (1.4 mg metazachlor/L, added in ethanol) and the growth was measured by increase of chlorophyll and packed-cell volume (pcv). Chlorophyll increase was inhibited by 73% and pcv by 43%. Since the conditions deviated too much from the usual standard test, the result was not considered to be relevant.
2 Results based on nominal concentrations. Algae were pre-incubated with metazachlor (3 concentrations, added in ethanol) for 30 min. Then ^{14}C-labelled oleic acid was added and incubation continued for 3 h. Thereafter the incorporation of oleic acid into the non-lipid fraction was measured. The EC50 was estimated by the evaluator. The endpoint is not considered to be relevant for MTR derivation.
3 Results based on nominal concentrations. Test carried out in micro-wells. The initial density of the cells was not reported. The incubation period was too short . Ri = 3.
4 Results based on nominal concentrations. Tested in 25 mL medium. The initial frond number was not reported. Yield was measured by the increase of the area of the fronds.
5 Measured concentrations were >80% of nominal. The incubation period was too short.
6 Results based on nominal concentrations. Test carried out according to OECD Guideline 221. Results of log logistic regression used, probit analysis also presented in paper.
7 Measured concentrations were >80% of nominal. Results based on nominal concentrations. Test carried out according to OECD Guideline 203.
8 Measured concentrations were >80% of nominal (with a few exceptions at test initiation). Results based on nominal concentrations. Test carried out according to OECD Guideline 203.
9 Measured concentrations were >80% of nominal. Results based on nominal concentrations. Test carried out according to OECD Guideline 202.
10 Measured concentrations were >80% of nominal. Results based on nominal concentrations. Test carried out according to OECD Guideline 201.
11 Results were based on mean measured concentrations. Test carried out according to OECD Guideline 201.
12 Measured concentrations were >80% of nominal. Results based on nominal concentrations. Test carried out according to Guidelines ASTM E 1415-91 and EPA 850.4400.
13 The plants were potted in a natural standard soil except for Ca. palustris, S. aloides and Ce. demersum, which were free floating within the test vessels. Plants were placed either in tall glass beakers or in small aquaria. These were filled with a 1:1 mixture of 6 x AAP medium and water from a natural lake. Mean concentrations were 99%-109% at test initiation, 84%-119% after 7 days and 61%-98% after 14 days. A correction for loss of test substance was not deemed necessary. Results were based on nominal concentrations.
14 Overall recoveries in the fresh and renewed media were 62% – 90%. The results were based on mean measured concentrations. Test carried out according to Guidelines ASTM E 1415-91 and EPA 850.4400. Test duration 14 days, 7-days endpoints have been used in line with OECD 221.
15 Results were based on initially measured concentrations. Test carried out according to OECD Guideline 201.
16 No analytical verification. Results were based on nominal concentrations. Test carried out according to OECD Guideline 201.
17 Measured concentrations were >80% of nominal. Results based on nominal concentrations. Test carried out according to draft Guideline 221. The biomass (AUGC) was based on log transformed frond numbers.
18 Test carried out largely according to Guideline OECD 221, but filtered mesocosm water was used as medium. Exposure to variable environmental conditions in a mesocosm hall. The test is not considered to be reliable. Ri = 3.
19 The test was carried out according to a BBA guideline proposal with standard OECD sediment on the bottom. The endpoint value was based on the mean measured concentrations in the water phase.
20 Study carried out in 1979. Test carried out according to Methods for Toxicity Tests with Aquatic Organisms (1975) (pre-OECD test).
21 Carried out according to BBA 33. Study carried out in 1979.
22 Carried out according to "the EPA guideline". Study carried out in 1979.
23 Carried out according to BBA 13. Study carried out in 1979 / 1980.
24 Carried out according to OECD 221. Measured concentrations 92-95% of nominal at start, 73-75% at end, result based on mean measured.
25 Test concentrations adapted to simulate exposure profile, 2.5-80 µg/L over 0-24 h, 0.32-11.2 µg/L over 24-48 h, fresh medium thereafter until assessment after 7 d. Measured concentrations >80% of nominal, endpoint based on initial nominal concentration. Exposure duration too short (48 h) and test concentrations not constant. Test is scientifically reliable, but not useful for standard derivation.
26 Test concentrations adapted to simulate single peak, 25-400 µg/L over 0-24 h, fresh medium thereafter until assessment after 7 d. Measured concentrations >80% of nominal, endpoint based on initial nominal concentration. Exposure duration too short (24 h). Test is scientifically reliable, but not useful for standard derivation.

Table A1.2. Chronic toxicity of metazachlor to freshwater organisms

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [µg a.s./L]	Ri	Notes	Ref.
Cyanobacteria																
Anabaena flos-aquae	3 x 10^4 cells/mL	Y	S	a.s.	98.7	am	7.5-7.7	23-25		96 h	EC10	growth rate	13900	2	3	EC, 2005 (Kubutza, 1998b)
Anabaena flos-aquae	3 x 10^4 cells/mL	Y	S	a.s.	98.7	am	7.5-7.7	23-25		96 h	EC10	biomass (AUGC)*	6200	2	3	EC, 2005 (Kubutza, 1998b)
Algae																
Chlorella fusca	2 x 10^4 cells/mL	N	S	a.s	90-95	am	7.1	20		96 h	NOEC	growth rate	340	2		Panman and Linders, 1990
Navicula pelliculosa	1 x 10^4 cells/mL	Y	S	a.s.	97.7	am	7.9-8.0	23±2		72 h	NOEC	growth rate	320	2	3	EC, 2005 (Scheerbaum, 2000)
Navicula pelliculosa	1 x 10^4 cells/mL	Y	S	a.s.	97.7	am	7.9-8.0	23±2		72 h	NOEC	biomass (AUGC)*	1000	2	3	EC, 2005 (Scheerbaum, 2000)
Pseudokirchneriella subcapitata	1 x 10^4 cells/mL	Y	S	a.s.	98.7	am	8.0	21-23		72 h	EC10	growth rate	6.1	2	3	EC, 2005 (Kubitza, 1998a)
Pseudokirchneriella subcapitata	1 x 10^4 cells/mL	Y	S	a.s.	98.7	am	8.0	21-23		72 h	EC10	biomass (AUGC)*	3.6	2	3	EC, 2005 (Kubitza, 1998a)
Pseudokirchneriella subcapitata	1 x 10^4 cells/mL	Y	S	BAS 479 22 H	43.5 (m/m)	am	8.0	22±1		72 h	EC10	growth rate	5.4	2	4	EC, 2005 (Kubitza, 2000)
Pseudokirchneriella subcapitata	1 x 10^4 cells/mL	Y	S	BAS 479 22 H	43.5 (m/m)	am	8.0	22±1		72 h	EC10	biomass (AUGC)*	3.6	2	4	EC, 2005 (Kubitza, 2000)
Scenedesmus subspicatus	1 x 10^4 cells/mL	Y	S	a.s.	97.7	am	7.9-8.1	23±2		72 h	NOEC	growth rate, biomass (AUGC)*	1.8	2	4	EC, 2005 (Scheerbaum, 2000)
Scenedesmus subspicatus	1 x 10^4 cells/mL	Y	S	FSG 02094 H	45.4 (m/m)	am	7.3-8.3	22.0-23.0		72 h	EC10	growth rate	15	2	3	EC, 2005 (Scheerbaum, 1997)
Scenedesmus subspicatus	1 x 10^4 cells/mL	Y	S	FSG 02094 H	45.4 (m/m)	am	7.3-8.3	22.0-23.0		72 h	EC10	biomass (AUGC)*	5	2	3	EC, 2005 (Scheerbaum, 1997
Scenedesmus vacuolatus		Y	S	a.s.	98	am		28		24 h	NOEC	biomass	15.3	3	1	Junghans et al., 2003
Macrophyta																
Callitriche palustris	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	NOEC	biomass (wwt)	5	2	9	EC 2005 (Kubitza and Dohmen, 2002)
Ceratophyllum demersum	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	NOEC	biomass (wwt), length	10	2	9	EC 2005 (Kubitza and Dohmen, 2002)

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [µg a.s./L]	Ri	Notes	Ref.
Elodea canadensis	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	NOEC	biomass (wwt), length	31.5	2	9	EC 2005 (Kubitza and Dohmen, 2002)
Lemna gibba	14 fronds/ vessel	Y	S	a.s.	98.7	am	6.6-9.1	25-26		7 d	NOEC	growth rate (frond #)	0.6	2	5	EC, 2005 (Dohmen, 1998b)
Lemna gibba	3 fronds/plant, 4 plants	Y	R	a.s.	97.7	am	7.5±0.1	25±2		7 d	NOEC	growth rate (frond #)	0.193	2	6	EC, 2005 (Scheerbaum, 2000)
Lemna gibba	3 fronds/plant, 4 plants	Y	R	a.s.	97.7	am	7.5±0.1	25±2		7 d	NOEC	biomass (AUGC)*	0.1	2	6	EC, 2005 (Scheerbaum, 2000)
Lemna gibba	4 fronds/plant, 3 plants	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.5-8.9	24-25		7 d	EC10	growth rate (frond #)	0.36	2	7	EC, 2005 (Junker, 2003)
Lemna gibba	4 fronds/plant, 3 plants	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.5-8.9	24-25		7 d	EC10	biomass	0.07	4	7,20	EC, 2005 (Junker, 2003)
Lemna gibba	9 fronds/vessel	Y	Sc	metazachlor 50 SC	500 g/L	am	7.5-8.5	22-24		7 d	NOEC	growth rate (frond #)	2.48	1	16	Juckeland, 2011
Lemna gibba	9 fronds/vessel	Y	Sc	metazachlor 50 SC	500 g/L	am	7.5-8.5	22-24		7 d	NOEC	growth rate (dwt)	0.245	1	16	Juckeland, 2011
Lemna gibba	9 fronds/vessel	Y	Sc	metazachlor 50 SC	500 g/L	am	7.5-8.5	22-24		7 d	NOEC	yield (frond #)	2.48	1	16	Juckeland, 2011
Lemna gibba	9 fronds/vessel	Y	Sc	metazachlor 50 SC	500 g/L	am	7.5-8.5	22-24		7 d	NOEC	yield (dwt)	0.772	1	16	Juckeland, 2011
Lemna minor	3 fronds/plant, 4 plants	N	S	a.s.	ag	am	5.5	24±2		7 d	EC10	growth rate (frond #)	0.6	2	2	Müller et al., 2010
Lemna minor	3 fronds/plant, 4 plants	N	S	a.s.	ag	am	5.5	24±2		7 d	EC10	growth rate (frond area)	0.8	2	2	Müller et al., 2010
Lemna minor	3 fronds/plant, 4 plants	N	S	a.s.	ag	am				7 d	EC10	growth rate (frond #)	1.5	3	19	Müller et al., 2010
Lemna minor	3 fronds/plant, 4 plants	N	S	a.s.	ag	am				7 d	EC10	growth rate (frond area)	1.1	3	19	Müller et al., 2010

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [µg a.s./L]	Ri	Notes	Ref.
Lemna minor	4 fronds/plant, 3 plants	Y	R	FUEGO SC	45.3 (m/m)	am	6.5±0.2	24±2		7 d	NOEC	growth rate (frond #), biomass (AUGC)*, dwt	1.4	2	8	EC, 2005 (Scheerbaum, 2004)
Myriophyllum heterophyllum	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	NOEC	biomass (wwt)	20	2	9	EC 2005 (Kubitza and Dohmen, 2002)
Potamogeton natans	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	NOEC	biomass (wwt)	≥200	2	9	EC 2005 (Kubitza and Dohmen, 2002)
Stratiotes aloides	Submerged	Y	S	BAS 479 22 H	43.5 (m/m)	am	7.8-8.2	19.4-20	165	14 d	NOEC	biomass (wwt)	5	2	9	EC 2005 (Kubitza and Dohmen, 2002)
Crustacea																
Daphnia magna	<24 h	Y	R	a.s.	≥90	rw	7.9-8.2	19-21	263-297	21 d	NOEC	reproduction, mortality	6250	2	13	EC, 2005 (Jatzek and Bias, 1990)
Daphnia magna	2-24 h	Y	R	a.s.	97.7	rw		20±2	160-180	21 d	NOEC	reproduction	100	2	14	EC, 2005 (Noack, 2000)
Daphnia magna	<24 h	Y	R	BAS 479 14 H	43.5 (m/m)	rw	7.7-8.1	18.9-21.5	270±50	21 d	NOEC	reproduction	22000	2	13	EC, 2005 (Elendt-Schneider, 1991b)
Insecta																
Chironomus riparius	larvae, 2-3 d old	Y	S	a.s.	97.9	rw	6.6-8.1	18.8-21.7		28 d	NOEC	emergence	5700	2	15	EC, 2005 (Scheerbaum, 2000)
Pisces																
Oncorhynchus mykiss	5 cm, 1.4 g	Y	R	a.s.	97.7	dtw	7.2-7.6	12.7-15.2	77	28 d	NOEC	growth	2500	2	11	EC, 2005 (Scheerbaum, 2000)
Oncorhynchus mykiss	6.1 cm, 2.4 g	Y	F	a.s.	98.7		7.7-8.1	14-16	230-240	28 d	NOEC	growth	2150	2	10	EC, 2005 (Munk and Kirsch, 1990)

* AUGC = Area Under the Growth Curve

Notes

1 Measured concentrations were >80% of nominal. Results were based on nominal concentrations. The incubation period was too short. Because better tests are available the results are not considered for the risk assessment.
2 Results based on nominal concentrations. Test carried out according to OECD Guideline 221. Results of log logistic regression used, probit analysis also presented in paper.
3 Measured concentrations were >80% of nominal. Results based on nominal concentrations. Test carried out according to OECD Guideline 201.
4 Results were based on mean measured concentrations. Test carried out according to OECD Guideline 201.
5 Measured concentrations were >80% of nominal. Results were based on nominal concentrations. Test carried out according to Guidelines ASTM E 1415-91 and EPA 850.4400.
6 Overall recoveries in the fresh and renewed media were 62% – 90%. The results were based on mean measured concentrations (except for the growth rate). Test carried out according to Guidelines ASTM E 1415-91 and EPA 850.4400.

7 Measured concentrations were >80% of nominal. Results were based on nominal concentrations. Test carried out according to Guidelines ASTM E 1415-91 and EPA 850.4400. The EC10 values were calculated by the evaluator.

8 Measured concentrations were >80% of nominal. Results were based on nominal concentrations. Test carried out according to draft Guideline 221. The biomass (AUGC) was based on log transformed frond numbers.

9 The plants were potted in a natural standard soil except for Ca. palustris, S. aloides and Ce. demersum, which were free floating within the test vessels. Plants were placed either in tall glass beakers or in small aquaria. These were filled with a 1:1 mixture of 6 x AAP medium and water from a natural lake. Mean concentrations were 99%-109% at test initiation, 84%-119% after 7 days and 61%-98% after 14 days. A correction for loss of test substance was not deemed necessary. Results were based on nominal concentrations.

10 Measured concentrations were 74%-111% of nominal. Results were based on nominal concentrations. Test carried out according to OECD Guideline 204.

11 Measured concentrations were 80%-110% of nominal, except for the lowest test concentration (77%). Results were based on nominal concentrations. Test carried out according to OECD Guideline 204.

12 Measured concentrations were >80% of nominal. Results were based on nominal concentrations. Test carried out according to Guideline 204.

13 Measured concentrations were >80% of nominal. Results were based on nominal concentrations. Test carried out according to Guideline EEC XI/681/86.

14 Measured concentrations were >80% of nominal, except for the lowest test concentration (66-82%). Results were based on nominal concentrations. Test carried out according to OECD Guideline 211.

15 The test was carried out according to a BBA guideline proposal with standard OECD sediment on the bottom. The endpoint value was corrected for mean measured concentrations in the water phase.

16 Carried out according to OECD 221. Measured concentrations 92-95% of nominal at start, 73-75% at end, result based on mean measured.

17 Test concentrations adapted to simulate exposure profile, 2.5-80 μg/L over 0-24 h, 0.32-11.2 μg/L over 24-48 h, fresh medium thereafter until assessment after 7 d. Measured concentrations >80% of nominal, endpoint based on initial nominal concentration. Exposure duration too short (48 h) and test concentrations not constant. Test is scientifically reliable, but not useful for standard derivation.

18 Test concentrations adapted to simulate single peak, 25-400 μg/L over 0-24 h, fresh medium thereafter until assessment after 7 d. Measured concentrations >80% of nominal, endpoint based on initial nominal concentration. Exposure duration too short (24 h). Test is scientifically reliable, but not useful for standard derivation.

19 Test carried out largely according to Guideline OECD 221, but filtered mesocosm water was used as medium. Exposure to variable environmental conditions in a mesocosm hall. The test is not considered to be reliable. Ri = 3.

20 Not clear which parameter endpoint refers to. Results are presented for inhibition of frond number, which would imply yield, but endpoint is denoted as EbC50 for biomass, which could be measured as area under the growth curve.

Annex 2 Field studies

Study 1

Species/Population/Community	Zooplankton, phytoplankton, periphyton
Test Method	Outdoor microcosms
System properties	2.48 m x 1.65 m x 1.25 m
Formulation	Not specified
Analyzed	Y
Exposure regime	Single application
Experimental time	49 d
Criterion	NOEC
Test endpoint	Abundance
Value [µg/L]	Not derived
Ri	3
Reference	Noack et al., 2003

Description

Test system.
8 outdoor microcosms (polyethyleen, 2.48 m x 1.65 m x 1.25 m, surface 4 m^2) were filled with natural ground water (4 weeks before test substance application at August 2, 2000). The zoo- and phytoplankton were introduced into the microcosms with communities from a natural surface water.
Cosms were treated once at 0.001, 0.032, 0.1, 3.2 and 10 mg a.s./L, and an untreated control. One pond per treatment, three replicates for control. In one of the untreated control ponds, a massive bleu-green alga bloom was observed at day 40. Therefore, this pond was excluded from the analyses.
Analytical sampling.
Water was sampled at day 0, 1, 7, 14, 28 and 49 after application. The two cosms with the highest phytoplankton density were used for the highest dose and as untreated control.
Effect sampling.
Abiotic parameters (O_2, pH, temperature, turbidity and conductivity) were assessed 4 times during the first week and weekly thereafter. Phytoplankton was sampled three times per week with a 1 L integrated water column sampler. Chlorophyll-a fluorescence was measured immediately after sampling. Zooplankton was sampled weekly using a 1 L Rüttner sampler, and at the end of the experiment with a net (55 µm). Counting and taxonomic determination were done with an inverse microscope in 2 mL plankton chambers. Further details about level of determination were not available. Zooplankton data are regarded as semi quantitative. Phytoplankton growth was determined on glass slides (7.5 x 2.5 0.2 cm). 20 slides per pond at 30 cm water depth. Periphyton and chlorophyll-a were analysed on day 0, 1, 7, 14, 21, 28 and 49 after application.
Statistical analysis
Statistical analysis was not possible because the number of replicates was too low.

Results

Chemical analysis.
Analysis of metazachlor confirmed nominal concentrations. At day 0 concentrations in the 0-50 cm layer were higher than nominal (196-435% of nominal), but after 1 day concentrations were 93-124% of nominal. During the experiment, concentrations slowly decreased to 20-66% of nominal after 7 weeks.
Abiotic parameters
pH increased during the study from 7-8 to 9-10. Oxygen increased in the first week, and then dropped to 50% in the two ponds with the highest dosage. At day 16 oxygen concentrations were dose related for the three highest dosages (low oxygen at higher dosage).

Biological observations.

Phytoplankton
For phytoplankton, density remained at low levels for all cosms incl. the control till day 30. After day 30 phytoplankton density increased. In the highest dosage the most strong decrease was found and recovery started latest. Green algae seemed to be less abundant in the highest dosages, in line with laboratory data.

Periphyton
In the highest dose no periphyton growth was observed till day 30. Periphyton growth was presented as chlorophyll-a only. No treatment related effects were observed. The control cosms showed a very high variation.

Zooplankton
Biodiversity was low. Only *Keratella quadrata*, *Chydorus thienemanni*, *Daphnia pullex* and *Polyarthra remata* were found frequently. *Keratella quadrata* was the most dominant species. No treatment related response was observed.

Evaluation of the scientific reliability of the field study
Criteria for a suitable (semi)field study
1. Does the test system represent a realistic freshwater community? No. Biodiversity is poor, no macrophytes or macro-invertebrates were included.
2. Is the description of the experimental set-up adequate and unambiguous? No, unclear what the constitution of the added water was, what organisms were introduced, how mixing between ponds was done, etc.
3. Is the exposure regime adequately described? Yes.
4. Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Partly, algae are sensitive. Macrophytes are however sensitive as well and were not incorporated in the cosms study.
5. Is it possible to evaluate the observed effects statistically? No, only one replicate for treatments, large variation between controls, no data provided in the paper.

This results in an overall assessment of the study reliability, -> Ri 3, not reliable. The only result that could be used for comparison with other studies is that at 10 mg/L clear effects were observed, with a tendency to recover after 30 d.

Study 2

Species/Population/Community	Zooplankton, phytoplankton, macrophytes
Test Method	Indoor ponds and streams
System properties	Ponds: 6.90 m x 3.35 m x 2.50 m; Streams 106 m long, 1 m wide, 4 pool sections of 3 m and 1.2 m wide
Formulation	Not specified
Analyzed	Y
Exposure regime	Single application
Experimental time	140 d
Criterion	NOEC
Test endpoint	Abundance
Value [µg/L]	5 µg/L , < 5 µg/L
Ri	2
Reference	Mohr et al., 2007,2008

Description

Test system.
Ponds: Eight indoor mesocosms (lxwxh = 6.90 m x 3.35 m x 2.50 m) were filled with 100,000 kg of sand (0.2 mm grains size), slope 1:2, 15 m^3 water, max water depth 1.25 m. Mesocosms were set up in February 2002. The sand surface was covered with sediment from an uncontaminated lake.
Streams: Eight indoor streams (106 m long, 1 m wide, with 4 pool sections of 3 m and 1.2 m wide, 0.45 m water, 12 cm sand and sediment as in the cosms). Water volume 40 m^3.
Ponds were planted with 5 *Polygonum amphibium*, 21 *Potamogeton natans*, *14 Myriophyllum verticillatum* and bunches *Chara vulgaris*. In addition *Carex* spec, *Iris pseudocorus* and *Myosotis palustris* were planted.
In the streams, in two of the four pool sections in each stream 15 *P. natans* and 15 *M. verticilatum* were introduced. *Elodea canadensis*, three *Berula erecta*, two *Nymphoides peltata* and *Callitriche palustris* have also been introduced.
All systems were equally stocked on several occasions with plankton and macrozoobenthos from nearby oligotrophic lakes and rivers, including Porifera, Hydridae, *Lymnaea stagnalis*, Planorbidae, Bivalvia, Hirudinea, Hydrachnellae, *Asellus aquaticus*, Hydrocorisae, Zygoptera, Anisoptera, Notonectidae, Gerridae, Dytiscinae. In addition, each stream received 320 *Gammarus pulex* and *Gammarus tigrinus* as well as 12 kg wet weight of watered foliage (*Alnus glutinosa*) for these shredder feeders. Further more 500 g *Tubifex* and 3000 *Chaoborus* were introduced in all systems, in the ponds 2000 *Culex* and in the streams 670 g *Chironomus.*
Systems were spiked with 5, 20, 80 200 and 500 µg/L metazachlor at 2 June 2003. Metazachlor, 98.2% pure was sprayed on the water surface and mixed during 5 min. One system per treatment, three replicates for the untreated control.
Analytical sampling.
Concentrations were measured for 140 d in the ponds and for 170 d in the streams.
Effect sampling.
Abiotic parameters (O_2, pH, temperature, turbidity, conductivity and current velocity) in the streams were measured on-line. In addition, every four days standard parameters (pH, conductivity, alkalinity, silicate, phosphate and nitrite and nitrate) and plankton were measured in all systems. Growth of macrophytes was assessed every four days using a photo and analyses of these images. At

the end of the experiment plants were harvested and biomass was determined (wet weight and dry weight and ash free dry weight). Phytoplankton was sampled at 50 cm depth in the ponds and at 10 cm in the streams and chlorophyll-a fluorescence was measured immediately after sampling. Zooplankton was sampled weekly using a depth-integrated sampler. Effects were evaluated till 140 d after exposure.
Statistical analysis
No ANOVA or multivariate approach could be applied due to a limited number of cosms. EC50 values were calculated using a log-logistic model.

Results

Chemical analysis.
Analysis of metazachlor confirmed nominal concentrations. Recovery immediately after dosing was 93-119% of nominal. The DT_{50} for dissipation in water was determined as 37-48 d in ponds and 27-44 d in streams. At the end of the experiment concentrations were 4.2-15.7% of nominal.
Abiotic parameters
Physicochemical parameters reflected the growth of biomass and algae. Conductivity decreased in cosms with the highest growth. Differences were present till the end of the experiment.

Biological observations.

Macrophytes
Floating leaves of *P. natans* in the mesocosms showed a decrease in the two highest dosages (200 and 500 µg/L). In the 5 and 80 µg/L dosages an effect on growth appeared to be present, however the 20 µg/L treatment showed a higher growth than the control. Growth in the streams was much lower, so that it was also more difficult to detect differences between treatments.
For biomass, a decrease was found with an increase of the dose. In the streams, clear effects on total macrophyte biomass were present at 20 µg/L and higher.

Phytoplankton
During the experiment, 71 species were identified in the stream and 61 in the ponds. Before application, a peak of chlorophytes occurred in the ponds. At the time of application, cosms were dominated by cryptophytes. In the ponds the PRC showed significant differences between treatment and control. *Cryptomonas erosa* and *R. minuta* were positively affected, most phytoplankton species were unaffected.
In the streams phytoplankton communities mainly consisted of chlorophytes and cyanobacteria. In the streams the community changed with increasing metazachlor concentrations. Chlorophytes and diatoms were found in the control and the 5 µg/L treatment, in the higher treatments chlorophytes were absent. In the PRC the 5 µg/L showed an opposite trend as compared to the higher concentrations and the control.
For filamentous algae, the effects were clear from the 20 µg/L dose and higher both in the ponds and the streams.

Zooplankton
A total of 49 and 34 zooplankton species were identified in the streams and ponds, respectively. Before application, communities were very similar. The PRC did not show significant effects, but a clear dose related response was seen. Individual species (*Keratella quadrata, Polyarthra dolichoptera, Ceriodaphnia quadrangular*) *indicated* a response even in the lowest dose.

Evaluation of the scientific reliability of the field study
Criteria for a suitable (semi)field study

1. Does the test system represent a realistic freshwater community? Yes. Macrophytes, phytoplankton and zooplankton were present. Macro-invertebrates were not reported. Given the long period before application, a stable ecosystem appears to be present.
2. Is the description of the experimental set-up adequate and unambiguous? Yes.
3. Is the exposure regime adequately described? Yes.
4. Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Yes, algae and macrophytes included, but most sensitive species from laboratory studies (*Lemna* sp.) is not present. Since macro-invertebrates were not reported, eventual indirect effects could not be studied.
5. Is it possible to evaluate the observed effects statistically? PRC results are given. However only one replicate for treatments, large variation between controls, no data provided in the paper.

This results in an overall assessment of the study reliability, -> Ri 2, less reliable. From the study it is clear that at 20 µg/L effects are found. However even in the lowest dose (5 µg/L) for some groups effects are found, but effects were not significant. Given the variation in the control, results should be handled with care. Because effects were often yes or no effects (species present or not) it is not possible to calculate a reliable EC10 value.

Study 3

Species/Population/Community	Zooplankton, phytoplankton, periphyton, macrophytes, macrozoobenthos
Test Method	Outdoor mesocosm
System properties	Diameter 143 cm, depth 150 cm, waterbody 120 cm
Formulation	Fuego 500 SC
Analyzed	Y
Exposure regime	Single application
Experimental time	82 d
Criterion	NOEC
Test endpoint	Abundance
Value [µg/L]	2 µg/L
Ri	2
Reference	Hommen et al., 2007 in DAR (EC, 2005; addendum)

Description

Test system.
Fifteen outdoor mesocosms (diameter 143 cm, depth 150 cm, waterbody 120 cm) were pressed into the sediment of an artificial pond, sediment layer 10-15 cm. Start 43 d before application on June 8, 2006. Ponds were established in November 2005. Cosms contained various macrophytes. Three individuals of *Stratoites aloides* were introduced 21 days before application and 40 individuals of *Lemna* sp. were introduced one day before application.
pH of the water 7.07-9.80, OC content of sediment 1.1%.

Systems were treated with Fuego 500 SC, 511.8 g/L metazachlor. Systems treated with 2.0, 5.0, 7.9, 12.5 and 31.3 µg a.s./L. Two systems per treatment, five replicates for control. Application 15 cm below water surface by moving the outlet of the funnel in a circular way.
Analytical sampling.
Concentrations were measured on day 0, 2, 7, 12, 26, 54 and 82. Sediment samples in the same days from the highest dose only (and 2 controls).
Effect sampling.
Physical parameters were measured weekly, chemical parameters biweekly. Zooplankton was sampled by depth-integrated sampling, rotatoria, Crustacea and Insecta were identified and counted as groups, Crustacea were identified to species level as far as possible without preparation. Macrozoobenthos traps were sampled before application, and after application for 2 weeks. Living species were identified and replaced in the appropriate enclosure. Phytoplankton by depth integrated sampling, identified to genus level. Periphyton on glass plates placed 43 d before application, only chlorophyll-a analyses. Macrophytes were visually observed and mapped (day 5 and 12 and every 2 weeks until the end of study). Lemna was counted on day 2, 6 and 11 and then once a week. Fresh weight of introduced *Stratoites aloides* was monitored weekly or biweekly.
Statistical analysis
Community level: PRC, diversity and similarity indices, Williams-test for NOEC.
Population level: Williams-test for NOEC, non-linear regression for EC50.

Results

Chemical analysis.
Analysis of metazachlor confirmed nominal concentrations. On day 1 and 2 measured values were 136-166% of nominal, after one week this was 93-110%. Mean DT50 for dissipation from the water phase was 33 d. At the end of the experiment (day 82) concentrations were 23-28% of nominal. Although in the sediment the concentrations were calculated below LOQ, metazachlor was found in one of the two enclosures of the highest treatment.
Abiotic parameters
Oxygen concentration was significantly increased in all treatments, on one sampling date, and on more sampling dates in the two highest treatments. pH and conductivity were significantly affected from the 7.9 µg/L treatment and higher.

Biological observations.

Macrophytes
Most sensitive responses: *Ceratophyllum demersum* and *Chara intermedia*: affected at 7.9 µg/L nominal and higher, and *Lemna* spec at 5.0 µg/L nominal and higher.

Phytoplankton
Significant effects on phytoplankton community were found in the two highest treatments (12.5 and 31.3 µg a.s./L) for total cells, but for other parameters in the highest treatment only. For dominant classes, effects in the two highest treatments were found for Bacillariophyceae and Chlorophyceae, and for others in the highest dose only. For individual species the most sensitive are Chrysophyceae, showing a significant effect on one sampling occasion in the 7.9 µg/L treatment.

Periphyton
Significant effects in highest treatment only.

Zooplankton
Main zooplankton groups *Phyllopoda*, *Branchiura* and *Daphnia* were significantly affected in the two highest treatments. *Diaptomidae* sum were affected in all treatments on one sampling date.

Macrozoobenthos
Total abundance increased in all treatments on 1 sampling date. Annelida probably caused this increase, of the individual species *Tubificidae* showed significant effects in all treatments. *Helobdella stagnalis* showed a significant increase at day 42 and 56 at all dosages.

The evaluators in the DAR conclude that 2.0 μg/L can be considered as the overall NOEC. Effects at this concentration are considered very minor, short-lived and not considered to be treatment related.

Evaluation of the scientific reliability of the field study
Criteria for a suitable (semi)field study

1. Does the test system represent a realistic freshwater community? Yes. Macrophytes, phytoplankton, periphyton, zooplankton and macroinvertebrates were present.
2. Is the description of the experimental set-up adequate and unambiguous? Yes.
3. Is the exposure regime adequately described? Yes.
4. Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Yes, algae and macrophytes included.
5. Is it possible to evaluate the observed effects statistically? Yes, different kind of analyses are reported. No data provided in the paper.

This results in an overall assessment of the study reliability, -> Ri 2, less reliable. From the study, it is clear that at 5 μg/L effects are found. However even in the lowest dose (2 μg/L) some effects are found. These effects are however not deemed treatment related. There are some effects on macro-invertebrates (mainly increases), while laboratory studies and the mode of action of the substance indicate that macrophytes and algae belong to the most sensitive groups. According to the authors, the observed differences are not related to concentration and are mainly due to low numbers in the control group. Therefore, they are not considered to be treatment related. This explanation is accepted. Given the small effects on the potentially most sensitive species it is not very likely that indirect effects on macro-invertebrates have occurred.

Summary of field study results

The results of the field studies are summarized below:

	NOEC (µg a.s./L initial)	Ri
Field study 1	<10000	3
Field study 2	< 5	2
Field study 3	2	2

From the results it is concluded that 2 µg/L can be used as the NOEC from the field studies. The lowest laboratory NOEC was generated for *Lemna gibba.* Following the recommendations of Brock et al. (2011), the test duration of 7 d is used to calculate the time weighted average exposure concentration in the mesocosm study for derivation of the MPC, since the 7-days NOEC of *Lemna gibba* is the most sensitive endpoint from laboratory tests. Given the DT50 of 33 d the NOEC expressed as TWA is rounded to 1.9 µg/L. For derivation of the MAC, the 48/72 hours TWA concentration is used, which is rounded to 2.0 µg/L.

Annex 3 SCOPUS profile

1. TITLE-ABS-KEY(effect* OR bioassay* OR toxic* OR ecotoxic* OR mortalit* OR sensitiv* OR phytotox* OR assessment* OR reproduct* OR lethal* OR response* OR growth OR teratogen*) OR TITLE-ABS-KEY(ec50* OR ec20* OR ec10* OR lc50* OR lc20* OR lc10* OR noec* OR loec* OR matc OR tlm OR chv OR ecx OR bioassay*) OR TITLE-ABS-KEY(bioconcentrat* OR bioaccumulat* OR uptake OR depuration OR food-web OR trophic OR biomagnificat* OR BCF* OR BAF* OR FWMF* OR TMF* OR BMF* OR BSAF*) OR TITLE-ABS-KEY(sorpt* OR adsorpt* OR freundlich OR koc* OR kd* OR kp* OR kf* OR partition-coefficient*)

2. TITLE-ABS-KEY(metazachlor OR metazachloor) OR CASREGNUMBER(67129-08-2)

#1 AND #2

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