VSP Adviesrapport 14413d01

Azoxystrobine
Afleiding van het JG-MKN- en MAC-MKN-water

R. van Herwijnen, F. de Jong, P.L.A. van Vlaardingen

11-10-2017

Opdrachtgegevens

VSP rapportnummer	14413d01
Projectnummer	E/124016/03/EC
Opdrachtgever	Ctgb
Ctgb briefnummer	201704140143
Ctgb aanvraagnummer	20170054 MTR
Datum opdracht	14 april 2017
Datum rapportage	11 oktober 2017
Auteur(s)	R. van Herwijnen, F. de Jong, P.L.A. van Vlaardingen
Toetser	M.H.M.M. Montforts, datum toetsing: 3 juli 2017 Toetsing petit comité (schriftelijke ronde): 1-7-2017 to 1-8-2017
Opdracht	Dit adviesrapport betreft de afleiding van de waterkwaliteitsnormen JG-MKN en MAC-MKN (AA-EQS en MAC-EQS) voor azoxystrobine.
Opmerkingen	Versie d01 bevat twee aanpassingen. In paragraaf 3.3.5 is de waarde van de AA-EQS_{fw} en AA-EQ_{sw} gecorrigeerd. Daar stonden nog oude waarden (0.28 µg/L en 0.028 µg/L); deze zijn vervangen door de juiste waarden: 0.20 µg/L en 0.020 µg/L. In de commentaartabel van het petit comité (opm. bij §3.2.2. over *unbound values*) is de verwijzing naar de aanpassing in de tekst geactualiseerd.

Kwaliteitsprocedures en beoordelingskader

De afleiding van de waterkwaliteitsnormen in dit rapport is opgesteld in overeenstemming met de vigerende VSP kwaliteitsprocedures. De afleiding is beoordeeld door de leden van de Wetenschappelijke Klankbordgroep Normstelling water en lucht.

Contents

Introduction

1.1 General

Azoxystrobin is a fungicide that is approved as plant protection product. For the Netherlands, an indicative MPC for surface water is available of 0.056 µg/L [66]. The Ctgb commissioned RIVM to update the dataset on azoxystrobin with new literature and derive EQSs according to the WFD-methodology. The resulting values can be used by Ctgb in the re-registration process when monitoring data have to be compared with water quality standards according to the agreed procedures.

1.2 Standards considered

Under the WFD, two types of EQSs are derived to cover both long- and short-term effects resulting from exposure:

- an Annual Average EQS (AA-EQS) – a long-term standard, expressed as an annual average concentration (AA-EQS) which should protect the ecosystem against adverse effects resulting from long-term exposure, and
- a Maximum Acceptable Concentration EQS (MAC-EQS) for aquatic ecosystems – the concentration protecting aquatic ecosystems from effects due to short-term exposure or concentration peaks.

Concentrations below the AA-EQS should not result in risks due to direct toxicity, secondary poisoning and/or risks for human health aspects. The latter two aspects are therefore also addressed in the AA-EQS, when triggered by the characteristics of the compound (i.e. human toxicology and/or potential to bioaccumulate). The MAC-EQS is based on direct ecotoxicity only. In the context of authorisation of plant protection products, only freshwater EQSs are used. However, for the purpose of EQS derivation, toxicity data on salt water species data are used as well. The total available dataset allows for derivation of freshwater and saltwater EQSs and since these values may be used for other purposes as well, standards for the saltwater environment are also derived in this report.

For authorisation of plant protection products, transient ecotoxicological effects may be considered acceptable under certain conditions if the potential for recovery is demonstrated. However, the quality standards in the context of the WFD refer to the absence of any impact on community structure of aquatic ecosystems. Hence, long-term undisturbed function is the protection objective under the WFD. Therefore, recovery in a test situation, after a limited exposure time, is not included in the derivation of the AA- and MAC-EQS.

1.3 Methodology

1.3.1 Guidance documents

The methodology used for ERL derivation is in accordance with the European guidance document for derivation of environmental quality standards under the WFD [15]. This document is

further referred to as the WFD-guidance. For those aspects that may not be fully covered by the WFD-guidance, additional information can be found in national guidance documents [4, 58, 53].

1.3.2 Data sources

For the derivation of the quality standards for azoxystrobin, studies used in the Draft Assessment Report (DAR) prepared within the context of Commission Regulation 737/2007 are used as basis [14]. Additionally a literature search was performed with SCOPUS (www.scopus.com) to find additional literature not included in the DAR. In 2016 a Swiss EQS derivation was published [19], the studies used in the Swiss report were checked for completeness of the dataset in this report. In the conclusion of the present report, the outcomes of the Swiss study are compared to our results.

1.3.3 Data evaluation

The new ecotoxicological data, not used in the DAR, were evaluated with respect to the validity (scientific reliability) of the study. Reliability indices (Ri) of 1 to 4 were assigned according to Klimisch et al. [38], with Ri 1: fully reliable, Ri 2: reliable with restrictions, Ri 3: not reliable and Ri 4: not assignable. A detailed description of the evaluation procedure is given in WFD-guidance [15].

The data used in the DAR, physico-chemical and ecotoxicological endpoints, are not re-evaluated and included in the report with a reliability of Ri 2. In some cases additional data is obtained from the original study report to provide sufficient information on the studies.

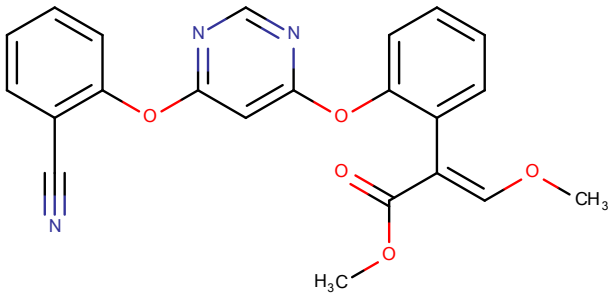
Occasionally, endpoints reported in the DAR exceed the water solubility as reported in Table 2 of this report. According to the WFD guidance, endpoints exceeding the water solubility with more than a factor 2 should not be used for the EQS derivation. This factor could be increased to 3 when the available data on the water solubility has a variation higher than a factor 2. The available endpoints on water solubility of azoxystrobin range from 5.9 to 6.7 mg/L depending of the pH (see Table 2), therefore the cut off value is set at 11.8 or 13.4 mg/L depending on the pH of the medium. Test media with a pH higher than 7 have been assessed against the appropriate value of 11.8 mg/L, test media with a pH of ≤7 against the value of 13.4 mg/L. It should be noted that choice of the value for the water solubility did not affect the rejection of endpoints because there were no endpoints in between the values of 11.8 and 13.4 mg/L.

Endpoints based on nominal test concentrations are accepted as sufficiently reliable for EQS derivation because azoxystrobin has characteristics that indicate that the substance is not likely to dissipate rapidly from the water phase: it has low vapour pressure, moderate to low hydrophobicity (relatively high water solubility), it does not hydrolyse and does not photolyse rapidly. This is reflected when reviewing the water only studies with static set up (see toxicity data tables in Annex 2 and footnotes), the concentration reduction is low (<20%) for the majority of studies up to a duration of 7 days. Nevertheless, because nominal concentrations give some uncertainty about the actual endpoints, endpoints based on nominal concentrations are given a reliability score of Ri 2. Endpoints for formulations are only included in the ERL derivation when these do not differ for more than a factor 10 from those for the active substance tested with the same species.

2 Information on the substance

2.1 Identity

Table 1. Substance identification

Name	azoxystrobin
Chemical name	methyl (E)-2-{2[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
CAS number	131860-33-8
Molecular formula	C_{22} H_{17} N_3 O_5
Molar mass	403.4 g/mol
EC number	not allocated
Structural formula	
SMILES code	COC=C(C(=O)OC)c1ccccc1Oc3cc(Oc2ccccc2C#N)ncn3
Use class	fungicide
Mode of action	electron transport inhibitor; β-methoxyacrylate strobilurin type. Binds to bc1 segment of mitochondrial respiratory chain

2.2 Physico-chemical properties

Table 2. Physico-chemical properties

Parameter	Unit	Value	Remark	Ref.
Water solubility	[g/L]	6.7×10^{-3}	pH 5.2, 20°C	[14]
	[g/L]	6.7×10^{-3}	pH 7.0, 20°C	[14]
	[g/L]	5.9×10^{-3}	pH 9.2, 20°C	[14]
pK_a	n.a.		does not dissociate	[14]
log K_{ow}	[-]	2.5	20°C, method not reported	[14]
Vapour pressure	[Pa]	1.1×10^{-10}	at 20°C	[14]
Henry's law constant	[Pa.m^3/mol]	7.4×10^{-9}	temperature not reported	[14]
Melting point	[°C]	116		[14]
Boiling point	[°C]	n.a.	substance is a solid	[14]

n.a. = not applicable.

2.3 Classification

A harmonised CLP classification (Annex VI of Regulation (EC) Nr 1272/2008) for azoxystrobin is available [16], which is presented in Table 3.

Table 3. Azoxystrobin: harmonised classification

Hazard Class and Category Codes	Hazard Statement Codes	Concentration limits, M-factors
Acute Tox. 3	H331	–
Aquatic Acute 1	H400	–[a]
Aquatic Chronic 1	H410	–[a]

[a] M-factors of 10 for acute and chronic aquatic toxicity are proposed under the current registration of the substance as biocidal active substance.

2.4 Fate and behaviour

2.4.1 Behaviour in the environment

Selected environmental properties of azoxystrobin are given in Table 4.

Table 4. Selected environmental properties of azoxystrobin

Parameter	Name/Unit	Value	Remark	Ref.
log K_{oc}	log [L/kg]	2.63[a]	see footnote	[14, 17]
Hydrolysis half-life	DT_{50} [d]	not reported	hydrolytically stable	[14]
Photolysis half-life	DT_{50} [d]	8.7	^{14}C-pyrimidinyl	[14]
Photolysis half-life	DT_{50} [d]	11.9	^{14}C-phenylacrylate	[14]
Photolysis half-life	DT_{50} [d]	13.9	^{14}C-cyanophenyl	[14]
Biodegradation in water/sediment systems	$DT_{50\ system}$[d]	234	20°C; 'Old Basing'	[14]
Biodegradation in water/sediment systems	$DT_{50\ system}$[d]	180	20°C; 'Virginia'	[14]

Footnotes

[a] Based on a K_{oc} of 423 L/kg. Footnote from EFSA [17]: 'Whilst a $K_{f\ oc}$ of 427 was accepted for use in the modelling the correct mean K_{foc} was 423 L/kg.'

2.4.2 Bioconcentration and biomagnification

Since log K_{ow} is < 3, the trigger for bioconcentration and biomagnification is not exceeded. Derivation of EQSs based on secondary poisoning ($EQS_{sp,\ water}$) is not triggered.

Human toxicology

Human toxicological threshold limits and carcinogenicity

There is no classification that triggers the inclusion of human health in risk limit derivation. Therefore the derivation of the $QS_{water,\ hh\ food}$ is not required.

3 Derivation of water quality standards

3.1 Ecotoxicological effect data

3.1.1 Aggregated laboratory toxicity data

The available acute and chronic ecotoxicity data for freshwater and marine organisms are summarised in Annex 2. The data selected for EQS derivation are reported in the tables below. When for chronic endpoints EC10 as well as NOEC values are available, preference is given to EC10 values as these are based on interpolation of the results for the different test concentration while the NOEC is dependent on the spacing between the test concentrations. In addition to the data presented below, [39] enhanced mortality in acute range finding tests under elevated UV light intensity has been reported. However, the details on these tests are limited and the effect was not observed in long term exposure effects. Therefore the extent of a potential effect of UV light cannot be interpreted and these data are not used in the EQS derivation.

Table 5. Acute ecotoxicity of azoxystrobine for freshwater aquatic organisms

Endpoints	L(E)C_{50} [mg/L]	Remark	Ref.
Bacteria			
Pseudomonas putida	>3.2		[14]
Fungi			
Saprolegnia sp.	<0.5	EC50 on the basis of a EC100	[10]
Algae / Diatoms			
Navicula pelliculosa	0.146	Most sensitive endpoint growth rate for exposure of 72 h	[14]
Raphidocelis subcapitata	1.47	Most sensitive endpoint for growth rate and exposure of 72 h, only endpoints considered where it is reported if they were determined for growth rate or biomass	[14]
Macrophyta			
Lemna gibba	3.2	Most sensitive endpoint for frond number	[14]
Rotifera			
Brachionus calyciflorus	>4.0		[14]
Mollusca			
Lymnea stagnalis	>4.0		[14]
Musculium lacustre	>1.0		[48]
Crustacea			
Asellus aquaticus	>4.0		[14]
Chydorus sphaericus	0.37		[40]
Daphnia galeata	0.095		[40]
Daphnia magna	0.19	Geometric mean of 0.28, 0.23, 0.19, 0.27, 0.53, 0.277, 0.071, 0.098, 0.19 and 0.11 mg/L	[14, 69]
Daphnia pulex	0.2		[14]
Gammarus fossarum	0.0908		[71]
Gammarus pulex	0.27	Most sensitive endpoint for 96 h exposure	[1]
Macrocyclops fuscus	0.15	Geometric mean of 0.13 and 0.18 mg/L	[14]
Insecta			
Chaoborus crystallinus	2.2	Geometric mean of 1.6 and 2.9 mg/L	[14]
Chaoborus flavicans	>6		[40]
Chironomus riparius	0.23	Geometric mean of 0.21 and 0.25 mg/L	[14, 23]
Cloeon dipterum	3.4	Geometric mean of 3.2 and 3.56 mg/L	[14, 40]
Hydropsyche angustipennis	>6		[14]

Endpoints	L(E)C_{50} [mg/L]	Remark	Ref.
Ischnura elegans	>4		[14]
Notonecta glauca	>4		[14]
Pisces			
Ctenopharyngodon idella	0.549		[41]
Cyprinus carpio	1.0	Geometric mean of 1.6 and 0.64 mg/L	[14]
Lepomis macrochirus	1.1	Geometric mean of 1.1 and 1.2 mg/L	[14]
Misgumus anguillicaudatus	1.56		[14]
Oncorhychus mykiss	0.45	Geometric mean of 0.47, 0.57, 0.56 and 0.28 mg/L	[14]
Oryzias latipes	1.30		[14]
Salmo salar	>0.352		[46]
Amphibia			
Rana temporania	0.13<x<0.5		[33]

Table 6. Acute ecotoxicity of azoxystrobin for saltwater aquatic organisms

Endpoints	L(E)C_{50} [mg/L]	Remark	Ref.
Bacteria			
Vibrio fischeri	6.96		[54]
Algae / Diatoms			
Isochrysis galbana	0.030	Geometric mean of 0.031 and 0.029 mg/L	[54]
Nannochloropsis gaditana	0.27	Geometric mean of 0.298 and 0.243 mg/L	[54]
Phaeodactylum tricornutum	2.997		[54]
Rhodomonas lens	2.406		[54]
Skeletonema costatum	0.3		[14]
Thalassiosira weisflogii	4.309		[54]
Rotifera			
Brachionius plicatillis	>6.8		[54]
Mollusca			
Crassostrea gigas	1.3		[14]
Gibbula umbilicallis	0.015	Geometric mean of 0.013 and 0.017 mg/L	[54]
Rissoa parva	0.118		[54]
Crustacea			
Artemia franciscana	0.66	Geometric mean of 0.345 and 1.256 mg/L	[54]
Americamysis bahia	0.055		[14]
Pisces			
Cyprinodon variegatus	0.66		[14]
Solea senegalensis	0.94	Geometric mean of 0.698 and 1.271 mg/L	[54]

Table 7. Chronic ecotoxicity of azoxystrobin for freshwater aquatic organisms

Endpoints	NOEC/EC_{10} [mg/L]	Remark	Ref.
Bacteria			
microbial community	0.014		[72]
Cyanobacteria			
Anabaena flos-aquae	8.5		[14]
Algae /Diatoms			
Navicula pelliculosa	0.02		
Raphidocelis subcapitata	0.024	Most sensitive endpoint for 120 h of exposure	[14]
Macrophyta			
Lemna gibba	0.8	Most sensitive endpoint: frond number	[14]
Mollusca			
Lampsilis siliquoidea	>0.028		[39]
Crustacea			
Ceriodaphnia dubia	0.0029		[39]

Endpoints	NOEC/EC_{10} [mg/L]	Remark	Ref.
Cyclops vicinus	0.01		[40]
Daphnia galeata	0.01	Most sensitive endpoint: length	[40]
Daphnia magna	0.04	Most sensitive endpoint: development time of neonates	[40]
Eudiaptomus graciloides	0.002	Most sensitive endpoint: litter size	[40]
Hyalella azteca	0.0035	Most sensitive endpoint: reproduction at 42 days of exposure	[39]
Insecta			
Chironomus dilutus	0.0086	Most sensitive endpoint: emergence	[39]
Chironomus riparius	0.8		[14]
Pisces			
Danio rerio	0.002	Most sensitive endpoint: gonad histopathology	[7]
Oncorhynchus mykiss	0.16	Most sensitive endpoint: toxic symptoms	[14]
Pimephales promelas	0.147	Most sensitive endpoint: growth	[14]
Amphibia			
Rana temporaria	≥0.010		[33]

Table 8. Chronic ecotoxicity of azoxystrobin for saltwater aquatic organisms

Endpoints	NOEC/EC_{10} [mg/L]	Remark	Ref.
Algae /Diatoms			
Thalassiosira weisflogii	1.934		[54]
Skeletonema costatum	0.010		[14]
Rhodomonas lens	2.24		[54]
Crustacea			
Americamysis bahia	0.00954		[14]

3.1.2 Toxicity data on fungi

Dijksterhuis *et al*. [12, 13] tested the effect of azoxystrobin to several aquatic fungi. MIC (minimal inhibitory concentration) or NOEC values for growth were determined in 96 wells microtiter plates and in agar (Table 9).

Table 9. NOEC and MIC values of azoxystrobin for eight aquatic fungal species. Data from Dijksterhuis *et al*. [12] except rightmost column.

Species	Code	Test type	Duration [d]	NOEC [mg/L]	LOEC[a] [mg/L]	MIC [mg/L]	geomean[b] (LOEC;MIC)
Cryptococcus flavescens	*CF*	microtiter, MM[a]	7	0.46	0.92	235	15
Trichoderma hamatum	*TH*	microtiter, MM[a]	7	0.46	0.92	59	7.4
Trichoderma hamatum	*TH*	microtiter, MEB[a]	7	0.9	1.8	117	14.5
Mucor hiemalis	*MH*	microtiter, MM[a]	7	0.23	0.46	15	2.6
Mucor hiemalis	*MH*	microtiter, MEB[a]	7	0.014	0.029	235	2.6
Fusarium sporotrichioides	*FS*	microtite, MM[a]	7	0.029	0.057	117	2.6
Fusarium sporotrichioides	*FS*	microtiter, MEB[a]	7	0.12	0.23	>235	
Pythium spp isolate 1	*Py*	agar	6	0.002	0.040	5.0	0.45
Pythium spp isolate 2	*Py*	agar	6	0.002	0.040	0.10	0.1
Helicoon richonis	*Hr*	agar	14	>5.0			
Helicodendron tubulosum	*HT*	agar	14	>5.0			

MEB = malt extract broth medium, MM = minimal medium.
[a]taken from Appendix III of Dijksterhuis *et al*. [12].
[b]geometric mean of LOEC and MIC was calculated by RIVM to derive a 'pseudo EC50' for the purpose of the ERL derivation.

Four fungi isolated from Dutch freshwater bodies were tested in the microtiter tests: *Cryptococcus flavescens, Trichoderma hamatum, Mucor hiemalis and Fusarium sporotrichioides*. In microtiter tests, growth was measured after 4 and 7 days at 24°C. DMSO was used as solvent. For agar tests the following four species were used: two *Pythium* species and two aero-aquatic species: *Helicoon richonis* and *Helicodendron tubulosum*. MICs and NOECs were determined for *Pythium* after 6 days of growth and for the aero-aquatic fungi after 14 days of growth. The latter two species are slow growers. DMSO was also used as solvent for agar tests.

MIC is defined as the concentration at which no growth was observed during 7 days (microtiter), 6 days (Py species, agar) or 14 days (HR and HT species, agar). The MIC thus represents an "EC100" value. NOEC values are those concentrations where no effect on growth was visible, for the tabulated incubation times.

Dijksterhuis *et al*. did not report EC50 values. However, the lowest value of the MIC data (i.e. total inhibition of growth) is 0.1 mg/L, the next higher values being 5.0 and 15 mg/L. These MIC values are in the same order of magnitude as most EC50 values for acute toxicity (Table 5). This means that EC50 values for these species can be expected to be lower than the EC50 values for the majority of other aquatic species. A 'pseudo EC50' for fungi was calculated from the Dijksterhuis data by taking the geometric mean of LOEC and EC100 (MIC) data. These values are shown in the rightmost column of Table 9. These 'pseudo EC50 values' were plotted with the EC50 values for both freshwater and marine species (taken from Table 5 and Table 6, respectively), see Figure 1. Unbounded EC50 values (EC50 >) shown in the data tables were not included in the SSD. For the fungi, one 'pseudo EC50 value' per species (the lowest) is included.

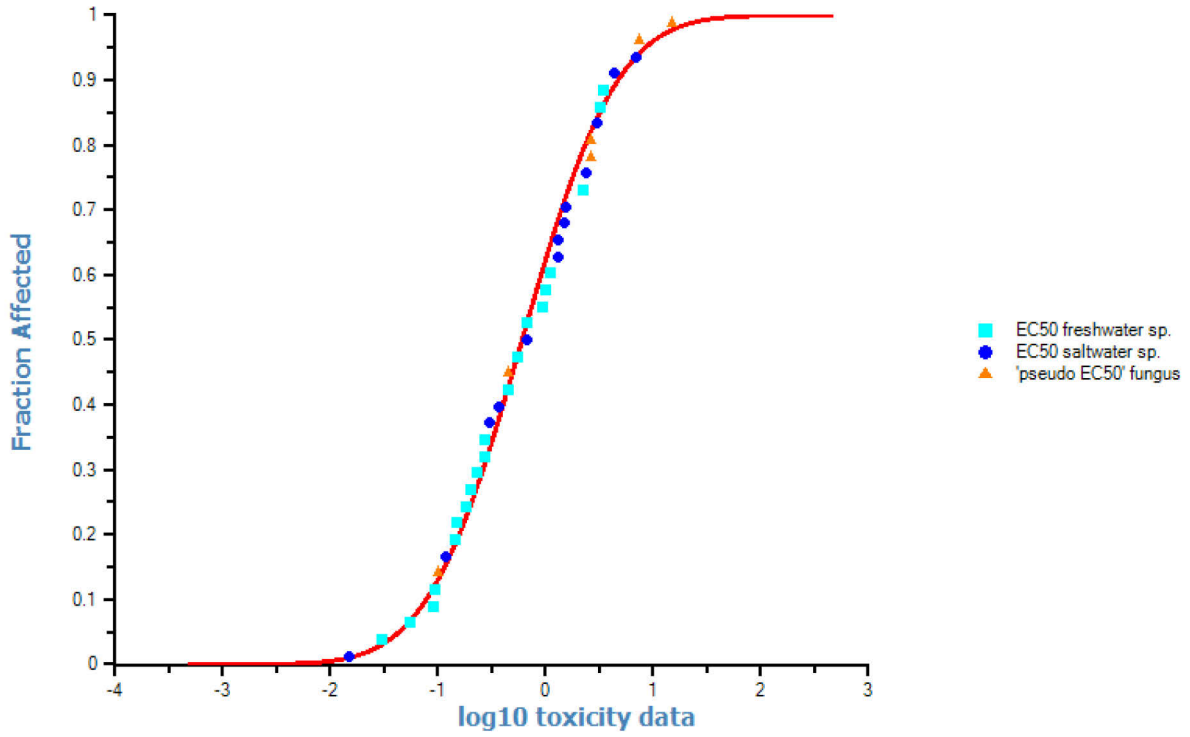


Figure 1. Cumulative density function (CDF) plot of EC50 values from acute toxicity studies with azoxystrobin (freshwater and marine species) and "pseudo EC50" values for aquatic fungi.

A single EC50 value for a fungal species was reported: <0.5 mg/L for *Saprolegnia* sp.. The HC50 of the SSD in Figure 1 is 0.6 mg/L and 18 of the 39 data points are <0.5 mg/L. This provides support for the statement that the current acute toxicity dataset covers the most sensitive species. An assessment factor of 10 to derive the $MAC_{eco, water}$ is therefore considered acceptable.

3.1.3 Mesocosm and semi-field studies

In Annex 1, mesocosm studies are assessed with the aim of deriving endpoints that could be used for deriving an MPC. In study 1 [68] a chronic exposure was mimicked, and the study results in a NOEC of 1 µg/L. Study 2 [70] used other exposure regimes in the study design of study 1. Since effects were found in all dosages, including the lowest of 10 µg/L, it is not possible to derive a NOEC. Study 3 [31] was conducted in Sweden, and used relatively small microcosms. In the outdoor cosms effects were found in the lowest dose (16 µg a.i./L). Additional indoor experiments showed effects at 3 µg a.i./L. Study 4 [8] and 5 [44] were conducted in the UK and had the same design. In study 4 effects were found in the lowest dose (10 µg a.i./L, based on a nominal, initial concentration). In study 5 only two low dosages were tested (1 and 2.85 µg a.i./L, based on nominal, initial concentrations) and no statistically significant effects were found. Because of the lack of a dosage with effects in study 5, this study is difficult to interpret.

Overall, the 5 mesocosm studies show rather consistent results. In all cases dosages of 10 µg a.i./L show statistically significant treatment related effects. In Study 1 a NOEC of 1 µg/L was found. The results of study 5, showing no effect at nominal concentrations of 1 and 2.85 µg/L, are not contradicting this result.

3.2 Derivation of the MAC-EQS

3.2.1 Assessment factor approach

Valid acute toxicity data for freshwater organisms including unbound values are available for 29 species from nine taxa covering algae, crustaceans and fish. Therefore a complete base set is available. Apart from data for freshwater organisms data for salt water organisms is also available for 15 species from six taxa. The available data do not show a difference in sensitivity between the freshwater and saltwater organisms (p = 0.05), therefore fresh- and saltwater data can be pooled for the purpose of derivation of risk limits. The MAC-$QS_{fw, eco}$ is derived from the lowest acute toxicity value available, the EC50 of 0.015 mg/L for the marine gastropod mollusk *Gibbula umbilicallis*. An assessment factor of 10 is applied because the substance has a known mode of action and representatives of the presumed most sensitive taxonomic groups (primary producers and fungi) are included in the dataset. The MAC-$QS_{fw, eco}$ is 0.015 / 10 = 0.0015 mg/L = 1.5 µg/L.

3.2.2 Species sensitivity distribution

For azoxystrobin a broad dataset for acute toxicity is available, therefore the option to perform an SSD is examined. Below, the criteria of the WFD guidance are copied with the representative species from the present dataset:

1. Fish (species frequently tested include salmonids, minnows, bluegill sunfish, channel catfish, etc.):
   – *Oncorhynchus mykiss*, family Salmonidae
2. A second family in the phylum Chordata (e.g. fish, amphibian, etc.):
   – *Cyprinus carpio*, family Cyprinidae
3. A crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.):
   – *Daphnia magna*
4. An insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.):
   – *Chironomus riparius*, midge, order Diptera
5. A family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.):
   – *Brachionus calyciflorus*, phylum Rotifera (unbound EC50);
   – *Lymnea stagnalis and Musculium lacustre*, phylum Mollusca (both are unbound EC50s);
6. A family in any order of insect or any phylum not already represented:
   – E.g. *Ischnura elegans*, damselfly, order Odonata
   – *P. putida*, phylum Eubacteria (unbound EC50)
   – *V. fischeri*, in the phylum Eubacteria.
7. Algae:
   – *Raphidocelis subcapitata*
8. Higher plants:
   – *Lemna gibba*.

The criteria for refined effect assessment (construction of an SSD) are met, both when unbound EC50 values are included and excluded.
The SSD determined with ETX 2.1 software [67] is shown in Figure 2. The calculated HC5 is 0.041 mg/L, with a two sided 90% confidence interval of 0.019-0.074 mg/L. The goodness of fit with a log normal distribution is accepted at all levels of significance using the three statistical tests available in the program.

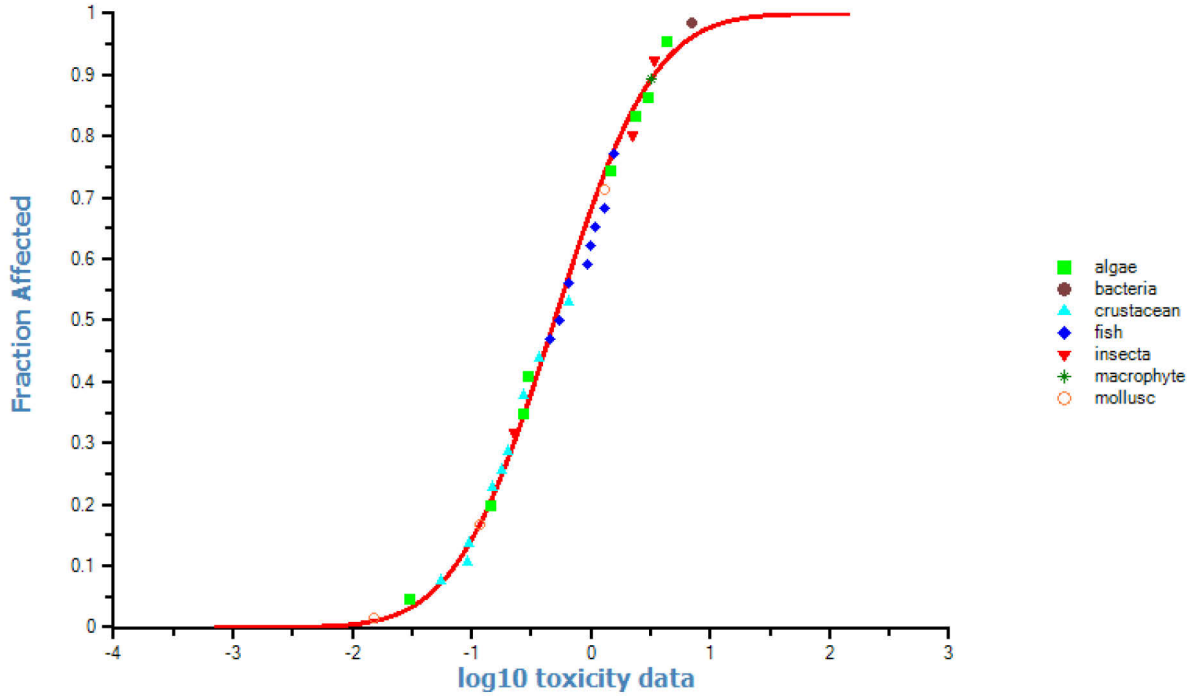


Figure 2. Species Sensitivity Distribution for azoxystrobin (acute data) determined with ETX (excluding unbound values).

The default assessment factor of 10 to derive the MAC-$QS_{fw, eco}$ is considered acceptable. This results in a MAC-$QS_{fw, eco}$ of 0.041 / 10 = 0.0041 mg/L = 4.1 µg/L.

3.2.3 SSD for acute toxicity data including unbound values

Although we prefer to use SSDs including unbound data points (results with > or <) for the effect assessment there is, at present, no agreed methodology at EU level nor at national level that underpins the statistical rationale for the inclusion of unbound data points. The derivation of HC5 values using MOSAIC_SSD software, which allows for the inclusion of unbound values, is added for the purpose of comparison.

The dataset includes unbound data (e.g. EC50 values >) for 12 species, these are reliable values but can generally not be used in risk assessment because the exact level of the toxicity is unknown. The program ETX [67] as used above does also not offer the possibility to include these data in the SSD approach. In the MOSAIC SSD tool available on the website "http://pbil.univ-lyon1.fr/software/mosaic/ssd/" this option is available.

Assuming a log-logistic distribution, an HC5 of 0.033 mg/L is calculated (95% confidence interval: 0.014 - 0.08 mg/L and log-likelihood: -58.3) and assuming a log normal distribution, the HC5 is 0.042 mg/L (95% confidence interval: 0.019 - 0.088 mg/L and log-likelihood: -57.6). The SSD curves determined with MOSAIC are presented in **Figure 3**.

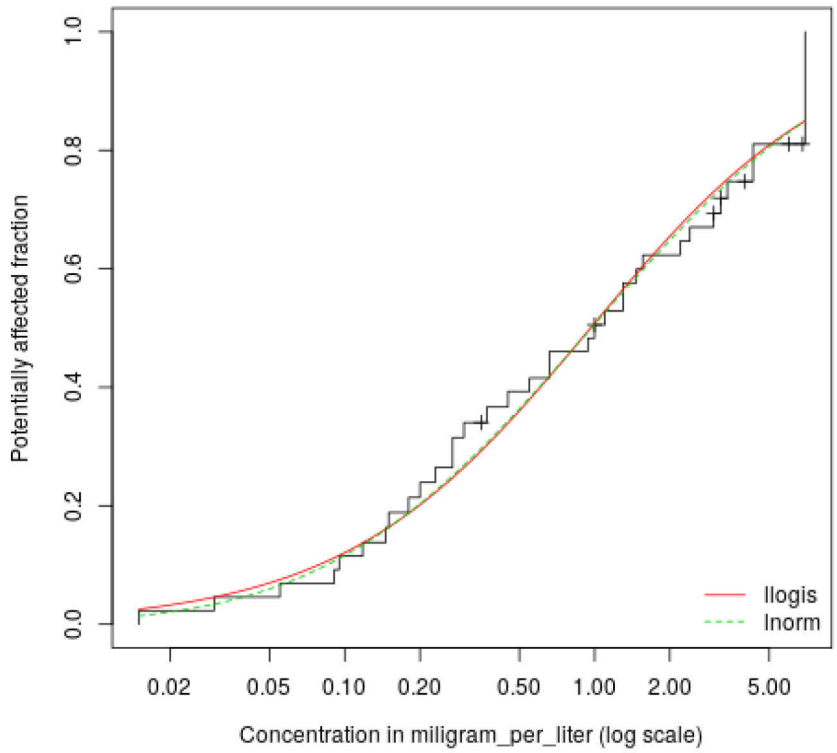


Figure 3. Species Sensitivity Distribution for azoxystrobin (acute data) determined with Mosaic (including unbound values).

The HC5 values calculated with MOSAIC are in the same order of magnitude as those calculated with ETX and both distributions show the same fit through the data points.

3.2.4 Selection of the MAC-EQS

The MAC-$QS_{fw, eco}$ derived with the assessment factor approach is 1.5 µg/L, the SSD-based MAC-$QS_{fw, eco}$ is 4.1 µg/L. Because the latter value represents a more robust approach towards ecosystem effects, the final MAC-$QS_{fw, eco}$ is set to 4.1 µg/L.

The MAC-$QS_{sw, eco}$ is derived on the basis of the combined freshwater and saltwater dataset. However, no acute data from additional marine taxa (see WFD guidance [15], section 3.3.2.1 for explanation) are available, therefore an additional assessment factor of 10 is applied to the MAC-$QS_{fw, eco}$. This results in a MAC-$QS_{sw, eco}$ of 0.41 µg/L.

3.3 Derivation of the AA-EQS

3.3.1 Ecotoxicity – $QS_{fw, eco}$ and $QS_{sw, eco}$

The available data do not show a difference in sensitivity between the freshwater and saltwater organisms (p = 0.05), therefore fresh- and saltwater data can be pooled for the purpose of derivation of risk limits.

3.3.1.1 Assessment factor approach

NOECs are available for 18 freshwater species from nine taxa, covering the base set. Additionally there are NOECs for four marine species from two taxa. The $QS_{fw, eco}$ is derived by applying an assessment factor of 10 to the lowest NOEC (0.002 mg/L for the freshwater copepod *Eudiaptomus graciloides*), resulting in a $QS_{fw, eco}$ of 0.002 / 10 = 0.0002 mg/L = 0.2 µg/L.

3.3.1.2 Species sensitivity distribution

For azoxystrobin a broad dataset for chronic toxicity is available, therefore the option to perform an SSD is examined. Below, the criteria of the WFD guidance are copied with the representative species from the present dataset:

1. Fish (species frequently tested include salmonids, minnows, bluegill sunfish, channel catfish, etc.):
   – *Oncorhynchus mykiss*, family Salmonidae;
2. A second family in the phylum Chordata (e.g. fish, amphibian, etc.):
   – *Danio rerio*, family Cyprinidae;
   – *Rana temporaria*, family Ranidae;
3. A crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.):
   – *Daphnia magna;*
4. An insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.):
   – *Chironomus riparius*, midge;
5. A family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.):
   – *Lampsilis siliquoidea*, Mollusca (unbound NOEC);
6. A family in any order of insect or any phylum not already represented:
   – *Anabaena flos-aquae*, Phylum Eubacteria as well as microbial community
7. Algae:
   – *Raphidocelis subcapitata*
8. Higher plants:
   – *Lemna gibba*.

Based on the checklist above it is concluded that the criteria for construction of an SSD are not met. Following WFD guidance, the AA-EQS should be derived using the assessment factor approach (section 3.3.1.1).

However, when unbound toxicity values are included, the criteria for refined effect assessment are met. For comparative purposes, we will present the outcome of the construction of SSDs, derivation of HC5 and AA-EQS in a separate section (3.3.2).

3.3.1.3 Selection of the $QS_{fw, eco}$ and $QS_{sw, eco}$

The $QS_{fw, eco}$ derived with the assessment factor approach is 0.2 µg/L. The available information on mesocosm studies, provided a lowest NOEC value of 1 µg/L. The $QS_{fw, eco}$ using the assessment factor approach therefore covers the effects seen in the mesocosm studies. Therefore, the final AA-EQS_{fw} is set to 0.20 µg/L.

The $QS_{sw, eco}$ is derived on the basis of the combined freshwater and saltwater datasets. Since there are no chronic data from specific marine taxa, an additional assessment factor of 10 is applied to derive the $QS_{sw, eco}$. This results in a $QS_{sw, eco}$ of 0.020 µg/L (20 ng/L).

3.3.2 SSD with chronic data – comparative analysis

As concluded in section 3.3.1.2, the chronic data set does not meet the criteria for refined effect assessment using the SSD approach. For comparative purposes we include the results of an SSD analysis, which may help interpret the robustness of the $QS_{fw, eco}$ derived using the assessment factor approach.

The SSD determined with ETX software [67] is shown in Figure 4. The calculated HC5 is 0.00060 mg/L, with a two sided 90% confidence interval of 0.000094-0.0021 mg/L. The goodness of fit with a log-normal distribution is accepted at the 0.025 level by the Anderson-Darling test and the 0.05 level by the Kolmogorov-Smirnov and the 0.01 level by the Cramer von Mises tests available in the program.

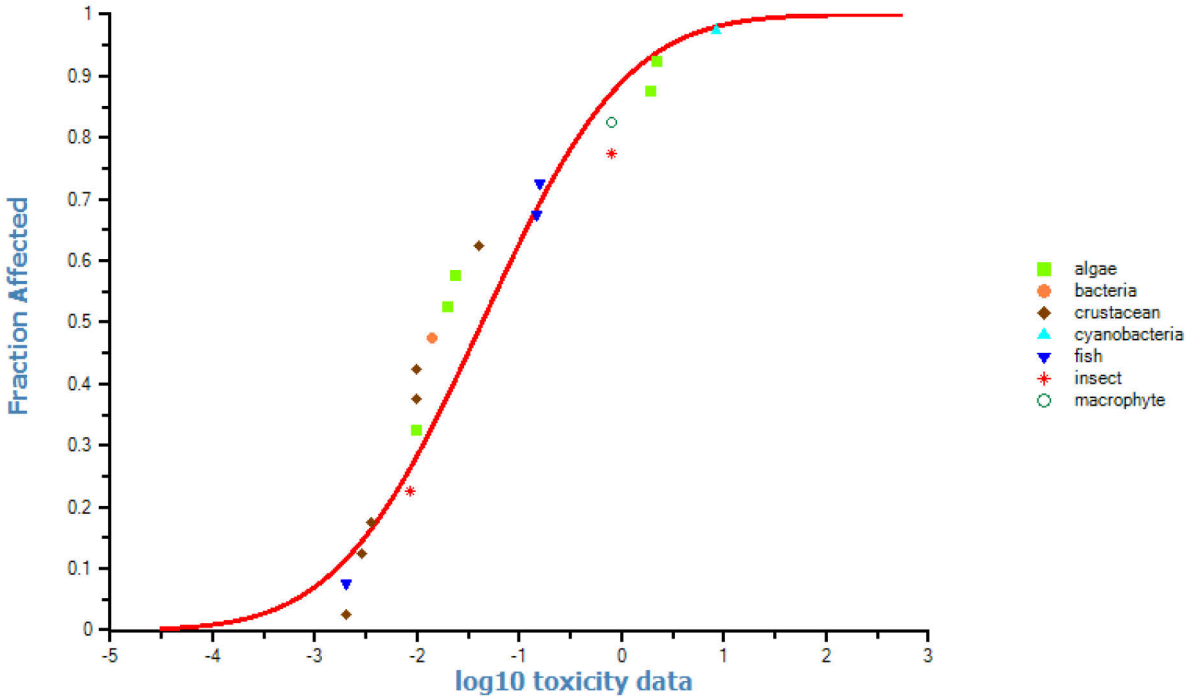


Figure 4. Species Sensitivity Distribution for azoxystrobin (chronic data) determined with ETX (excluding unbound values).

It is noted that the completeness of the data set for the SSD approach includes unbound data that cannot be used in the ETX program [67]. With the Mosaic_SSD tool available on the website http://pbil.univ-lyon1.fr/software/mosaic/ssd/, assuming a log-logistic distribution, a HC5 of 0.00053 mg/L is calculated (95% confidence interval: 0.00024 – 0.002 mg/L and log-likelihood: 14.7) and for a log normal distribution the HC5 is 0.00085 mg/L (95% confidence interval: 0.0004 – 0.0027 mg/L and log-likelihood: 15.4). The SSD curves determined with Mosaic are presented in **Figure 5**.

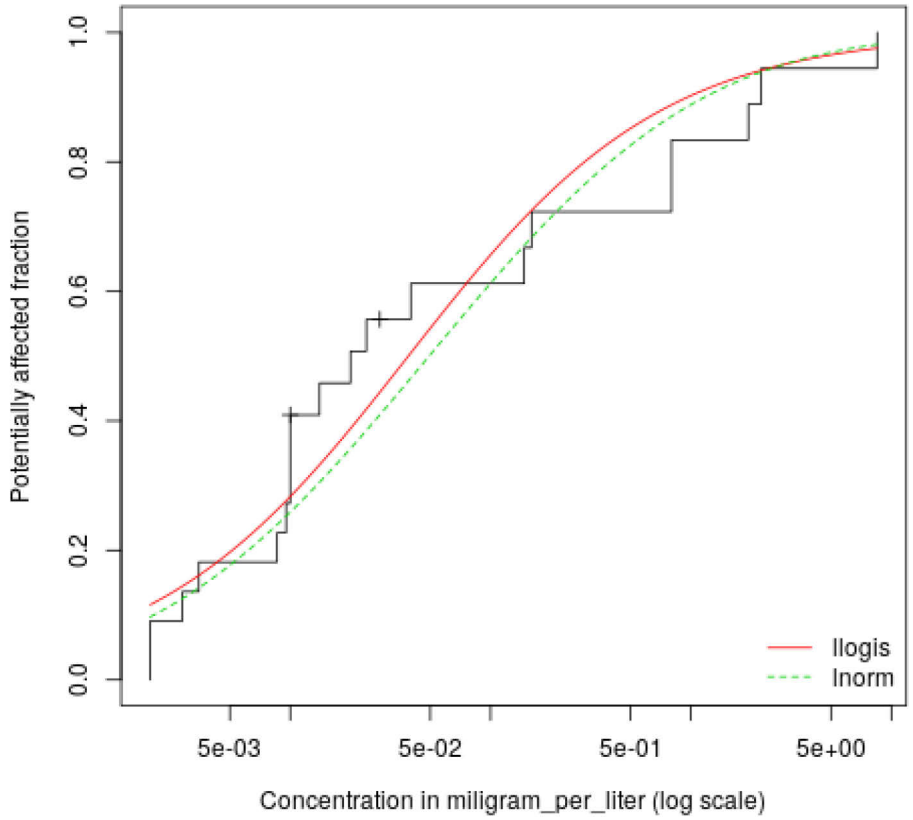


Figure 5. Species Sensitivity Distribution for azoxystrobin (chronic data) determined with Mosaic (including unbound values).

These calculations are in the same order of magnitude as the outcome of the ETX calculations.

3.3.2.1 Assessment factor – comparative analysis

The SSDs are based on data that result from true chronic studies or studies covering sensitive life stages. The chronic toxicity data set including unbound NOECs contains 19 species, divided over 9 taxonomic groups (minimum requirement 10 NOECs over 8 taxa). The three HC5 estimates range from 0.53 to 0.85 µg/L. The NOEC derived from evaluation of the mesocosm and field studies is 1 µg/L (section 3.1.3). This is in the same range. However, one of the most sensitive species in the SSD is a fish. Fish were not present in the mesocosm studies (fish are normally not present in mesocosm studies). Chronic laboratory toxicity data on the target species (fungi) are absent. Although intended as a fungicide, the mode of action of azoxystrobin is likely to affect primary producers as well. These are well represented in the data set (macrophyta, bacteria, cyanobacteria, algae), however, information on the difference in sensitivity between primary producers and fungi upon chronic exposure is not available.
The statistical tests in ETX as well as visual inspection of Figure 4 show that the fit of the log normal distribution using ETX methodology is relatively poor.
In view of the above considerations, it would seem reasonable to reduce the assessment factor that is applied to the HC5 to 3. In this it is considered that fungi and fish (sensitive taxon) were not included in the mesocosms, and the data for fungi discussed in Section 3.1.2 do not include chronic studies. Moreover, the AF of 3 would covers for the uncertainty in the HC5 estimate due to the relatively poor fit. This would have resulted in a $QS_{fw, eco}$ of 0.00085 / 3 = 0.00028 mg/L = 0.28 µg/L using the MOSAIC methodology and a $QS_{fw, eco}$ of 0.00053 / 3 = 0.00018 mg/L = 0.18 µg/L.

We conclude that the $QS_{fw, eco}$ of 0.20 µg/L (section 3.3.1.3) is supported by this comparative analysis that investigated potential outcome using the refined effect assessment method.

3.3.3 $QS_{fw, secpois}$ – secondary poisoning

Since the log K_{ow} is < 3, derivation of a $QS_{fw, secpois}$, covering for effects of secondary poisoning is not required.

3.3.4 Human fish consumption – $QS_{water, hh food}$

Azoxystrobin does not meet any of the triggers that require the inclusion of human health in risk limit derivation (see section 1.3.2 in part 3 of [53]. The derivation of the $QS_{water, hh food}$ is not required.

3.3.5 Selection of the AA-EQS

The AA-EQS_{fw} is determined by the $QS_{fw, eco}$: 0.20 µg/L.

The AA-EQS_{sw} is determined by the $QS_{sw, eco}$: 0.020 µg/L (20 ng/L).

4 Conclusion

The MAC-EQS_{fw} for azoxystrobin is 4.1 µg/L, the MAC-EQS_{sw} is 0.41 µg/L.
The AA-EQS_{fw} for azoxystrobin is 0.20 µg/L, the AA-EQS_{sw} is 0.020 µg/L.

These quality standards are comparable to the outcome of the Swiss ESQ derivation [19]. For the MAC-EQS (Swiss freshwater standard is 0.55 µg/L), there is a small difference, the main reason is the inclusion of multiple endpoints published by Rodriguez et al. in 2017 [54]. This study was published after the Swiss study was finalised. The AA-EQS for Swiss freshwater standard is 0.2 µg/L, which is equal to the $QS_{fw, eco}$ derived in this report.

5 References

1. Beketov, M.A., Liess, M. (2008) Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates. Arch Environ Contam Toxicol. 55: 247-253.
2. Boeri, R.L., Magazu, J.P., Ward, T.J. (1997) Azoxystrobin: Chronic Toxicity to Mysid Shrimp (*Mysidopsis bahia*). T.R. Wilbury Laboratories Inc., Marblehead, Massachusetts, 1350-ZE.
3. Bony, S., Gaillard, I., Devaux, A. (2010) Genotoxicity assessment of two vineyard pesticides in zebrafish. Int J Environ Anal Chem. 90: 421-418.
4. Brock, T.C.M., Arts, G.H.P., Ten Hulscher, T.E.M., De Jong, F.M.W., Luttik, R., Roex, E.W.M., Smit, C.E., Van Vliet, P.J.M. (2011) Aquatic effect assessment for plant protection products; Dutch proposal that addresses the requirements of the Plant Protection Product Regulation and Water Framework Directive. Alterra, Wageningen, the Netherlands, 2235.
5. Brock, T.C.M., Heger, W., Giddings, J.M., Heimbach, F., Maund, S.J., Norman, S., Ratte, H.-T., Schäfers, C., Steloke, M. (2000) Guidance document on Cummunity Level Aquatic Studies - Interpretation Criteria. Proceedings of the SEATC-Europe/OECD/EC Workshop held at Schmallenberg, Germany, 30 May - 2 June 1999. SETAC, Brussels.
6. Campbell, P.J., Arnold, D.J.S., Brock, T.C.M., Grandy, N.J., Heger, W., Heimbach, F., Maund, S.J., Streloke, M. (1999) Guidance document on higher-tier aquatic risk assessment for pesticides (HARAP), from the SETAC-Europe/OECD/EC Workshop, La-canau Océan, France. SETAC-Europe, Brussels.
7. Cao, F., Zhu, L., Li, H., Yu, S., Wang, C., Qiu, L. (2016) Reproductive toxicity of azoxystrobin to adult zebrafish (*Danio rerio*). Environ Pollut. 219: 1109-1121.
8. Cole, J.F.H., Everett, C.J., Gentle, W., Ashwell, J.A., Goggin, U. (2000) Azoxystrobin: An outdoor pond microcosm study. Zeneca Agrochemicals, Bracknell, Berkshire, RJ 2857B.
9. [redacted] (1993) CIA5504: Acute Toxicity To Rainbow Trout (*Oncorhynchus mykiss*). [redacted]
10. Dawoud, M., Bundschuh, M., Goedkoop, W., McKie, B.G. (2017) Interactive effects of an insecticide and a fungicide on differentorganism groups and ecosystem functioning in a stream detritalfood web. Aquat Toxicol. 186: 215-221.
11. De Jong, F.M.W., Brock, T.C.M., Foekema, E.M., Leeuwangh, P. (2008) Guidance for summarizing and evaluating aquatic micro- and mesocosm studies. A guidance document of the Dutch Platform for the Assessment of Higher Tier Studies. RIVM, Bilthoven, 601506009.
12. Dijksterhuis, J., van Doorn, T., Postma, J. (2009) De gevoeligheid van oppervlaktewaterschimmels blootgesteld aan azxolen en strobilurines die worden toegepast in de landbouw. Centraalbureau voor schimmelcultures/Fungal Biodiversity Centre and Ecofide.
13. Dijksterhuis, J., van Doorn, T., Samson, R., Postma, J. (2011) Effects of Seven Fungicides on Non-Target Aquatic Fungi. Water, Air, and Soil Pollution. 222: 421-425.
14. EC (2009) Council Directive 91/414/EEC. Azoxystrobin. Report and proposed decision of the United Kingdom made to the European Commission under Commission Regulation 737/2007. Rapporteur Member State: United Kingdom.
15. EC (2011) Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 27. Technical Guidance For Deriving Environmental Quality Standards. European Commission, Brussels, Belgium, Technical Report - 2011 - 055.
16. ECHA (2017) C&L Inventory. European Chemicals Agency (ECHA),. https://echa.europa.eu/information-on-chemicals/cl-inventory-database
17. EFSA (2010) Conclusion on the peer review of the pesticide risk assessment of the active substance azoxystrobin. EFSA Journal. 8: 1542.
18. EFSA (2013) Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal. 11: 3290.
19. EPFL, E.-. (2016) EQS - Vorslag des Oekotoxzentrums für: Azoxystrobin. Schweizerisches Zentrum für angewandte Ökotoxicologie Eawag-EPFL, Lausanne.
20. Farrelly, E., Kearson Jr., L.L., Rapley, J.H., Hamer, M.J. (1995) ICIA5504: Comparative Toxicity of the Technical Material in Solution and a 500 g kg-1 WG Formulation to the Damselfly Nymph *Ischnura elegans*. Zeneca Agrochemicals, Bracknell, Berkshire, UK, RJ1794B.

21. Farrelly, E., Kearson Jr., L.L., Rapley, J.H., Hamer, M.J. (1995) ICIA5504: Comparative Toxicity of the Technical Material in Solution and a 500 g kg-1 WG Formulation to the Freshwater Copepod *Macrocyclops fuscus*. Zeneca Agrochemicals, Bracknell, Berkshire, UK, RJ1793B.
22. Farrelly, E., Kearson Jr., L.L., Rapley, J.H., Hamer, M.J. (1995) ICIA5504: Comparative Toxicity of the Technical Material in Solution and a 500 g Kg-1 WG Formulation to the Freshwater Shrimp *Gammarus pulex*. Zeneca Agrochemicals, Bracknell, Berkshire, UK, RJ1782B.
23. Farrelly, E., Kearson Jr., L.L., Rapley, J.H., Hamer, M.J. (1995) ICIA5504: Comparative Toxicity of the Technical Material in Solution and a 500 g kg-1 WG Formulation to the Larvae of the Midge *Chironomus riparius*. Zeneca Agrochemicals, Bracknell, Berkshire, UK, RJ1790B.
24. Farrelly, E., Kearson Jr., L.L., Rapley, J.H., Hamer, M.J. (1995) ICIA5504: Comparative Toxicity of the Technical Material in Solution and a 500 g kg-1 WG Formulation to the Mayfly Nymph *Cloeon dipterum*. Zeneca Agrochemicals, Bracknell, Berkshire, UK, RJ1795B.
25. Farrelly, E., Kearson Jr., L.L., Rapley, J.H., Hamer, M.J. (1995) ICIA5504: Comparative Toxicity of the Technical Material in Solution and a 500 g kg-1 WG Formulation to the Phantom Midge Larva *Chaoborus crystallinus*. Zeneca Agrochemicals, Bracknell, Berkshire, UK, RJ1792B.
26. Farrelly, E., Kearson Jr., L.L., Rapley, J.H., Hamer, M.J. (1995) ICIA5504: Comparative Toxicity of the Technical Material in Solution and a 500 g kg-1 WG Formulation to the Pond Snail *Lymnaea stagnalis*. Zeneca Agrochemicals,, Bracknell, Berkshire, UK, RJ1796B.
27. Farrelly, E., Kearson Jr., L.L., Rapley, J.H., Hamer, M.J. (1995) ICIA5504: Comparative Toxicity of the Technical Material in Solution and a 500 g kg-1 WG Formulation to the Rotifer *Brachionus calyciflorus*. Zeneca Agrochemicals, Bracknell, Berkshire, UK, RJ1791B.
28. Farrelly, E., Kearson Jr., L.L., Rapley, J.H., Hamer, M.J. (1995) ICIA5504: Comparative Toxicity of the Technical Material in Solution and a 500 g kg-1 WG Formulation to the Water-Louse *Asellus aquaticus*. Zeneca Agrochemicals, Bracknell, Berkshire, UK, RJ1789B.
29. Friberg-Jensen, U., Nachman, G., Christoffersen, K.S. (2010) Early signs of lethal effects in *Daphnia magna* (Branchiopoda,Cladocera) exposed to the insecticide cypermethrin and the fungicide azoxystrobin. Environ Toxicol Chem. 29: 2371-2378.
30. Gentle, W.E., Rapley, J.H. (1997) Azoxystrobin: BBA Toxicity Test with Sediment-Dwelling *Chironomus riparius*. Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, UK, RJ2293B.
31. Gustafsson, K., Blidberg, E., Elfgren, I.K., Hellström, A., Kylin, H., Gorokhova, E. (2010) Direct and indirect effects of the fungicide azoxystrobin in outdoor brackish water microcosms. Ecotoxicology. 19: 431-444.
32. Hu, X.-G., Liu, L., Hu, K., Yang, X.-L., Wang, G.-X. (2013) In vitro screening of fungicidal chemicals for antifungal activity against Saprolegnia. J World Aquacult Soc. 44: 528-535.
33. Johansson, M., Piha, H., Kylin, H., Merilä, J. (2006) Toxicity of six pesticides to common frog (*Rana temporaria*) tadpoles. Environ Toxicol Chem. 25: 3164-3170.
34. [redacted] (1994) ICIA5504 Technical: 96h Acute Toxicity Study using Killifish. [redacted].
35. Kent, S., Sankey, S.A., Caunter, J.E., Grinell, A.J. (1994) ICIA5504: Acute Toxicity to Larvae of the Pacific Oyster (*Crassostrea gigas*). Brixham Environmental Laboratory, Zeneca Ltd, Brixham, BL4842/B.
36. [redacted] (1994) ICIA5504: The 28 Day NOEC to Rainbow Trout (*Oncorhynchus mykiss*). [redacted].
37. Kent, S., Sankey, S.A., Grinell, A.J. (1993) ICIA5504: Acute Toxicity to Mysid Shrimp (*Mysidopsis bahia*). Brixham Environmental Laboratory, Zeneca Ltd, Brixham, BL4785/B.
38. Klimisch, H.J., Andreae, M., Tillman, U. (1997) A systematic approach for evaluating the quality of experimetnal toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology. 25: 1-5.
39. Kunz, J.L., Ingersoll, C.G., Smalling, K.L., Elskus, A.A., Kuivila, K.M. (2017) Chronic toxicity of azoxystrobin to freshwater amphipods, midges, cladocerans, and mussels in water-only exposures. Environ Toxicol Chem. 36: 2308-2315.
40. Lauridsen, T.L., Friberg-Jensen, U., Cristoffersen, K. (2003) Effekter af cypermethrin, azoxystrobin og bentazon pa° limniske invertebrater. Miljøstyrelsen, Miljøministeriet, Copenhagen, Denmark, 76.
41. Liu, L., Jiang, C., Wu, Z.-Q., Gong, Y.-X., Wang, G.-X. (2013) Toxic effects of three strobilurins (trifloxystrobin, azoxystrobin and kresoxim-methyl) on mRNA expression and antioxidant enzymes in grass carp (*Ctenopharyngodon idella*) juveniles. Ecotoxicol Env Saf. 98: 297-302.

42. Liu, L., Zhu, B., Wang, G.-X. (2015) Azoxystrobin-induced excessive reactive oxygen species (ROS) production and inhibition of photosynthesis in the unicellular green algae *Chlorella vulgaris*. Environ Sci Pollut Res. 22: 7766-7775.
43. [redacted] (1994) ICIA5504 Technical: 96h Acute Toxicity Study using Loach. [redacted]
44. Maund, S.J., Kearson Jr., L.L. (1995) ICIA5504: An aquatic mesocosm study to assess the effects of a 500 g kg-1 WG formulation on filter-feeding zooplankton. Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, UK, RJ1941B.
45. Ochoa-Acuña, H.G., Bialkowski, W., Yale, G., Hahn, L. (2009) Toxicity of soybean rust fungicides to freshwater algae and *Daphnia magna*. Ecotoxicology. 18: 440-446.
46. Olsvik, P.A., Kroglund, F., Finstad, B., Kristensen, T. (2010) (2010): Effects of the fungicide azoxystrobin on Atlantic salmon (*Salmo salar* L.) smolt. Ecotoxicol Env Saf. 73: 1852-1861.
47. Padilla, S., Corum, D., Padnos, B., Hunter, D.L., Beam, A., Houck, K.A., Sipes, N., Kleinstreuer, N., Knudsen, T., Dix, D.J., Reif, D.M. (2012) Zebrafish developmental screening of the ToxCastTM Phase I chemical library. Reprod Toxicol. 33: 174-187.
48. Pickervance, J.C. (2002) Azoxystrobin (ICIA5504), applied as a 250 g ai suspension concentrate A12705A: acute toxicity to the freshwater bivalve *Musculium lacustre* (Sphaeridae). Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, UK, RJ3337B.
49. Rapley, J.H., Farrelly, E., Hamer, M.J. (1994) ICIA5504: Chronic Toxicity to *Daphnia magna*. Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, UK, RJ1493B.
50. Rapley, J.H., Farrelly, E., Hamer, M.J. (1995) ICIA5504: Acute Toxicity of the Technical Material to First Instar *Daphnia magna*. Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, UK, RJ1385B.
51. Rapley, J.H., Kearson Jr., L.L., Hamer, M.J. (1995) ICIA5504: Comparative Toxicity of the Technical Material in Solution and a 500 g kg-1 WG Formulation to the Water-Boatman *Notonecta glauca*. Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, UK, RJ1799B.
52. [redacted] (1994) Early Life Stage Toxicity of ICIA5504 to the Fathead Minnow (*Pimephales promelas*) Under Flow Through Conditions. [redacted]
53. RIVM (2015) Guidance for the derivation of environmental risk limits. National Institute for Public Health and the Environment, Bilthoven, the Netherlands, http://www.rivm.nl/rvs/Normen/Milieu/Milieukwaliteitsnormen/Handleiding_normafleiding.
54. Rodrigues, E.T., Pardal, M.Â., Gante, C., Loureiro, J., Lopes, I. (2017) Determination and validation of an aquatic Maximum Acceptable Concentration-Environmental Quality Standard (MAC-EQS) value for the agricultural fungicide azoxystrobin. Environ Pollut. 221: 150-158.
55. [redacted]. (1993) ICIA5504: Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*). [redacted]
56. [redacted]. (1993) ICIA5504: Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*). [redacted]
57. SETAC-Europe (1992) Guidance Document on Testing Procedures in Freshwater Static Ecosystems. SETAC-Europe, Brussels.
58. Smit, C.E., Arts, G.H.P., Brock, T.C.M., Ten Hulscher, T.E.M., Luttik, R., Van Vliet, P.J.M. (2013) Aquatic effect and risk assessment for plant protection products. Evaluation of the Dutch 2011 proposal. Alterra Wageningen UR (University & Research centre), , Wageningen, The Netherlands, 2463.
59. Smyth, D.V., Kent, S.J., Graig, N.C.D., Sankey, S.A., Stanley, R.D. (1993) ICIA5504: Toxicity to the Green Alga *Selenastrum capricornutum*. Brixham Environmental Laboratory, Zeneca Ltd, Brixham, Devon, UK, BL4800/B.
60. Smyth, D.V., Kent, S.J., Sankey, S.A., Johnson, P.A. (1994) ICIA5504 - Toxicity to the marine alga *Skeletonema costatum*. Brixham Environmental Laboratory, Zeneca Ltd, Brixham, Devon, UK, BL5053/B.
61. Smyth, D.V., Kent, S.J., Sankey, S.A., Shearing, J.M. (1994) ICIA5504: Toxicity to the Blue-Green Alga *Anabaena flos-aquae*. Brixham Environmental Laboratory, Zeneca Ltd, Brixham, Devon, UK, BL5054/B.

62. Smyth, D.V., Kent, S.J., Sankey, S.A., Shearing, J.M. (1994) ICIA5504: Toxicity to the freshwater diatom *Navicula pelliculosa*. Brixham Environmental Laboratory, Zeneca Ltd, Brixham, Devon, UK, BL5087/B.
63. Smyth, D.V., Kent, S.J., Sankey, S.A., Stanley, R.D. (1994) ICIA5504: Toxicity to the Duckweed *Lemma gibba*. Brixham Environmental Laboratory, Zeneca Ltd, Brixham, Devon, UK, BL5000/B.
64. Špalková, M., Beňová, K., Dvořák, P., Falis, M., Andrej Renčko, A. (2015) Study of interaction of low doses of ionizing radiation with selected pesticides on *Artemia franciscana*. Acta Vet Brno. 84.
65. [redacted]. (1993) ICIA5504: Acute Toxicity to Mirror Carp (*Cyprinus carpio*). [redacted]
66. van de Plassche, E.J. (2002) Een ad-hoc Maximaal Toelaatbaar Risiconiveau voor bestrijdingsmiddelen. Haskoning Nederland bv, Nijmegen, 9M0255.01/R0001/EVDP/Nijm.
67. Van Vlaardingen, P., Traas, T.P., Wintersen, A.M., Aldenberg, T. (2014) EtX 2.1. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. National Institute for Public Health and the Environment., Bilthoven, The Netherlands, 601501028.
68. Van Wijngaarden, R.P.A., Belgers, D.J.M., Zafar, M.I., Matser, A.M., Boerwinkel, M.-C., Arts, G.H.P. (2014) Chronic aquatic effect assessment for the fungicide azoxystrobin. Environ Toxicol Chem. 33.
69. Warming, T.P., Mulderu, G., Christoffersen, K.S. (2009) Clonal variation in physiological responses of *Daphnia magna* to the strobilurin fungicide azoxystrobin. Environ Toxicol Chem. 28: 374-380.
70. Zafar, M.I., Belgers, J.D.M., Van Wijngaarden, R.P.A., Matser, A., Van den Brink, P.J. (2012) Ecological impacts of timevariable exposure regimes to the fungicide azoxystrobin on freshwater communities in outdoor microcosms. Ecotoxicology. 21: 1024-1038.
71. Zubrod, J.P., Baudy, P., Schulz, R., Bundschuh, M. (2014) Effects of current-use fungicides and their mixtures on the feedingand survival of the key shredder *Gammarus fossarum*. Aquat Toxicol. 150: 133-143.
72. Zubrod, J.P., Englert, D., Wolfram, J., Wallace, D., Schnetzer, N., Baudy, P., Konschak, M., Schulz, R., Bundschuh, M. (2015) Waterborne toxicity and diet-related effects of fungicides in the key leaf shredder *Gammarus fossarum* (Crustacea: Amphipoda). Aquat Toxicol. 169: 105-112.

6 List of abbreviations

AA-EQS	Annual Average – Environmental Quality Standard (In Dutch: JG-MKN, jaargemiddelde milieukwaliteitsnorm)
ADI	Acceptable Daily Intake
BCF	Bioconcentration Factor
BMF	Biomagnification Factor
EC_x	Concentration at which x% effect is observed
$EQS_{sp, water}$	Environmental Quality Standard based on the exposure of birds and mammals feeding on aquatic organisms
ERL	Environmental Risk Limit
LC_{50}	Concentration at which 50% mortality is observed
MAC-EQS	Maximum Acceptable Concentration – Environmntal Quality Standard for ecosystems (In Dutch: MAC-MKN)
Marine species	Species that are representative for marine and brackish water environments and that are tested in water with salinity > 0.5 ‰.
MIC	Minimal Inhibitory Concentration: lowest concentration at which no growth of fungi is observed
NOEC	No Observed Effect Concentration
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NOEAEC	No Observed Ecosystem Adverse Effect Level
$QS_{water, hh food}$	Quality Standard for surface water based on the human consumption opf fishery products
SSD	Species Sensitivity Distribution
TGD	Technical Guidance Document
TWA	Time Weighted Average
WFD	Water Framework Directive (2000/60/EC)

Annex 1 Summaries of mesocosm studies

Study 1	Chronic aquatic effect assessment for the fungicide azoxystrobin
Reference	Van Wijngaarden et al., 2014 [68]
Species; Population; Community	Phytoplankton, zooplankton, invertebrates, introduced: *Elodea nuttallii*
Test Method	Mesocosm
System properties	Outdoor mesocosm, water volume 1270 L, Ø 1.8 m, 0.8 m depth
Test compound	Azoxystrobin as Amistar (250 g a.i./L soluble concentrate formulation)
Exposure regime	Nominal chronic 0, 0.33, 1, 3.3, 10 and 33 µg a.i./L 42 D TWA measures 0.31, 0.98, 3.09, 9.35 and 32.77 µg a.i./L
Analysed	Y
Temperature [°C]	water temperature not reported
pH range	9 – 9.8
Salinity [‰]	Not reported
Exposure time	42 d
Criterion	NOEC
Test endpoint	Abundance of copepod and cladocera
Value [µg/L]	1 µg/L (measured chronic concentration)
GLP	N
Guideline	Not specified
Notes	
Ri	2

Description

Test system

The aim of the study was to study the effects of chronic exposure of azoxystrobin on the aquatic ecosystem. Circular mesocosms (19), diameter 1.8 m; depth 0.8 m; water volume 1270 L, 50-cm water column, lined with a water-tight nontoxic layer of black polyethylene were used. Mesocosms were located in Renkum, the Netherlands. Natural sediment 8 cm (fine clay, from a mesotrophic lake. Water from a supply basin with a mixture of rain and groundwater and housing a freshwater community. On 75% of the sediment surface of each cosm 100 shoots of *Elodea nuttallii* were planted. Other macrophytes developed from the diaspora during the experiment.

In the 3 months pre-treatment period invertebrates were added from uncontaminated mesotrophic ditches in the surrounding of the test facility. Dominant species were crustaceans (*Asellus aquaticus*, *Gammarus pulex*, *Cladocera* and *Copepoda*), insects (*Cloeon dipterum*, *Chaoborus sp.*, *Plea minutissima*, Chironomidae, Odonata, and Trichoptera), Hirudinea (*Erpobdella* sp.) and Gastropoda (*Valvata* sp.). Water was circulated during 2 weeks in the pre-treatment period to enhance a homogenous distribution between cosms. The date of the start of the experiment could not be found in the paper. Duration of the experiment 42 days.

Dosing and analytical measurements

Azoxystrobin was applied as Amistar (250 g a.i./L soluble concentrate formulation, purity 99.5%). Nominal concentrations: 0 µg a.i./L, 0.33 µg a.i./L, 1 µg a.i./L, 3.3 µg a.i./L, 10 µg a.i./L, and 33 µg a.i./L. Actual concentration was measured every 1-3 d. In order to keep the exposure

at the chronic treatment level on days 2, 9, 16, 20, 27, 32, and 37 additional product was added when needed.

Abiotic measurements

Dissolved oxygen, pH, electric conductivity, and temperature were measured according to Table 10. Alkalinity and concentrations of ammonia, nitrate, nitrite, total nitrogen, orthophosphate, and total phosphate were determined 5 d before the treatments started and at the end (day 42) of the experiment.

Table 10. Summary of sampling days and methods used for the sampling of the investigated endpoints in the microcosms

Compartment/Community Sampling	days
Water quality parameters[a]	-5, 2, 9, 16, 23, 32, 42
Dosing of water	-1, 2, 9, 16, 20, 27, 23, 37
Nutrients[b]	0, 42
Zooplankton sampling	-5, 2, 9, 16, 23, 32, 42
Phytoplankton sampling	-5, 2, 9, 16, 23, 32, 42
Phytoplankton chlorophyll-a	-5, 2, 9, 16, 23, 32, 42
Periphytic chlorophyll-a	-5, 2, 9, 16, 23, 32, 42
Maycrophyte species composition	-1, 14, 42
Myriophyllum spicatum	-3, 14, 42
Macroinvertebrates	
Pebble baskets	-7, 3, 10, 17, 42
Litter bags	-7, 3, 10, 17, 42
Decomposition	-7, 3, 10, 17, 42

[a]Dissolved oxygen, pH, alkalinity, electric conductivity, and temperature.
[b]Ammonia, nitrate, nitrite, total nitrogen, orthophosphate, and total phosphate.

Effect sampling

For the sampling scheme of the effect parameters see Table 10. The sampling methods which are shortly elaborated in the following were describes in other publications with the exception of the dosing of the water.

Zooplankton and phytoplankton were sampled using a Perspex (polymethyl methacrylate) tube and counted. Rotifers and cladocerans were identified to the lowest practical taxonomic level (i.e., genus or species level). Copepods were identified to the suborder by classifying them as calanoids or cyclopoids. A distinction was also made between nauplii and the more mature stages of the copepods.

Phytoplankton species composition was determined, the species being identified to the lowest practical taxonomic level. Zooplankton and phytoplankton data were expressed as numbers of individuals per liter.

Phytoplankton chlorophyll-*a* was sampled in parallel with the phytoplankton and zooplankton sampling. For periphyton, microscope glass plates were used that were placed 10 cm below the water surface and were allowed to be colonized for 14 d prior to sampling.

Macrophytes cover and abundance were determined three times. Biomass was determined at the end of the experiment. An additional bioassay was performed with *Myriophyllum spicatum*: in each cosm, at day -4, 12 pots with 3 shoots were placed. At day -3, 1 pot was harvested, and at day 14 and 42, 6 pots per cosm (note of the reviewer: is total of 13 per coms, which is not in accordance with the number of pots placed). Above and below ground dry weight was measured as well as number and total length of shoots.

Macroinvertebrates were sampled using two litterbags and two pebble bags per cosm, placed 2 weeks before start of the experiment. Invertebrates were identified and counted and released back in their original cosm.
Decomposition was determined using litterbags. Two litterbags were incubated for two weeks. (note of the reviewer: from the description in the paper it is unclear how two litterbags and a two week period matches with the sampling scheme of Table 10).

Statistical analysis
The macroinvertebrate data were transformed to Ln(2x+1), the zooplankton data to Ln(10x+1), and the phytoplankton data to Ln(x+1) before statistical analysis. NOEC was determined using Williams test. Multivariate analysis using PRC (Principal Response Curves) was applied to analyse the response in time of the macroinvertebrate and zooplankton community. Effects were classified according to De Jong et al. (2008).
For the bioassay with *M. spicatum*, EC50 and EC10 values were calculated using logistic regression.
Data for chronic toxicity from literature were used to apply a SSD, using the ETX software.

Results
Chemical analysis
Since dosing was adjusted according to the results of the analytical measurements, azoxystrobin concentrations (see Figure 6 below, copied from report) were close to the intended concentrations. The 42 d TWA is 0.31 µg a.i./L, 0.98 µg a.i./L, 3.09 µg a.i./L, 9.35 µg a.i./L and 32.77 µg a.i./L, which is between 95.2 and 99.3 % of intended.

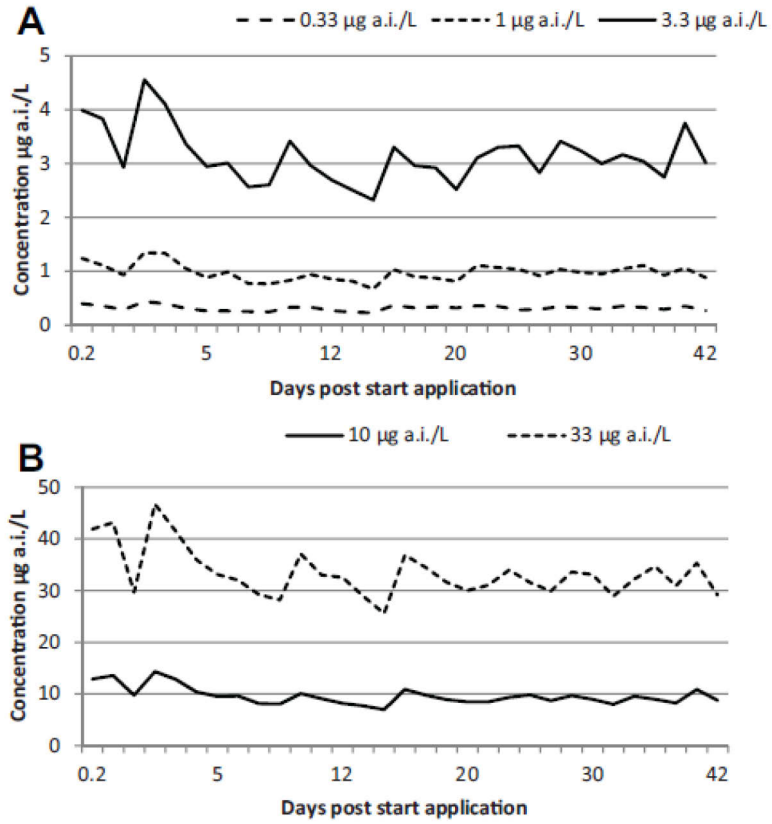


Figure 6. Measured concentration of azoxystrobin (A) 0.33 µg a.i./L, 1 µg a.i./L, and 3.3 µg a.i./L treatments, and for the (B) 10 µg a.i./L and 33 µg a.i./L treatments. Values are arithmetic means per treatment level.

Abiotic parameters
Values not reported (except pH), the paper stated that no consistent significant treatment-related effects were found on decomposition, alkalinity, nutrients, electrical conductivity, dissolved oxygen, temperature, (see Table 11) no further details reported. pH ranged from 9 – 9.8 (average 9.4-9.7). Relatively large differences in pH between treatments were present on day -7 (0.5 pH units). By the end of the experimental period, pH in treated microcosms was higher compared with controls resulting in a treatment related effect on pH and a NOEC for pH of 0.33 µg/L was estimated (difference between treatments and controls within 0.5 pH units).

Biological observations

Chlorophyll-a and decomposition
No consistent significant treatment-related effects were found on decomposition and periphyton/phytoplankton chlorophyll-a (see Table 11) no further details reported.

Phytoplankton
A number of 225 phytoplankton taxa were identified. According to the authors, the PRC diagram of the phytoplankton revealed deviations from the controls, these did not show a dose-effect relationship. PRC diagram not reported. According to the authors consistent negative treatment-related effects on the community were observed on days 16 and 23 in the 3.3 µg a.i./L treatment only. The NOEC for the phytoplankton community was set to 33 µg a.i./L (see Table 11). For some individual phytoplankton species, lower NOECs were calculated (see Table 11 and Figure 7). The authors argue that the results are difficult to interpret. The NOEC of 0.33 µg a.i./L calculated for *Cosmarium moniliferum* is based on low abundance and the difference were already found in the pre-treatment phase. For *C. crenulatum* (NOEC of 3.3 µg a.i./L) no clear concentration–response relationship was observed. The NOECs for *Ankyra sp*., *Chroococcales* 2-mm to 5-mm colony and *Cosmarium turpinii* (decrease) were all 10 µg a.i./L. The effects in the highest treatment concentration indicate that these species are relatively insensitive. Overall, consistent responses were mostly found within the first 23 d of the treatment.

Table 11 Consistent (bold font) no-observed-effect concentrations (NOECs) for azoxystrobin (mg a.i./L)[a]. Copied from Van Wijngaarden et al., 2014).

	Days after initiation chronic treatment					
	2–3	9–10	16	23	32	42
Physiochemical						
Water chemistry	≥33	≥33	≥33	≥33	≥33	≥33
Community metabolism	≥33	≥33	≥33	≥33	≥33	≥33
pH	≥33	≥33	≥33	≥33	3.3	0.33
Functional						
Decomposition	≥33	≥33	≥33	≥33	≥33	≥33
Zooplankton						
Community (PRC)	**10**	**10**	**10**	**10**	**10**	**10**
Copepoda						
Nauplii	**3.3**	**10**	**10**	**3.3**	**3.3**	**3.3**
Calanoida	**3.3**	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**
Cyclopoida	**3.3**	**10**	**3.3**	**10**	**3.3**	**10**
Cladocera						
Daphnia longispina group	**1.0**	**3.3**	**10**	**10**	**10**	**10**
Rotifera						
Synchaeta sp.	≥33(+)	≥33(+)	10(+)	≥33(+)	≥33(+)	≥33(+)
Cephalodella gibba	≥33(+)	≥33(+)	≥33(+)	≥33(+)	≥33(+)	≥33(+)
Phytoplankton						
Community (PRC)	≥33	≥33	≥33	≥33	≥33	≥33
Chlorophyll-*a*	≥33	≥33	≥33	≥33	≥33	≥33
Cosmarium moniliferum	≥33	**0.33**	**0.33**	**0.33**	—	≥33
Cosmarium crenulatum	≥33	**3.3**(+)	**3.3**(+)	**3.3**(+)	—	≥33
Cosmarium turpinii	**10**	**10**	≥33	≥33	—	≥33
Ankyra sp.	≥33	**10**(+)	**10**(+)	**10**(+)	—	≥33
Chroococales 2–5 μm	**10**	≥33	**10**	**10**	—	**10**
Periphyton						
Chlorophyll-*a*	≥33	≥33	≥33	≥33	≥33	≥33
Macrophytes						
Community (PRC)	≥33	≥33	≥33	≥33	≥33	≥33
Chara globularis	≥33	≥33	**1**	≥33	≥33	**10**
Elodea nuttallii	**10**	≥33	**10**	≥33	≥33	**10**
Macroinvertebrates						
Community (PRC)	≥33	≥33	≥33	≥33	≥33	≥33
Population level	N/A	N/A	N/A	N/A	N/A	N/A

[a]Concentrations > no-observed-effect concentration showed significant reductions or increases (+).
a.i. = active ingredient; PRC = principal response curves; — = not sampled; N/A = numbers were too low for evaluation.

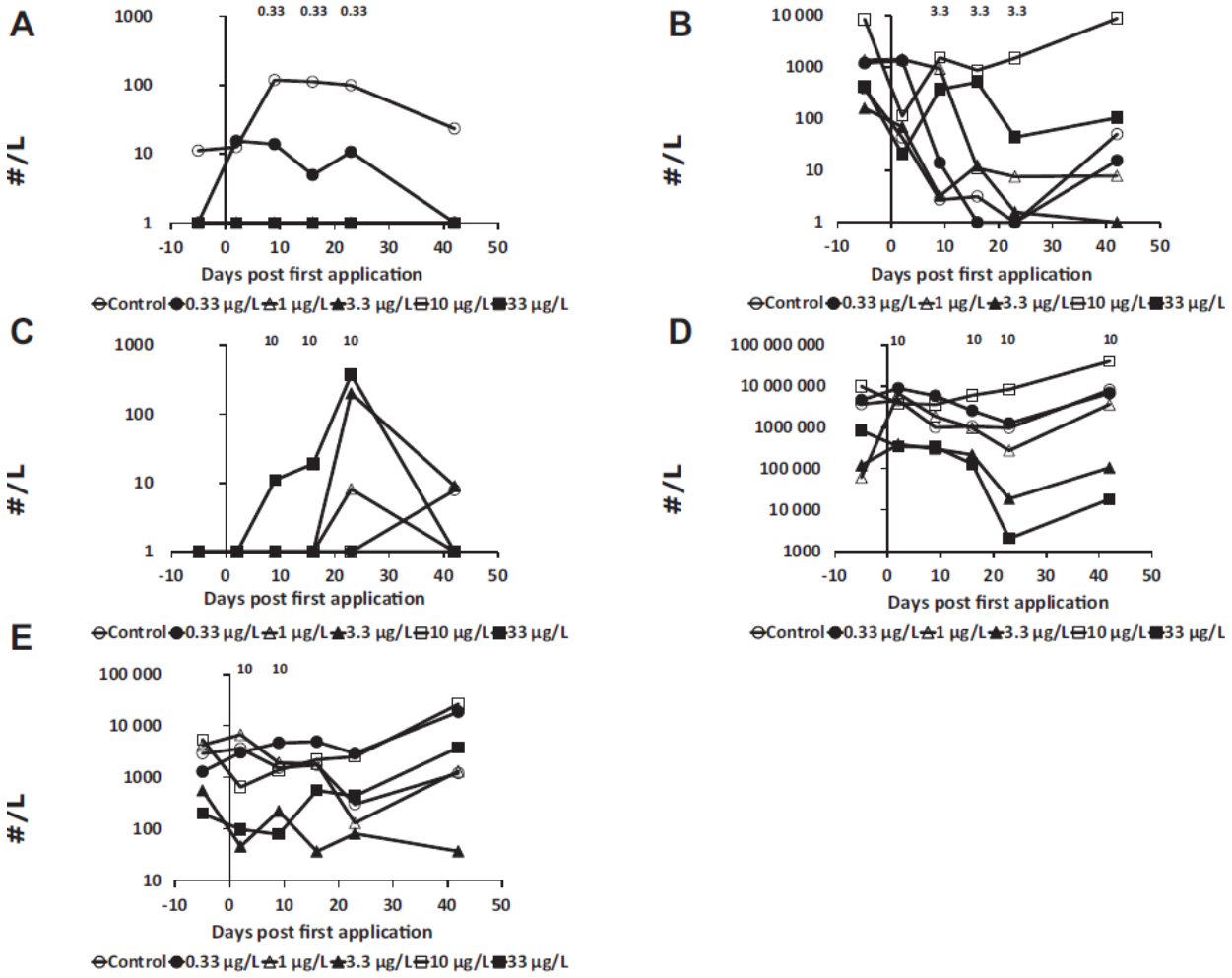


Figure 7. Dynamics of numbers (geometric means) of (A) *Cosmarium moniliferum*, (B) *Cosmarium crenulatum*, (C) *Ankyra* sp., (D) Chroococcales 2-mm to 5-mm colony and (E) *Cosmarium turpinii*. Numbers above the sampling dates indicate the no-observed-effect concentrations, derived following the Williams test, one-sided, a=0.05. Copied from Van Wijngaarden et al., 2014) [68].

Macrophytes

The results of the bioassays with *M. spicatum* show that the calculated EC50 was higher than the highest tested concentration, at 39 µg a.i./L for root dry weight and 50 µg a.i./L for shoot dry weight. On day 14, significant effects were found on the number of shoots, mean length of shoots, and dry weight of roots and shoots, with NOEC values of 1 µg a.i./L, 1 µg a.i./L, 10 µg a.i./L, and 10 µg a.i./L, respectively. Effects were not found on consecutive sampling dates. The PRC analyses for the community and the biomass at the end of the experiment did not show any significant treatment related effects.
For *Chara globularis* and *E. nuttallii* significant differences were found on some isolated sampling moments, with NOEC of 10 µg/L for *E. nuttallii* and 1 and 10 µg/L for *C. globularis*

Zooplankton

86 different zooplankton taxa were identified. Consistent negative treatment-related effects on the zooplankton communities were observed in the 33 µg a.i./L treatment on all sampling dates without recovery within the period of the experiment Figure 8 and Table 11). No significant differences between treatments and controls were found in the other treatments, the authors report a treatment related effect at the 3.3 µg a.i./L treatment on day 16, followed by recovery.

According to the reviewer this should be the 10 µg a.i./L treatment. At the zooplankton community level, a NOEC of 10 µg a.i./L was be deduced from the data (Table 11).

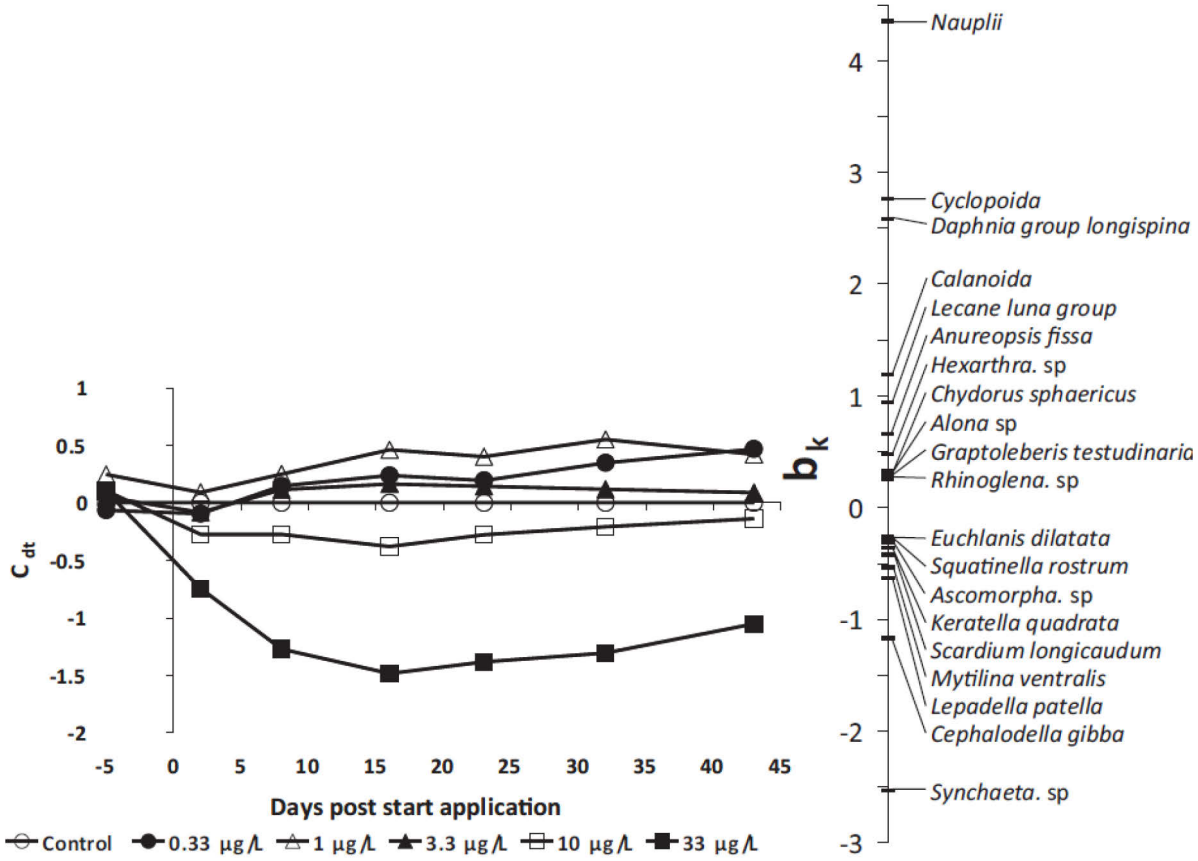


Figure 8. Principal response curves for the zooplankton dataset. Sixteen percent of all variance could be attributed to the sampling date (displayed on the horizontal axis). Thirty-one percent of all variance could be attributed to treatment level, 31% of which is displayed on the vertical axis. The lines represent the development of the treatments in time. The species weight (b_k) can be interpreted as the affinity of a taxon with the principal response curves (c_{dt}). Taxa with a species weight between 0.25 and -0.25 are not shown. A Monte Carlo permutation test indicated that the diagram displays a significant amount of the variance explained by the treatment (p=0.002).

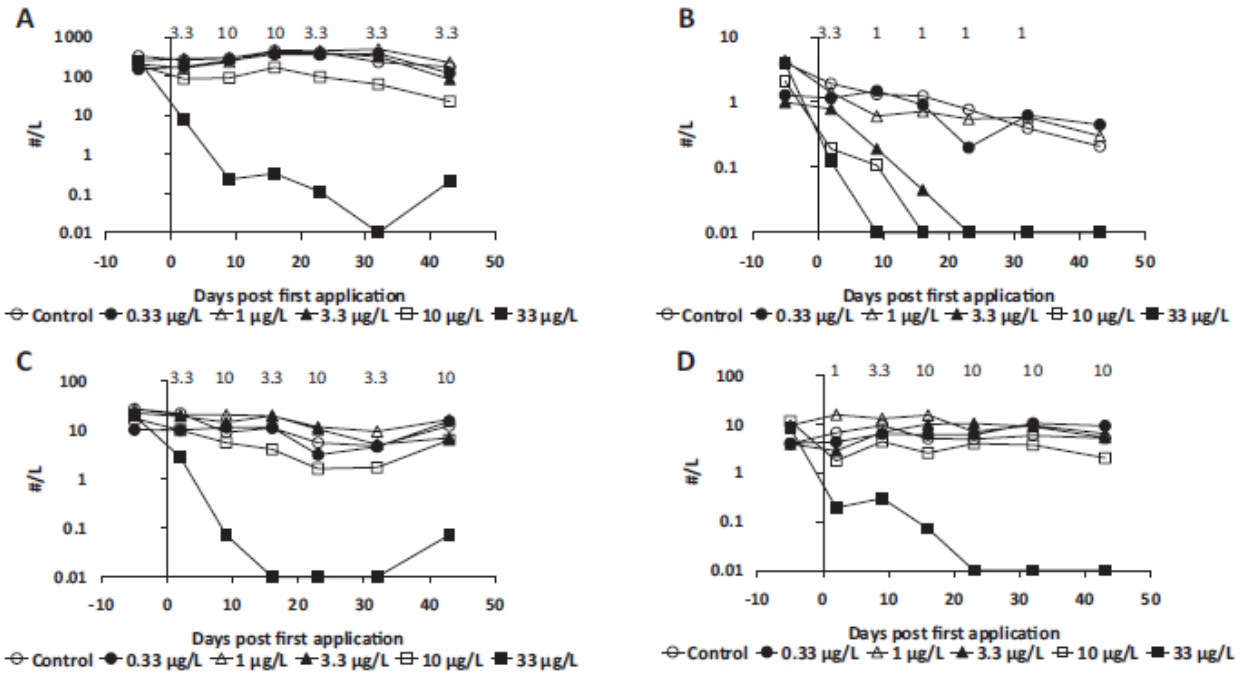


Figure 9. Dynamics of numbers (geometric means) of (A) copepod nauplii, (B) calanoid copepods, (C) cyclopoid copepods, and (D) *Daphnia longispina* group. Numbers above the sampling dates indicate the no-observed-effect concentrations, which are derived following the Williams test, one-sided, a=0.05.

Figure 9 shows taxa with the high positive species scores (bk>2) in the PRC diagram (copepod *nauplii*, calanoid copepods, cyclopoid copepods and *Daphnia longispina* group. Effects became apparent immediately after the azoxystrobin application. For all 4 taxa, statistically significant treatment-related effects on abundance were observed on consecutive sampling days at the highest treatment concentration of 33 µg a.i./L (Figure 9), lasting over the entire experimental period, without recovery. Clear effects also occurred at the 10 µg a.i./L treatment concentration, but these were less pronounced (nauplii, cyclopoids, *D. longispina* group) and were mostly followed by recovery for the cyclopoids and the *D. longispina* group. Calanoids showed statistically significant reductions at concentrations of 3.3 µg a.i./L and higher without recovery. Overall this results in NOEC values of 1, 3.3 and 10 µg a.i./L.

Macroinvertebrates
The PRC analysis did not show any significant effects (Table 11). At the species level, only 1 of the 86 species identified during the experiment showed significant treatment-related effects on consecutive sampling dates. For *Corixa* sp., NOEC values of 3.3 and 10 µg a.i./L were calculated on days 17 and 42, respectively. The densities of *Corixa* sp. were, however, very low in all microcosms, including controls (generally <1 individual per substrate). Further details are not presented in the paper.

Effect classes
Effects were classified according to De Jong et al., 2008 [11], see Table 12.

Table 12. Effects of azoxystrobin on ecosystem endpoints[a]

	Azoxystrobin (μg/L)				
Category	0.33	1.0	3.3	10	33
Water chemistry	1	1	1	1	1
Community metabolism	1	1	1	1	1
Decomposition	1	1	1	1	1
Zooplankton					
Community (PRC)	1	1	1	1	4
Copepoda	1	1	4(↓)	4(↓)	4(↓)
Cladocera	1	1	2(↓)	3A(↓)	4(↓)
Rotifera	1	1	1	1	1
Phytoplankton					
Community (PRC)	1	1	1	1	1
Chlorophyll-*a*	1	1	1	1	1
Desmids	1	1	1	3A(↓)	3A(↓↑)
Chlorophyta	1	1	1	1	3A(↑)
Cyanobacteria	1	1	1	1	4(↓)
Periphyton					
Chlorophyll-*a*	1	1	1	1	1
Macrophytes					
Community (PRC)	1	1	1	1	1
Macroinvertebrates					
Community (PRC)	1	1	1	1	1

[a]Effects are categorized into Effect Classes following De Jong et al. 2008 [11]. Themost sensitive endpoint (mostly species) within each category was chosen for effect evaluation.
↓decrease in endpoint; ↑increase in endpoint; 1=no effects; 2=slight effect;
3A=clear short-term effect, with full recovery observed within 8 wk;
4=clear effects, recovery of effects within 8 wk cannot be evaluated and/or
effects occurred late in study; PRC=principal response curves.

Evaluation of the scientific reliability of the field study

The following five criteria are used to assess if this (semi)field study is suitable, based on De Jong et al. [11] and EFSA [18].

1. *Does the test system represent a realistic marine community?*

Species composition and abundances were not specified in the paper. Natural populations of zooplankton, invertebrates, phytoplankton, and macrophytes were present. Fish were not present, but absence of vertebrates is common in mesocosm studies.

2. *Is the description of the experimental set-up adequate and unambiguous?*

Design and methods are clear. Since the results are presentented in a paper, raw data are not reported also only the results of the statistical evaluations are shown, without the underlying data.

3. *Is the exposure regime adequately described?*

Yes. Concentrations are measured regularly, in order to maintain a chronic constant concentration.

4. *Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound?*

Yes, potentially sensitive taxa such as invertebrates and algae were present. The mode of action of the a.i. is fungicidal; effects on fungi are not studied in the mesocosm. It is however uncommon to study fungi in mesocosms.

5. *Is it possible to evaluate the observed effects statistically?*
Yes/No. The data are analysed according to acceptable statistical methods. However, details (raw data, significancy, statistical output) are not presented in the paper.

Remarks
The pH of the study is relatively high (9.0 – 9.8). From the available information, no pH depended toxicity is indicated.

The aim of the paper was to present the study results not the underlying data. Therefore only few data are available. For that reason an RI 2 (less reliable) is assigned. Nevertheless from this mesocosm study it can be concluded that at a dose of 1 µg/L no effects were present on community and populations of zooplankton, phytoplankton and macroinvertebrates, and this endpoint can be used as NOEC from the mesocosm study.

Study 2	Ecological impacts of time-variable exposure regimes to the fungicide azoxystrobin on freshwater communities in outdoor microcosms
Reference	Zafar et al., 2012 [70]
Species; Population; Community	Phytoplankton, zooplankton, invertebrates, introduced: *Elodea nuttallii*
Test Method	Mesocosm
System properties	Outdoor mesocosm, water volume 1270 L, Ø 1.8 m, 0.8 m depth
Test compound	Azoxystrobin as Amistar (250 g a.i./L soluble concentrate formulation)
Exposure regime	Nominal chronic 0, 10 and 33 µg a.i./L, single application 33 µg a.i./L, 4 applications with 16 µg a.i./L. 42 D TWA measured 9.35 and 32.8 µg a.i./L, and 14.9 and 14.9 µg a.i./L respectively.
Analysed	Y
Temperature [°C]	water temperature not reported
pH range	9.1 – 9.9
Salinity [‰]	Not reported
Exposure time	42 d
Criterion	NOEAEC
Test endpoint	Abundance of cyclopoida
Value [µg/L]	< 10 µg/L (measured chronic concentration)
GLP	N
Guideline	Not specified
Notes	
Ri	2

In a study design that is similar to of the study of Van Wijngaarden et al, 2014 (Study 1) the effects of different exposure regimes is studied. The aim of the study was to investigate whether the TWA or the peak concentration is a better predictor for long-term effects. For this aim the effects of continuous exposure (42 d) with 10 and 33 µg a.i./L were compared to a single application with 33 µg a.i./L and four applications with 16 µg/L (10 d interval).
Clear treatment related effects where however found for all treatments on Cyclopoida.

The PRC results show clear effect on zooplankton for all treatments. The Monte Carlo permutation tests however show that these effects were not significant for the 10 µg a.i./L treatment. The authors conclude that the NOEAEC is 10 µg a.i./L. Since clear effects were found on Cyclopoida in the 10 µg a.i./L treatment, it is not possible to derive a NOEC from this mesocosm study.

Study 3	Direct and indirect effects of the fungicide azoxystrobin in outdoor brackish water microcosms
Reference	Gustafsson et al. (2010) [31]
Species; Population; Community	Phytoplankton, zooplankton, bacterial activity, decomposition
Test Method	Microcosm
System properties	Experiment 1: 24-L outdoor microcosms for 21 days Experiment 2: 4-L indoor microcosms for 12 days
Test compound	Azoxystrobin in aceton
Exposure regime	Experiment 1: 0, 15 and 60 µg/L (nominal) Experiment 2: 0, 3, 7.5, 15 µg/L (nominal)
Analysed	Y
Temperature [°C]	Experiment 1: 20.6 (mean over experiment) Experiment 2: 15 (controlled chamber)
pH range	Experiment 1: 8.8 (mean over experiment) Experiment 2: 8.09 (control, start of experiment)
Salinity [PSU]	Experiment 1: 5.8 (mean over experiment) Experiment 2: 5.5 (control, start of experiment)
Exposure time	Experiment 1: 21 d Experiment 2: 12 d
Criterion	NOEC
Test endpoint	Abundance of copepod and cladocera
Value [µg/L]	Experiment 1: < 15 µg/L Experiment 2: < 3 µg/L
GLP	N
Guideline	Not specified
Notes	
Ri	2

Description

Experiment 1

Test system

The aim of the study was to investigate the potential effects on the aquatic ecosystem of the Baltic Sea. Circular microcosms (18), white plastic 30-L containers, filled with 24 L water. Microcosms were located in Stockholm, Sweden. Sediment and water were collected from the Baltic sea on April 1, 2003), sifted through a 5 mm sieve and kept stored in the dark at 4ºC for 3 months. Aim was to maintain the pre-spring bloom characteristics of the sediment in order to conserve resting stages of plankton. Mid-June the sediment and water was transferred to an outdoor open tank. After two weeks 100 L unfiltered surface water and concentrated living plankton, obtained by sieving 40 L Baltic sea water was added to the tank. One week later, the microcosms were prepared in the containers by adding sediment (380 mL; 375 g dry weight) and water (14 L) from the hatching tank, and 10 L unfiltered surface water. The microcosms were placed outdoors in three rows of 6 microcosms. According to the authors the zooplankton community corresponded well with the community in the Baltic sea.

Dosing and analytical measurements

Azoxystrobin was dissolved in acetone (acetone was also applied to the control, no separate solvent control was performed) at initial nominal concentrations of 0, 15 and 60 µg/L on July 19 (day 0) and lasted for 21 days.

Abiotic measurements
Dissolved oxygen, pH, temperature and salinity were measured daily, nutrients at the end of the experiment. Concentrations were measured at the start and the end of the experiment.

Effect sampling
Zooplankton was sampled at day 3, 7, 16 and 20. Phytoplankton was sampled at day 7 and 20, primary production was estimated at day day 0,1, 2, 4, 8, 11, 14, 17 and 20). Chlorophyll-a concentration was measured at day 3, 7, 11, 15 and 20. Bacterial activity was estimated by determination of the ^{14}C leucine incorporation on day 1, 2, 4, 8 and 14. Biological degradation was determined by weighing stems and leaves of *Ranunculus baudotii* at day 0 and 21.

Statistical analysis
Analyses of variance (ANOVA) was performed on log-transformed data, exept for abundance of copepods and rotifers where on 4th root transformed data were used. When differences were significatn, Dunnett's test was used to compare treatments with controls. Homogenity of variance was tested according to Cochran's C test.
Multivariate analyses was performed on the structure of the zooplankton community and the phytoplankton community. Analysis of similarity (ANOSIM), was followed by pairwise comparisons between treatments and controls to investigate the differences between the communities. Bray–Curtis similarity measure and square root transformed data were used. Analyses of similarity percentages (SIMPER) were used to determine taxa contributing the most to the observed dissimilarities in the data sets.

Experiment 2
An almost analougous indoor experiment was conducted with 5 L containers. Treatment 0, 3, 7.5 and 15 µg a.i./L, five replicates. Zooplankton community was sampled after 12 days. No analyzes of the active ingredient.

Results
Significant effects were found for a number of endpoints in the 15 and 60 µg/L treatment of the microcosms of experiment 1. Therefore it is not possible to obtain a NOEC that can be used for EQS derivation.

In the indoor experiments (experiment 2) effects on copepods were found in all concentrations (lowest dose 3 µg a.i./L), so also from this study it is not possible to derive a NOEC. It should be noted that this study concerns an indoor experiment in a relatively small cosm, and the concentrations were not measured.

Study 4	From the DAR (2009) [14]: Azoxystrobin: an outdoor pond mesocosm study.
Reference	Cole et al. (2000) [8]
Species; Population; Community	Phytoplankton, zooplankton, macroinvertebrates, introduced: *Elodea nuttallii*
Test Method	Mesocosm
System properties	18 Mesocosms, diameter and depth 125 cm, 1230 L, 10 cm sediment
Test compound	Azoxystrobin (250 g a.i./L soluble concentrate formulation)

Exposure regime	0, 10, 30, 100, 300 and 1000 µg/L (nominal) Measured initial (21 h) of the 10 µg/L treatment is 115% of nominal
Analysed	Y
Temperature [°C]	14.2-19.6
pH range	7.4 - 0.4
Salinity [‰]	Not reported
Exposure time	91 d
Criterion	NOEC
Test endpoint	Abundance of zooplankton
Value [µg/L]	< 10 µg/L (initial nominal)
GLP	Y
Guideline	SETAC Europe, 1992 [57], Campbell et al, 1999 [6], Brock et al., 2000 [5]
Notes	
Ri	2

The study is summarized in the DAR [14].

At the 10 µg/L treatment, consistent and treatment related significant effects were found for a number of taxa, especially within the zooplankton. Only the initial concentration was measured. No NOEC could be derived from this study. The NOEC < 10 µg a.i./L can be used as supporting information.

Study 5	Assessed in original DAR (1997): An aquatic mesocosm study to assess the effects of a 500 g/kg WG formulation on filter feeding zooplankton
Reference	Maund and Kearson (1995)[44]
Species; Population; Community	Phytoplankton, zooplankton
Test Method	Mesocosm
System properties	15 Mesocosm, diameter and depth 125 cm, 1200 L, 10 cm sediment.
Test compound	Azoxystrobin (500 g a.i./kg water dispersible granule formulation)
Exposure regime	10, 30 g a.i./ha (intitial nominal), equivalent to spray drift into aquatic ecoysystems at 4% and 12% of a 250 g a.i./ha treatment. Calculated initial 1 and 2.85 µg a.i./L. Measured 0.75 and 2.2 µg a.i./L, 1 day after application.
Analysed	Y
Temperature [°C]	15.9-21.7
pH range	7.4 – 9.8
Salinity [‰]	Not reported
Exposure time	28 d
Criterion	NOEC
Test endpoint	Abundance of zooplankton
Value [µg/L]	2.2 µg/L (measured 1 day after application)
GLP	Y
Guideline	SETAC Europe, 1992 [57]
Notes	
Ri	2

The study was originally assessed in the original DAR (1997) for Annex I inclusion.

Study 5 has the same design as study 4. Dose is expressed as g a.i./ha, however calculated initial concentration and measured concentrations are only presented in Figure 10. From this figure it can be derived that initial concentration (calculated) is 1 and 2.85 µg a.i./L. Measured concentrations after 1 day are 0.75 and 2.2 µg a.i./L.

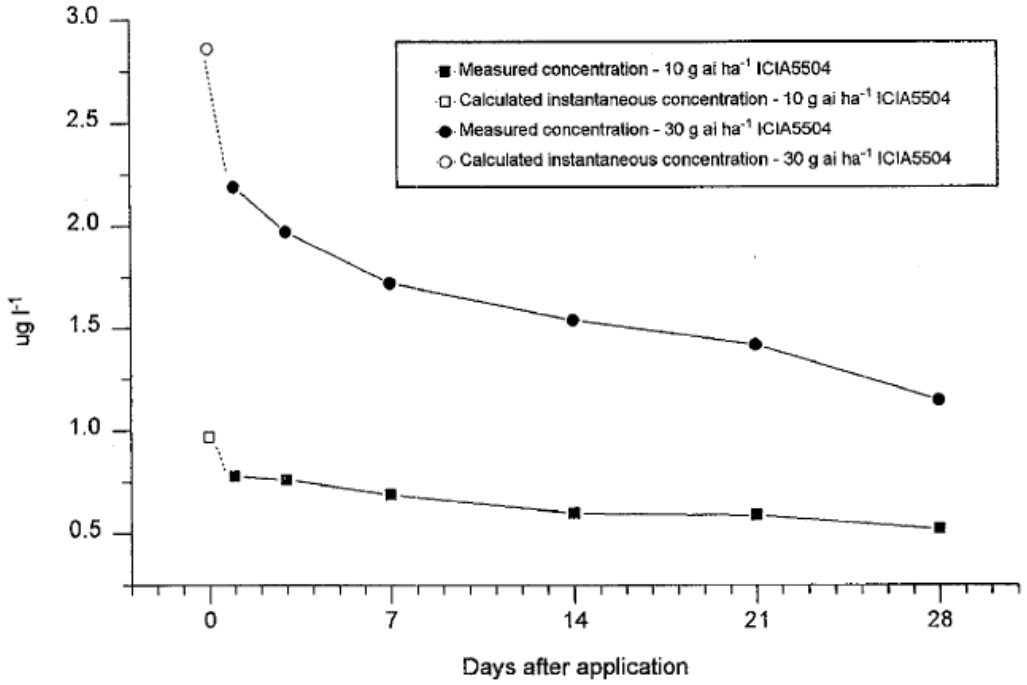


Figure 10. Measured and calculated concentrations of azoxystrobine

Effects are measured on zooplankton and phytoplankton population. According to the authors no significant treatment related effects could be observed. The calculated Minimum Significant Differences (MSD) for zooplankton and phytoplankton varied from 54-74%, indicating that the test system is a relatively sensitive. Before start of the experiment, the cosms were allocated to blocks, based on number of cladocerans and phytoplankton density. The results of individual species show considerable variance in the sampling date before application.
In the study only two concentrations has been studied. In both concentrations no effects were found, higher concentrations that could show an effect are not included. This renders it difficult to put the lack of effects in the tested concentrations into perspective. On the other hand is the lack of effects in line with the findings in study 1 for the 1 µg a.i./L treatment in study 1. In study 5 only one application is applied and the concentration over the study period is lower (see Figure 10). For the 2.85 µg a.i./L concentration the measured concentration is 2.2 µg a.i./L. In study 1 effects om some species were found at 3.3 µg a.i./L (chronic exposure). In the indoor experiment (2) in study 3 shows clear effects at 3 a.i./L. The results of study 5 thus underpins the NOEC of 1 µg a.i./L.

Annex 2 Aquatic toxicity data

The abbreviations used in the toxicity data tables in this Annex are explained in the Dutch guidance on EQS derivation [53], part 3, section 2.1.1.

Table A2.1. Acute toxicity of azoxystrobin to freshwater organisms

Species	Species properties	Test type	A	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg a.s./L]	Ri	Notes	Ref	Study id.
Bacteria																	
Pseudomonas putida		S	N							6 h	NOEC		>3.2	2		[14]	Table B.9.2.1
Pseudomonas putida		S	N							6 h	EC50		>3.2	2		[14]	Table B.9.2.1
Cyanobacteria																	
Anabaena flos-aquae	strain CCAP 1403/13A	S	Y	tg	96.2	am	7.2-7.6	23.8-24.4	15	120 h	EC50	growth rate	>21	3	21	[14, 61]	P 627
Anabaena flos-aquae	strain CCAP 1403/13A	S	Y	tg	96.2	am	7.2-7.6	23.8-24.4	15	72 h	EC50	growth rate	13.9	3	21	[14, 61]	P 627
Fungi																	
leaf litter fungi; micro-cosm	predation by *A. aquaticus*	R	N	ag	n.r.	am (M7)		11-12		13 d	LOEC	biomass	2.6	3	58	[10]	
Saprolegnia sp.	strain JL, No. HM637287	S	N		98	am		25		48 h	MIC (EC100)	growth	0.5	2	26	[32]	
Saprolegnia sp.	spores; strain JL, No. HM637287	S	N		98	agar plates		25		72 h	MFC=LOEC	growth	0.13	3	62	[32]	
Saprolegnia sp.	strain JL, No. HM637287	S	N		98	agar plates		25		48 h	EC50	growth	0.212	3	27	[32]	
Algae																	
Chlorella vulgaris		S	Y	a.s.	98.5	am				96 h	EC50	biomass	0.51	3	42	[42]	
Navicula pelliculosa	strain Utex 667	S	Y	tg	96.2	am	7.5-8.6	23.9-24.3	15	120 h	EC50	growth rate	>0.320	2	14	[14, 62]	P 627
Navicula pelliculosa	strain Utex 667	S	Y	tg	96.2	am	7.5-8.6	23.9-24.3	15	72 h	EC50	growth rate	0.146	2	14	[14, 62]	P 627
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	72 h	EC50	growth rate	1.47	2	16	[14, 59]	
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	72 h	EC50	biomass	0.183	2	16	[14, 59]	
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	96 h	EC50	growth rate	2.0	2	16	[14, 59]	
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	96 h	EC50	biomass	0.36	2	15	[14, 59]	Table B.9.2.1
Raphidocelis subcapitata		S	N	a.s.		am		25		72 h	EC50	biomass	0.23	3	42	[45]	
Raphidocelis subcapitata		S	N	250 g/L SC formulation						72 h	EC50		0.063	2	44	[14]	Table B.9.2.2
Raphidocelis subcapitata		S	N	250 g/L SC formulation						72 h	EC50		0.16	2	44	[14]	Table B.9.2.2
Raphidocelis subcapitata		S	N	250 g/L SC formulation						120 h	EC50		0.054	2	44	[14]	Table B.9.2.2
Macrophyta																	
Lemna gibba	strain G3	R	Y	tg	92.6	am	4.5-6.0	25±1	700	14 d	EC50	frond number	3.2	2	20	[14, 63]	P 630
Lemna gibba	strain G3	R	Y	tg	92.6	am	4.5-6.0	25±1	700	14 d	EC50	dry weight	>6.4	2	20	[14, 63]	P 630
Rotifera																	
Brachionus calyciflorus	neonates <24 h; ROTXKIT F	S	Y	tg	92.6	50%dtw,50%dw		25±1	163	24 h	LC50	mortality	>4.00	2	35	[14, 27]	Table B.9.2.1
Brachionus calyciflorus	neonates <24 h; ROTXKIT F	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw		25±1	163	24 h	LC50	mortality	>4.00	2	53	[14, 27]	Table B.9.2.2
Mollusca																	
Lymnea stagnalis	15 mm; exp. pond	S	Y	tg	92.6	50%dtw,50%dw	7.4-7.8	20.2-20.9	163	48 h	LC50	mortality	>4.00	2	39	[14, 26]	Table B.9.2.1
Lymnea stagnalis	15 mm; from experimental pond	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	7.4-7.8	20.2-20.9	163	48 h	LC50	mortality	>4.00	2	45	[14, 26]	Table B.9.2.2
Musculium lacustre	ad, 4-6 mm, exp. pond	S	Y	250 g/L SC form. (A-12705)	25	dtw+dw	7.3-8.5	18.6	172	48 h	LC50	mortality	>1.0	2	31	[48]	

Species	Species properties	Test type	A	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg a.s./L]	Ri	Notes	Ref	Study id.
Crustacea																	
Asellus aquaticus	juv, 5 mm, exp. pond	S	Y	tg	92.6	50%dtw,50%dw	8.0-8.3	20.6-20.9	163	48 h	LC50	mortality	>4.00	2	32	[14, 28]	Table B.9.2.1
Asellus aquaticus	non breeding females; 6-8 mm; field collected	R	N	ag	n.r.	am (M7)		11-12		13 d	LOEC	mortality	2.6	3	57	[10]	
Asellus aquaticus	juv, 5 mm, exp. pond	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	8.0-8.3	20.6-20.9	163	48 h	LC50	mortality	>4.00	2	46	[14, 28]	Table B.9.2.2
Chydorus sphaericus	0.25 mm	S	N		>80	am (ADaM)	7.8±0.2		250	48 h	NOEC	immobility	0.15	2	8	[40]	
Chydorus sphaericus	0.25 mm	S	N		>80	am (ADaM)	7.8±0.2		250	48 h	EC50	immobility	0.37	2	8	[40]	
Daphnia galeata	lab culture; 1.41 mm	S	N		>80	am (ADaM)	7.8±0.2		250	48 h	NOEC	immobility	<0.08	2	4	[40]	
Daphnia galeata	lab culture; 1.41 mm	S	N		>80	am (ADaM)	7.8±0.2		250	48 h	EC50	immobility	0.095	2	3	[40]	
Daphnia galeata	field collected; 1.69 mm	S	N		>80	am (ADaM)	7.8±0.2		250	48 h	NOEC	immobility	0.08	3	7	[40]	
Daphnia galeata	field collected; 1.69 mm	S	N		>80	am (ADaM)	7.8±0.2		250	48 h	EC50	immobility	0.18	3	7	[40]	
Daphnia magna	<24 h	S	Y	tg	92.6	50%dtw,50%dw	7.8-8.0	20.2-20.6	160-180	48 h	NOEC	mortality	0.126	2	43	[14, 49]	Table B.9.2.1
Daphnia magna	<24 h	S	Y	tg	92.6	50%dtw,50%dw	7.8-8.0	20.2-20.6	160-180	48 h	LC50	mortality	0.28	2	43	[14, 49]	Table B.9.2.1
Daphnia magna		S	Y							48 h	EC50		0.23	2	61	[14]	Table B.9.2.1
Daphnia magna		S	Y							48 h	EC50		0.19	2	59	[14]	Table B.9.2.1
Daphnia magna		S	N							48 h	EC50		0.82	3	60	[14]	Table B.9.2.1
Daphnia magna		S	N							48 h	NOEC		0.125	2		[14, 50]	Table B.9.2.1
Daphnia magna		S	N							48 h	EC50		0.27	2		[14, 50]	Table B.9.2.1
Daphnia magna	ad. ♀ with eggs in pouch; 8 d; 2.55 mm	S	N	ag	99.6	am (ADaM)	6.1-7.8	20±0.2	250	24 h	NOEC	heart activity	1	2	10	[29, 40]	
Daphnia magna	ad. ♀ with eggs in pouch; 8 d; 2.55 mm	S	N	ag	99.6	am (ADaM)	6.1-7.8	20±0.2	250	24 h	NOEC	filter activity	1	2	10	[29, 40]	
Daphnia magna	ad. ♀ with eggs in pouch; 8 d; 2.55 mm	S	N	ag	99.6	am (ADaM)	6.1-7.8	20±0.2	250	24 h	NOEC	mandible activity	1	2	10	[29, 40]	
Daphnia magna	ad. ♀ with eggs in pouch; 8 d; 2.55 mm	S	N	ag	99.6	am (ADaM)	6.1-7.8	20±0.2	250	24 h	NOEC	focal spine activity	1	2	10	[29, 40]	
Daphnia magna	lab culture; juv 24 h; 0.91 mm	S	N		>80	am (ADaM)	7.8±0.2	20	250	48 h	NOEC	immobility	0.404	2	5	[40]	
Daphnia magna	lab culture; juv 24 h; 0.91 mm	S	N		>80	am (ADaM)	7.8±0.2	20	250	48 h	EC50	immobility	0.53	2	5	[40]	
Daphnia magna	lab culture; ad with eggs; 2.4 mm	S	N		99.6	am (ADaM)	7.8±0.2	20	250	48 h	NOEC	immobility	0.9	3	9	[40]	
Daphnia magna	lab culture; ad with eggs; 2.4 mm	S	N		99.6	am (ADaM)	7.8±0.2	20	250	48 h	EC50	immobility	1.57	3	9	[29, 40]	
Daphnia magna	neonates; clone LD	S	N			am	7.5-7.7	20		48 h	LC50	mortality	0.277	2	17	[69]	
Daphnia magna	neonates; clone GM	S	N			a.m.	7.5-7.7	20	250	48 h	LC50	mortality	0.071	**2**	17	[69]	
Daphnia magna	neonates; clone HG	S	N			a.m.	7.5-7.7	20	250	48 h	LC50	mortality	0.098	**2**	17	[69]	
Daphnia magna	neonates; 1 d old	S	N	a.s.		am		22	112	96 h	LC50	mortality	0.31	2	41	[45]	
Daphnia magna		S	N	500 g/kg WG formulation						48 h	NOEC		<0.00039	2	44	[14]	Table B.9.2.2
Daphnia magna		S	N	500 g/kg WG formulation						48 h	EC50		0.00091	2	44	[14]	Table B.9.2.2
Daphnia magna		S	N	500 g/kg WG formulation						48 h	NOEC		0.00049	2	44	[14]	Table B.9.2.2
Daphnia magna		S	N	500 g/kg WG formulation						48 h	EC50		0.0055	2	44	[14]	Table B.9.2.2
Daphnia magna		S	Y	500 g/kg WG formulation						48 h	EC50		0.001	2	56	[14]	Table B.9.2.2
Daphnia magna		S	Y	500 g/kg WG formulation						48 h	EC50		0.08	2	54	[14]	Table B.9.2.2
Daphnia magna		S	N	250 g/L SC formulation						48 h	NOEC		0.13	2	44	[14]	Table B.9.2.2
Daphnia magna		S	N	250 g/L SC formulation						48 h	EC50		0.19	2	44	[14]	Table B.9.2.2
Daphnia magna		S	N	250 g/L SC formulation						48 h	NOEC		0.028	2	44	[14]	Table B.9.2.2
Daphnia magna		S	N	250 g/L SC formulation						48 h	EC50		0.11	2	44	[14]	Table B.9.2.2
Daphnia pulex		S	N							48 h	NOEC		0.062	2		[14]	Table B.9.2.1
Daphnia pulex		S	N							48 h	EC50		0.2	2		[14]	Table B.9.2.1
Daphnia pulex		S	N	500 g/kg WG formulation						48 h	NOEC		0.00024	2	44	[14]	Table B.9.2.2
Daphnia pulex		S	N	500 g/kg WG formulation						48 h	EC50		0.0058	2	44	[14]	Table B.9.2.2
Eudiaptomus graciloides	field collected; 1.08 mm	S	N		>80	am (ADaM)	7.8±0.2	20	250	48 h	NOEC	immobility	0.034	3	2	[40]	

Species	Species properties	Test type	A	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg a.s./L]	Ri	Notes	Ref	Study id.
Eudiaptomus graciloides	field collected; 1.08 mm	S	N		>80	am (ADaM)	7.8±0.2	20	250	48 h	EC50	immobility	0.038	3	2	[40]	
Gammarus fossarum	adult male, 6-8 mm, wild type	S	Y	SC formulation 'Ortiva'		am		20	125	7 d	EC50	feeding activity	0.0908	2	28	[71]	
Gammarus fossarum	adult male, 6-8 mm, wild type	S	Y	SC formulation 'Ortiva'		am		20	125	7 d	LC50	mortality	0.1484	2	28	[71]	
Gammarus pulex	juv, 5 mm, exp. pond	S	Y	tg	92.6	50%dtw,50%dw	8.0-8.3	20.6-20.9	163	48 h	NOEC	mortality	0.125	2	33	[14, 22]	Table B.9.2.1
Gammarus pulex	juv, 5 mm, exp. pond	S	Y	tg	92.6	50%dtw,50%dw	8.0-8.3	20.6-20.9	163	48 h	EC50	mortality	0.35	2	33	[14, 22]	Table B.9.2.1
Gammarus pulex	field collected from stream	S	N	ag	n.r.	am (M7)	7.4	n.r.	180	96 h	LC50	mortality	0.27	3	25	[1]	
Gammarus pulex	juv, 5 mm, exp. pond	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	8.0-8.3	20.6-20.9	163	48 h	NOEC	mortality	0.125	2	49	[14, 22]	Table B.9.2.2
Gammarus pulex	juv, 5 mm, exp. pond	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	8.0-8.3	20.6-20.9	163	48 h	EC50	mortality	0.38	2	49	[14, 22]	Table B.9.2.2
Macrocyclops fuscus	ad, 2 mm, exp. pond	S	Y	tg	92.6	50%dtw,50%dw	7.8-8.0	20.9-21.3	163	48 h	NOEC	mortality	0.062	2	36	[14, 21]	Table B.9.2.1
Macrocyclops fuscus	ad, 2 mm, exp. pond	S	Y	tg	92.6	50%dtw,50%dw	7.8-8.0	20.9-21.3	163	48 h	LC50	mortality	0.13	2	36	[14, 21]	Table B.9.2.1
Macrocyclops fuscus	ad, 2 mm, exp. pond	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	7.8-8.0	20.9-21.3	163	48 h	NOEC	mortality	0.125	2	48	[14, 21]	Table B.9.2.2
Macrocyclops fuscus	ad, 2 mm, exp. pond	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	7.8-8.0	20.9-21.3	163	48 h	LC50	mortality	0.18	2	48	[14, 21]	Table B.9.2.2
Insecta																	
Chaoborus crystallinus	larvae, exp. pond	S	Y	tg	92.6	50%dtw,50%dw	7.5-8.0	20.2-20.9	163	48 h	LC50	mortality	1.6	2	38	[14, 25]	Table B.9.2.1
Chaoborus crystallinus	larvae, exp. pond	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	7.5-8.0	20.2-20.9	163	48 h	LC50	mortality	2.9	2	51	[14, 25]	Table B.9.2.2
Chaoborus flavicans	field collected; 9.6 mm	S	N		>80	am (ADaM)	7.8±0.2	20	250	48 h	NOEC	immobility	>6	2	13	[40]	
Chironomus plumosus	field collected; 23 mm	S	N		>80	am (ADaM)	7.8±0.2	20	250	48 h	NOEC	immobility	0.25	3	6	[40]	
Chironomus plumosus	field collected; 23 mm	S	N		>80	am (ADaM)	7.8±0.2	20	250	48 h	EC50	immobility	0.54	3	6	[40]	
Chironomus riparius	2nd instar	S	Y	tg	92.6	50%dtw,50%dw	7.4-8.0	20.2-20.9	163	48 h	NOEC	mortality	0.125	2	37	[14, 23]	Table B.9.2.1
Chironomus riparius	2nd instar	S	Y	tg	92.6	50%dtw,50%dw	7.4-8.0	20.2-20.9	163	48 h	LC50	mortality	0.21	2	37	[14, 23]	Table B.9.2.1
Chironomus riparius	2nd instar	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	7.4-8.0	20.2-20.9	163	48 h	NOEC	mortality	0.125	2	52	[14, 23]	Table B.9.2.2
Chironomus riparius	2nd instar	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	7.4-8.0	20.2-20.9	163	48 h	LC50	mortality	0.25	2	52	[14, 23]	Table B.9.2.2
Cloeon dipterum	mymphs, 5 mm, exp.pond	S	Y	tg	92.6	50%dtw,50%dw	7.5-7.7	20.2-20.9	163	48 h	NOEC	mortality	0.125	2	34	[14, 24]	Table B.9.2.1
Cloeon dipterum	mymphs, 5 mm, exp.pond	S	Y	tg	92.6	50%dtw,50%dw	7.5-7.7	20.2-20.9	163	48 h	LC50	mortality	3.2	2	34	[14, 24]	Table B.9.2.1
Cloeon dipterum	field collected; 3.11 mm	S	N		>80	am (ADaM)	7.8±0.2	20	250	48 h	NOEC	immobility	1.5	2	11	[40]	
Cloeon dipterum	field collected; 3.11 mm	S	N		>80	am (ADaM)	7.8±0.2	20	250	48 h	EC50	immobility	3.56	2	11	[40]	
Cloeon dipterum	mymphs, 5 mm, exp.pond	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	7.5-7.7	20.2-20.9	163	48 h	NOEC	mortality	0.062	2	47	[14, 24]	Table B.9.2.2
Cloeon dipterum	mymphs, 5 mm, exp.pond	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	7.5-7.7	20.2-20.9	163	48 h	LC50	mortality	0.22	2	47	[14, 24]	Table B.9.2.2
Hydropsyche angustipennis	field collected; 8.9 mm	S	N		>80	am (ADaM)	7.8±0.2	20	250	48 h	NOEC	immobility	3	2	11	[40]	
Hydropsyche angustipennis	field collected; 8.9 mm	S	N		>80	am (ADaM)	7.8±0.2	20	250	48 h	EC50	immobility	>6	2	12	[40]	
Ischnura elegans	mymphs, 10 mm, exp.pond	S	Y	tg	92.6	50%dtw,50%dw	8.0-8.2	20.6-20.9	163	48 h	LC50	mortality	>4.00	2	32	[14, 20]	Table B.9.2.1
Ischnura elegans	mymphs, 10 mm, exp.pond	S	Y	500 g/kg WG form. (YF8287)	51	50%dtw,50%dw	8.0-8.2	20.6-20.9	163	48 h	LC50	mortality	>4.00	2	50	[14, 20]	Table B.9.2.2
Notonecta glauca	ad, exp. pond	S	Y	tg	92.6	50%dtw,50%dw	7.9-8.4	20.2-20.9	160-180	48 h	LC50	mortality	>4.00	2	40	[14, 51]	Table B.9.2.1
Notonecta glauca	ad, exp. pond	S	Y	500 g/kg WG formulation	51	50%dtw,50%dw	7.9-8.4	20.2-20.9	160-180	48 h	LC50	mortality	>4.00	2	55	[14, 51]	Table B.9.2.1
Pisces																	
Ctenopharyngodon idella	juv. < 10 d after hatching	R	N	a.s.	98.5%			25		48 h	LC50	mortality	0.549	2	22	[41]	
Cyrpinus carpio	1.12 g; 37 mm; at test end	F	Y	tg	92.6	dechlorinated t.w.	7.38-7.80	21.4-21.7	53-58	96 h	NOEC	mortality	0.98	2	19	[14, 65]	Table B.9.2.1
Cyrpinus carpio	1.12 g; 37 mm; at test end	F	Y	tg	92.6	dechlorinated t.w.	7.38-7.80	21.4-21.7	53-58	96 h	LC50	mortality	1.6	2	19	[14, 65]	Table B.9.2.1
Cyrpinus carpio		F	N	250 g/L SC formulation						96 h	NOEC		0.42	2	44	[14]	Table B.9.2.2
Cyrpinus carpio		F	N	250 g/L SC formulation						96 h	LC50		0.64	2	44	[14]	Table B.9.2.2
Lepomis macrochirus	0.82 g; 35 mm; at test end	F	Y	tg	92.6	dechlorinated t.w.	7.11-7.65	21.8=21.9	23-48	96 h	NOEC	mortality	0.5	2	18	[14, 56]	Table B.9.2.1
Lepomis macrochirus	0.82 g; 35 mm; at test end	F	Y	tg	92.6	dechlorinated t.w.	7.11-7.65	21.8=21.9	23-48	96 h	LC50	mortality	1.1	2	18	[14, 56]	Table B.9.2.1
Lepomis macrochirus		S	N	500 g/kg WG formulation						96 h	NOEC		0.91	2	44	[14]	Table B.9.2.2
Lepomis macrochirus		S	N	500 g/kg WG formulation						96 h	LC50		1.2	2	44	[14]	Table B.9.2.2

Species	Species properties	Test type	A	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg a.s./L]	Ri	Notes	Ref	Study id.
Misgumus anguillicauda-tus	8.47±0.09 g; 12.1±0.62 cm	R	N	tg	94.5	aerated gw	7.72-8.31	23±2	115	96 h	NOEC	mortality	1.20	2	23	[14, 43]	P 610
Misgumus anguillicauda-tus	8.47±0.09 g; 12.1±0.62 cm	R	N	tg	94.5	aerated gw	7.72-8.31	23±2	115	96 h	LC50	mortality	1.65	2	23	[14, 43]	P 610
Oncorhynchus mykiss	1.49 g, 47 mm	F	Y	tg	92.6	dechlorinated t.w.	7.41-7.68	14.6-14.9	58-64	96 h	NOEC	mortality	0.068	2	29	[14, 9]	Table B.9.2.1
Oncorhynchus mykiss	1.49 g, 47 mm	F	Y	tg	92.6	dechlorinated t.w.	7.41-7.68	14.6-14.9	58-64	96 h	LC50	mortality	0.47	2	29	[14, 9]	Table B.9.2.1
Oncorhynchus mykiss		S	N	500 g/kg WG formulation						96 h	NOEC		0.28	2	44	[14]	Table B.9.2.2
Oncorhynchus mykiss		S	N	500 g/kg WG formulation						96 h	LC50		0.57	2	44	[14]	Table B.9.2.2
Oncorhynchus mykiss		F	N	250 g/L SC formulation						96 h	NOEC		0.28	2	44	[14]	Table B.9.2.2
Oncorhynchus mykiss		F	N	250 g/L SC formulation						96 h	LC50		0.56	2	44	[14]	Table B.9.2.2
Oncorhynchus mykiss		F	N	250 g/L SC formulation						96 h	NOEC		0.074	2	44	[14]	Table B.9.2.2
Oncorhynchus mykiss		F	N	250 g/L SC formulation						96 h	LC50		0.28	2	44	[14]	Table B.9.2.2
Oryzias latipes	0.19±0.1 g; 2.83±0.05 cm	R	N	tg	94.5	aerated gw	7.55-7.80	25±1	115	96 h	NOEC	mortality	0.65	2	24	[14, 34]	P 608
Oryzias latipes	0.19±0.1 g; 2.83±0.05 cm	R	N	tg	94.5	aerated gw	7.55-7.80	25±1	115	96 h	LC50	mortality	1.30	2	23	[14, 34]	P 608
Salmo salar	2nd spring smolts, 21-23 cm, 100-128 g	S	Y	SC formulation 'Amistar'	25	n.w.	6.3	4.1	'soft'	96 h	LC50	mortality	>0.352	2	30	[46]	
Salmo salar	2nd spring smolts, 21-23 cm, 100-128 g	S	Y	SC formulation 'Amistar'	25	n.w.	6.3	4.1	'soft'	96 h	EC50	growth	>0.352	**2**	30	[46]	
Apmhibia																	
Rana temporania	tadpoles; Gosner stage 25	S	N		n.r.	rw		15	42	72 h	NOEC	mortality	0.13	2	1	[33]	
Rana temporania	tadpoles; Gosner stage 25	S	N		n.r.	rw		15	42	72 h	LC50	mortality	0.13<x<0.5	2	1	[33]	
Rana temporania	tadpoles; Gosner stage 25	S	N		n.r.	rw		15	42	72 h	NOEC	body length	0.13	2	1	[33]	
Rana temporania	tadpoles; Gosner stage 25	S	N		n.r.	rw		15	42	72 h	NOEC	weight	≥0.5	2	1	[33]	
Rana temporania	tadpoles; Gosner stage 25	S	N		n.r.	rw		15	42	72 h	NOEC	tail length	≥0.5	2	1	[33]	

Notes

1 Acetone used as solvent ≤330 µmol/L; control and solvent control included; 3 test concentrations, 5 replicates per concentration; control replicated 15 times; pH and purity not reported

2 Acetone used as solvent at 0.0006% (v/v); pilot trial showed no effect of acetone up to 0.1%; 7 concentrations, 4 replicates; control mortality 20% therefore Ri=3

3 Acetone used as solvent at 0.0055% (v/v); pilot trial showed no effect of acetone up to 0.1%; 7 concentrations, 4 replicates

4 Acetone used as solvent at 0.0055% (v/v); solvent control included; 7 concentrations, 4 replicates

5 Acetone used as solvent at 0.0075% (v/v); pilot trial showed no effect of acetone up to 0.1%; 9 concentrations, 4 replicates

6 Acetone used as solvent at 0.0080% (v/v); pilot trial showed no effect of acetone up to 0.1%; 9 concentrations, 10 replicates; dissolved oxygen 2.2 mg/L at t=48 h, this affects the health of the animals therefore Ri=3

7 Acetone used as solvent at 0.0086% (v/v); pilot trial showed no effect of acetone up to 0.1%; 8 concentrations, 4 replicates; control mortality 25% therefore Ri=3

8 Acetone used as solvent at 0.0122% (v/v); solvent control included; 6 concentrations, 4 replicates

9 Acetone used as solvent at 0.025% (v/v); pilot trial showed no effect of acetone up to 0.1%; 6 concentrations, 4 replicates; dissolved oxygen 2.2 mg/L at t=48 h, this affects the health of the animals therefore Ri=3

10 Acetone used as solvent at 0.025% (v/v); three test concentrations, three animals video recorded per test concentration; pilot trial showed no effect of acetone up to 0.1%; concentration not measured, results based on nominal concentrations; relevance of effect for population is unclear

11 Acetone used as solvent at 0.03% (v/v); pilot trial showed no effect of acetone up to 0.1%; 7 concentrations, 10 replicates

12 Acetone used as solvent at 0.03% (v/v); pilot trial showed no effect of acetone up to 0.1%; 7 concentrations, 10 replicates; EC50 based on no effects observed at highest test concentration

13 Acetone used as solvent at 0.03% (v/v); pilot trial showed no effect of acetone up to 0.1%; 7 concentrations, 4 replicates

14 Acetone used as solvent at 0.1% (v/v), solvent control included; measured initial concentrations 108-130% of nominal; mean measured concentrations were 109-130% of nominal; result based on nominal concentrations.

15 Acetone used as solvent at 0.1%; solvent control included; initial concentrations 100-130% of nominal; concentrations at test end 100-130% of nominal; results based on mean measured concentrations.

16 Acetone used as solvent at 0.1%; solvent control included; initial concentrations 100-130% of nominal; concentrations at test end 100-130% of nominal; results based on mean measured concentrations; only 96 h biomass endpoint presented in DAR, value taken over from original study
17 Acetone used as solvent, concentration of solvent: <0.000003%; control was solvent control, no control without solvent included
18 DMF used as solvent at 0.01% (v/v); solvent control included; measured concentrations 89-94% of nominal; results based on mean measured concentrations.
19 DMF used as solvent at 0.01% (v/v); solvent control included; measured concentrations 97-107% of nominal; results based on mean measured concentrations.
20 DMF used as solvent at 0.01% (v/v); solvent control included; measured concentrations 98-110% of nominal, results based on nominal concentrations
21 DMF used as solvent at 0.01%; solvent control included; initial concentrations 91-106% of nominal, mean measured concentrations 84-109% of nominal; result based on mean measured concentrations; endpoint exceeds two time the water solubility, therefore Ri=3
22 DMSO used as solvent up to 2.5‰ (v/v), solvent control included in test set-up; survival in the solvent control >95%; renewal every 6 h; there is an inconsistency between LC50 values reported in the actual text and those in table 1 of the publication, the endpoint from the table is taken over
23 DMSO used as a solvent; amount of solvent not reported; LC50 corrected for purity
24 DMSO used as a solvent; amount of solvent not reported; NOEC corrected for purity
25 DMSO used as solvent at <1%; not reported are number of concentrations, number of replicates, no analysis of test substance was performed, purity of test substance was not reported; no mentioning of solvent control, therefore Ri=3; control performance was not reported.
26 DMSO used as solvent; concentration in test not reported; 6 test concentrations tested in triplicate; each test repeated 3 times; only solvent control, no negative control included; microcosm study
27 Endpoint is mycelial growth of fungi colonised rapeseeds; use of solvent (or not) is not reported; 6 test concentrations tested, nr of replicates not reported; endpoint considered Ri3 because of agar
28 Endpoints based on nominal concentrations, mean measured concentrations within 80-110% of nominal; control performed; five concentrations, 30 replicates; aerated solutions
29 Fish loading in control 0.33 g/L; solvent: DMF at 0.1 5 (v/v); solvent control included; results based on mean measured concentrations
30 Formulation tested; no solvent used; negative control included; three concentrations, tested in duplicate; endpoint based on nominal concentrations confirmed by analysis
31 Measured concentrations 99-107% at t=0; 99-104% at t=48 h; result based on nominal concentrations
32 Methanol used as solvent, 0.05% (v/v); control was solvent control, no control without solvent included; concentrations 81-87% of nominal, results based on nominal concentrations
33 Methanol used as solvent, 0.05% (v/v); control was solvent control, no control without solvent included; concentrations 81-91% of nominal, not reported if results are based on nominal or measured concentrations
34 Methanol used as solvent, 0.05% (v/v); control was solvent control, no control without solvent included; concentrations 82-92% of nominal, endpoint based on nominal concentrations
35 Methanol used as solvent, 0.05% (v/v); control was solvent control, no control without solvent included; concentrations 85-89% of nominal; result based on nominal concentrations
36 Methanol used as solvent, 0.05% (v/v); control was solvent control, no control without solvent included; concentrations 85-93% of nominal, not reported if results are based on nominal or measured concentrations
37 Methanol used as solvent, 0.05% (v/v); control was solvent control, no control without solvent included; concentrations 87-92% of nominal at t=0; 83-102% at t=48 h, endpoint based on nominal concentrations
38 Methanol used as solvent, 0.05% (v/v); control was solvent control, no control without solvent included; concentrations 88-92% of nominal at t=0; 75-105% at t=48 h, not reported if results based on nominal or measured concentrations
39 Methanol used as solvent, 0.05% (v/v); control was solvent control, no control without solvent included; concentrations 88-92% of nominal, results based on nominal concentrations
40 Methanol used as solvent, 0.05% (v/v); control was solvent control, no control without solvent included; concentrations 95-98% of nominal, results based on nominal concentrations
41 No solvent used, endpoint based on nominal concentrations
42 No solvent used, endpoint based on nominal concentrations; test conducted in 96 well microplates (200 µL test solution+20 µL of algae), Ri3 because quality criteria cannot be checked in a microwell plate
43 No solvent; 1 mg/L solution in test water shaken overnight and filtered (10 µm); initial measured concentrations 53-71% of nominal, but stable during test. Results based on mean measured concentrations.
44 Result expressed in mg active/L
45 Result expressed in mg active/L, based on nominal concentrations; measured concentrations were 79-83% of nominal
46 Result expressed in mg active/L, based on nominal concentrations; measured concentrations were 80-91% of nominal
47 Result expressed in mg active/L, not reported if results are based on nominal or measured concentrations; measured concentrations were 79-87% of nominal
48 Result expressed in mg active/L, not reported if results are based on nominal or measured concentrations; measured concentrations were 81-102% of nominal
49 Result expressed in mg active/L, not reported if results are based on nominal or measured concentrations; measured concentrations were 87-91% of nominal
50 Result expressed in mg active/L, results based on nominal concentrations; measured concentrations were 80-91% of nominal
51 Result expressed in mg active/L; concentrations 79-83% of nominal at t=0; 72-82% at t=48 h, not reported if results based on nominal or measured concentrations
52 Result expressed in mg active/L; concentrations 79-86% of nominal at t=0; 71-94% at t=48 h, endpoint based on nominal concentrations
53 Result expressed in mg active/L; concentrations 81-102% of nominal; results based on nominal concentrations

54 result expressed in mg active/L; filtered test water
55 Result expressed in mg active/L; measured concentrations were 69-96% of nominal; particulate material present in higher test concentrations; results based on nominal concentrations since no effects were observed.
56 Result expressed in mg active/L; unfiltered test water
57 Single concentration tested; 10 replicates per concentration, control and solvent control; 2 animals per test concentration; 'microcosm' study; *A. aquaticus* fed on discs colonised with fungi on leaves; not analysed; acetone used as solvent at 0.1%.
58 Single concentration tested; 10 replicates per concentration, control and solvent control; 2 animals per test concentration; growth of fungi on leaves; not analysed; acetone used as solvent at 0.1%; extent of effect unclear therefore Ri3
59 Tested in soil/water, water phase; original reference not available; endpoint based on measured concentrations
60 Tested in soil/water, whole system, therefore Ri=3; endpoint based on nominal concentrations; original reference not available
61 Tested water alone; original reference not available; endpoint based on measured concentrations
62 Unclear how test substance was added to agar (with or without solvent); triplicate spore suspension spots per agar plate (=test concentration); nr of test concentrations not reported. MFC = lowest concentration preventing visible spore growth or germination; endpoint considered Ri3 because of agar

Table A2.2. Acute toxicity of azoxystrobin to saltwater organisms

Species	Species properties	Test type	A	Test compound	Purity [%]	Test water	pH	T [°C]	Salinity [‰]	Exp. time	Crit.	Test endpoint	Value [mg a.s./L]	Ri	Notes	Ref	Location in DAR
Bacteria																	
Vibrio fischeri		S	Y	a.s.	99.9	am		4		5 min	EC50	bioluminescence	6.96	2	1	[54]	
Vibrio fischeri		S	Y	SC formulation 'Ortiva'	25	am		4		5 min	EC50	bioluminescence	869	3	5	[54]	
Algae																	
Isochrysis galbana		S	Y	a.s.	99.9	am		20	33	72 h	EC50	growth	0.031	2	1	[54]	
Isochrysis galbana		S	Y	SC formulation 'Ortiva'	25	am		20	33	72 h	EC50	growth	0.029	2	4	[54]	
Nannochloropsis gaditana		S	Y	a.s.	99.9	am		20	33	72 h	EC50	growth	0.298	2	1	[54]	
Nannochloropsis gaditana		S	Y	SC formulation 'Ortiva'	25	am		20	33	72 h	EC50	growth	0.243	2	4	[54]	
Phaeodactylum tricornutum		S	Y	a.s.	99.9	am		20	33	72 h	EC50	growth	>5.90	2	1	[54]	
Phaeodactylum tricornutum		S	Y	SC formulation 'Ortiva'	25	am		20	33	72 h	EC50	growth	2.997	2	4	[54]	
Rhodomonas lens		S	Y	a.s.	99.9	am		20	33	72 h	EC50	growth	>5.60	2	1	[54]	
Rhodomonas lens		S	Y	SC formulation 'Ortiva'	25	am		20	33	72 h	EC50	growth	2.406	2	4	[54]	
Skeletonema costatum	strain CCAP 1077/IC	S	Y	tg	96.2	am	8.1-8.9	19.9-20.5	31.5	72 h	EC50	growth rate	0.3	2	12	[14, 60]	P620
Thalassiosira weisflogii		S	Y	a.s.	99.9	am		20	33	72 h	EC50	growth	>5.40	2	1	[54]	
Thalassiosira weisflogii		S	Y	formulation		am		20	33	72 h	EC50	growth	4.309	2	4	[54]	
Rotifera																	
Brachionius plicatillis		S	Y	a.s.	99.9	am		25	15	24 h	LC50	mortality	>6.80	2	1	[54]	
Brachionius plicatillis		S	Y	SC formulation 'Ortiva'	25	am		25	15	24 h	LC50	mortality	>6.20	2	4	[54]	
Mollusca																	
Crassostrea gigas	freshly fert. embr.	S	Y	tg	96.2	nsw, 0.2 µm filtered	8.19-8.31	20-21	32	48 h	NOEC	development	0.56	2	9	[14, 35]	P615
Crassostrea gigas	freshly fert. embr.	S	Y	tg	96.2	nsw, 0.2 µm filtered	8.19-8.31	20-21	32	48 h	EC50	development	1.3	2	10	[14, 35]	P615
Gibbula umbilicalis	6.5-8.1 mm	S	Y	a.s.	99.9	am		15	34	96 h	LC50	mortality	0.013	2	1	[54]	
Gibbula umbilicalis	6.5-8.1 mm	S	Y	SC formulation 'Ortiva'	25	am		15	34	96 h	LC50	mortality	0.017	2	4	[54]	
Rissoa parva	2.4-3.8 mm	S	Y	a.s.	99.9	am		15	34	96 h	LC50	mortality	0.118	2	1	[54]	
Crustacea																	
Artemia franciscana	freshly hatched nauplii	S	N			am	8.31	20	41	96 h	LC50	mortality	<0.1	2	11	[64]	
Artemia franciscana		S	Y	a.s.	99.9	am		25	35	24 h	LC50	mortality	0.345	2	3	[54]	
Artemia franciscana		S	Y	SC formulation 'Ortiva'	25	am		25	35	24 h	LC50	mortality	1.256	2	7	[54]	
Americamysis bahia	<24 h, in house culture	S	Y	tg	96.2	nsw+dist.w, filtered 10 µm	7.96-8.28	25.0-25.3	20.2-20.6	96 h	NOEC	mortality	0.032	2	13	[14, 37]	P612
Americamysis bahia	<24 h, in house culture	S	Y	tg	96.2	nsw+dist.w, filtered 10 µm	7.96-8.28	25.0-25.3	20.2-20.6	96 h	LC50	mortality	0.055	2	13	[14, 37]	P612
Pisces																	
Cyprinodon variegatus	0.93 g; 33 mm; at test end	F	Y	tg	96.2	nsw, filtered	7.82-8.07	21.8-21.9	35.0-35.2	96 h	NOEC	mortality	0.33	2	8	[14, 55]	P605
Cyprinodon variegatus	0.93 g; 33 mm; at test end	F	Y	tg	96.2	nsw, filtered	7.82-8.07	21.8-21.9	35.0-35.2	96 h	LC50	mortality	0.66	2	8	[14, 55]	P605
Solea senegalensis	newly hatched	S	Y	a.s.	99.9	am		20	35	48 h	LC50	mortality	0.698	2	2	[54]	
Solea senegalensis	newly hatched	S	Y	SC formulation 'Ortiva'	25	am		20	35	48 h	LC50	mortality	1.271	2	6	[54]	

Notes

1 Analysis used to confirm nominal concentration, reported endpoints based on geomean of measured and nominal; acetone used as solvent, 0.5% (v/v); test set-up contains negative and solvent control

2 Analysis used to confirm nominal concentration, reported endpoints based on geomean of measured and nominal; acetone used as solvent, 0.5% (v/v); test set-up contains negative and solvent control; mortality observed in the solvent control below 10%
3 Analysis used to confirm nominal concentration, reported endpoints based on geomean of measured and nominal; acetone used as solvent, 0.5% (v/v); test set-up contains negative and solvent control; mortality observed in the control below 10%
4 Analysis used to confirm nominal concentration, reported endpoints based on geomean of measured and nominal; test set-up only contains negative control
5 Analysis used to confirm nominal concentration, reported endpoints based on geomean of measured and nominal; test set-up only contains negative control; endpoint exceeds water solubility, therefore Ri=3
6 Analysis used to confirm nominal concentration, reported endpoints based on geomean of measured and nominal; test set-up only contains negative control; mortality observed in the solvent control below 10%
7 Analysis used to confirm nominal concentration, reported endpoints based on geomean of measured and nominal; test set-up only contains negative control; mortality observed in the control below 10%
8 DMF used as solvent at 0.01%; solvent control included; 20 fish per test concentration; each concentration tested without replicates (n=1); measured concentrations 93-100% of nominal; results based on mean measured concentrations
9 DMF used as solvent, ≤0.1%, solvent control included; mean measured concentrations 97-106% of nominal; results based on mean measured concentrations
10 DMF used as solvent, ≤0.1%, solvent control included; results based on mean measured concentrations
11 Only one concentration tested; resulting in 86% mortality compared to control; endpoint based on nominal concentration; no mentioning of solvent used; only negative control performed; control mortality 4%
12 Solvent: acetone at 0.1% in test; solvent control included; test duration 120 h, but control did not grow exponentially after 72 h; reported are results for day 0- 3 (72 h); initial concentrations 100-138% of nominal; mean measured concentrations 100-141% of nominal; result based on mean measured concentrations.
13 Test animals fed 10-20 *Artemia* nauplii per test animal per day; DMF used as solvent, 0.1%, solvent control included; mean measured concentrations 106-116% of nominal, results based on nominal concentrations

Table A2.3. Chronic toxicity of azoxystrobin to freshwater organisms

Species	Species properties	Test type	A	Test compound	Purity [%]	Test	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Ref.	Location in DAR
Bacteria																	
microbial community	natural inocculum	R	Y	formulation		a.m.		16		12 d	NOEC	leaf decomposition	0.014	2	31	[72]	
Cyanobacteria																	
Anabaena flos-aquae	strain CCAP 1403/13A	S	Y	tg	96.2	am	7.2-7.6	23.8-24.4	15	120 h	NOEC	growth rate	8.5	2	22	[14, 61]	P 625
Anabaena flos-aquae	strain CCAP 1403/13A	S	Y	tg	96.2	am	7.2-7.6	23.8-24.4	15	120 h	LOEC	growth rate	14	3	23	[14, 61]	P 625
Algae																	
Navicula pelliculosa	strain Utex 667	S	Y	tg	96.2	am	7.5-8.6	23.9-24.3	15	120 h	NOEC	growth rate	0.02	2	2	[14, 62]	p622
Navicula pelliculosa	strain Utex 667	S	Y	tg	96.2	am	7.5-8.6	23.9-24.3	15	120 h	LOEC	growth rate	0.04	2	2	[14, 62]	p622
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	72h	NOEC	growth rate	0.038	2	4	[59]	
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	72h	LOEC	growth rate	0.11	2	4	[59]	
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	72h	NOEC	biomass	0.038	2	4	[59]	
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	72h	LOEC	biomass	0.11	2	4	[59]	
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	96 h	NOEC	growth rate	0.038	2	4	[59]	
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	96 h	LOEC	growth rate	0.11	2	4	[59]	
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	96 h	NOEC	biomass	0.038	2	3	[14, 59]	Table B.9.2.1
Raphidocelis subcapitata	strain ATCC 22662	S	Y	tg	92.6	am	7.1-10.4	24.2-24.3	15	96 h	LOEC	biomass	0.11	2	4	[59]	
Raphidocelis subcapitata		S	N	250 g/L SC formulation						72 h	NOEC		0.026	2	36	[14]	Table B.9.2.2
Raphidocelis subcapitata		S	N	250 g/L SC formulation						72 h	NOEC		0.03	2	36	[14]	Table B.9.2.2
Raphidocelis subcapitata		S	N	250 g/L SC formulation						120 h	NOEC		0.024	2	36	[14]	Table B.9.2.2
Raphidocelis subcapitata		S	N	a.s.		a.m.		25		72 h	EC10	growth	0.032	3	32	[45]	
Macrophyta																	
Lemna gibba	strain G3	R	Y	tg	92.6	am	4.5-6.0	25±1	700	14 d	NOEC	frond number	0.8	2	21	[14, 63]	p628
Lemna gibba	strain G3	R	Y	tg	92.6	am	4.5-6.0	25±1	700	14 d	NOEC	dry weight	3.2	2	21	[14, 63]	p628
Mollusca																	
Lampsilis siliquoidea	juv. 9 wk post transf.; 0.15 mg	F	Y	ag	99.4	ww+dw	8.0-8.1	23±1	105-108	28 d	NOEC	survival	>0.028	2	40	[39]	
Lampsilis siliquoidea	juv. 9 wk post transf.; 0.15 mg	F	Y	ag	99.4	ww+dw	8.0-8.1	23±1	105-108	28 d	EC10	survival	>0.028	2	40	[39]	
Lampsilis siliquoidea	juv. 9 wk post transf.; 0.15 mg	F	Y	ag	99.4	ww+dw	8.0-8.1	23±1	105-108	28 d	NOEC	weight	>0.028	2	40	[39]	
Lampsilis siliquoidea	juv. 9 wk post transf.; 0.15 mg	F	Y	ag	99.4	ww+dw	8.0-8.1	23±1	105-108	28 d	EC10	weight	>0.028	2	40	[39]	
Lampsilis siliquoidea	juv. 9 wk post transf.; 0.15 mg	F	Y	ag	99.4	ww+dw	8.0-8.1	23±1	105-108	28 d	NOEC	biomass	>0.028	2	40	[39]	
Lampsilis siliquoidea	juv. 9 wk post transf.; 0.15 mg	F	Y	ag	99.4	ww+dw	8.0-8.1	23±1	105-108	28 d	EC10	biomass	>0.028	2	40	[39]	
Crustacea																	
Ceriodaphnia dubia	<24 h	R	Y	ag	99.4	ww+dw	8.3-8.4	25±1	106-109	7 d	NOEC	survival	>0.026	2	39	[39]	
Ceriodaphnia dubia	<24 h	R	Y	ag	99.4	ww+dw	8.3-8.4	25±1	106-109	7 d	EC10	survival	>0.026	2	39	[39]	
Ceriodaphnia dubia	<24 h	R	Y	ag	99.4	ww+dw	8.3-8.4	25±1	106-109	7 d	NOEC	reproduction	0.0065	2	39	[39]	
Ceriodaphnia dubia	<24 h	R	Y	ag	99.4	ww+dw	8.3-8.4	25±1	106-109	7 d	EC10	reproduction	0.0029	2	39	[39]	
Cyclops vicinus	lab culture; eggs	R	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	LOEC	reproduction	0.02	2	12	[40]	
Cyclops vicinus	lab culture; eggs	R	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	dev. time egg	0.01	2	11	[40]	
Cyclops vicinus	lab culture; eggs	R	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	dev. time nauplii	0.01	2	13	[40]	
Cyclops vicinus	lab culture; eggs	R	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	litter size	0.01	2	15	[40]	
Daphnia galeata	lab culture; 6-8 d	R	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	dev. time egg	0.08	2	9	[40]	
Daphnia galeata	lab culture; 6-8 d	R	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	dev. time neonate	0.02	2	9	[40]	
Daphnia galeata	lab culture; 6-8 d	R	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	litter size	0.06	2	10	[40]	
Daphnia galeata	lab culture; 6-8 d	R	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	length	0.01	2	9	[40]	

Species	Species properties	Test type	A	Test compound	Purity [%]	Test	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Ref.	Location in DAR
Daphnia magna	<24 h	R	Y	tg	92.6	dtw+dw	8.0-8.3	18.8-22.3	172	21 d	NOEC	reproduction	0.044	2	27	[14, 49]	Table B.9.2.3
Daphnia magna	<24 h	R	Y	tg	92.6	dtw+dw	8.0-8.3	18.8-22.3	172	21 d	LOEC	reproduction	0.084	2	27	[14, 49]	Table B.9.2.3
Daphnia magna	<24 h	R	Y	tg	92.6	dtw+dw	8.0-8.3	18.8-22.3	172	21 d	NOEC	mortality F0	0.084	2	28	[14, 49]	
Daphnia magna	<24 h	R	Y	tg	92.6	dtw+dw	8.0-8.3	18.8-22.3	172	21 d	LC50	mortality F0	0.15	2	28	[14, 49]	
Daphnia magna	<24 h	R	Y	tg	92.6	dtw+dw	8.0-8.3	18.8-22.3	172	21 d	NOEC	length	≥372	2	28	[14, 49]	
Daphnia magna	lab culture; juv 48-72 h	R	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	dev. time egg	≥0.28	2	17	[40]	
Daphnia magna	lab culture; juv 48-72 h	RS	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	mortality	0.24	2	16	[40]	
Daphnia magna	lab culture; juv 48-72 h	RS	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	dev. time neonate	0.04	2	11	[40]	
Daphnia magna	lab culture; juv 48-72 h	RS	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	litter size	≥0.28	2	14	[40]	
Daphnia magna	lab culture; juv 48-72 h	R	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	length	0.28	2	11	[40]	
Daphnia magna				formulation 'Ortiva'	25						NOEC		0.044	4		[71]	
Eudiaptomus graciloides	field collected egg-bearing ad	R	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	LOEC	reproduction	0.01	2	8	[40]	
Eudiaptomus graciloides	field collected egg-bearing ad	RS	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	dev. time nauplii	0.005	2	7	[40]	
Eudiaptomus graciloides	field collected egg-bearing ad	RS	N		>80	am (ADaM)	7.8±0.2	20	250	21 d	NOEC	litter size	0.002	2	7	[40]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	28 d	NOEC	survival	0.0073	2	37	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	28 d	EC10	survival	0.0083	2	37	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	28 d	NOEC	dry weight, ind.	>0.0095	2	37	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	28 d	EC10	dry weight, ind.	>0.0095	2	37	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	28 d	NOEC	biomass	0.0095	2	37	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	28 d	EC10	biomass	0.0069	2	37	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	42 d	NOEC	survival	0.0073	2	41	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	42 d	EC10	survival	0.0080	2	41	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	42 d	NOEC	dry weight, ind.	>0.0095	2	41	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	42 d	EC10	dry weight, ind.	>0.0095	2	41	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	42 d	NOEC	biomass	0.0073	2	41	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	42 d	EC10	biomass	0.0085	2	41	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	42 d	NOEC	reproduction	0.0037	2	41	[39]	
Hyalella azteca	7 d old; 0.0089 mg	F	Y	ag	99.4	ww+dw	7.9-8.1	23±1	108-112	42 d	EC10	reproduction	0.0035	2	41	[39]	
Insecta																	
Chironomus dilutus	7 d old; 0.033 mg	F	Y	ag	99.4	ww+dw	7.8-8.1	23±1	104-108	13 d	NOEC	survival	>0.041	2	38	[39]	
Chironomus dilutus	7 d old; 0.033 mg	F	Y	ag	99.4	ww+dw	7.8-8.1	23±1	104-108	13 d	EC10	survival	>0.041	2	38	[39]	
Chironomus dilutus	7 d old; 0.033 mg	F	Y	ag	99.4	ww+dw	7.8-8.1	23±1	104-108	13 d	NOEC	dry weight, ind.	>0.041	2	38	[39]	
Chironomus dilutus	7 d old; 0.033 mg	F	Y	ag	99.4	ww+dw	7.8-8.1	23±1	104-108	13 d	EC10	dry weight, ind.	>0.041	2	38	[39]	
Chironomus dilutus	7 d old; 0.033 mg	F	Y	ag	99.4	ww+dw	7.8-8.1	23±1	104-108	13 d	NOEC	biomass	>0.041	2	38	[39]	
Chironomus dilutus	7 d old; 0.033 mg	F	Y	ag	99.4	ww+dw	7.8-8.1	23±1	104-108	13 d	EC10	biomass	>0.041	2	38	[39]	
Chironomus dilutus	7 d old; 0.033 mg	F	Y	ag	99.4	ww+dw	7.8-8.1	23±1	104-108	50 d	NOEC	emergence	0.0077	2	38	[39]	
Chironomus dilutus	7 d old; 0.033 mg	F	Y	ag	99.4	ww+dw	7.8-8.1	23±1	104-108	50 d	EC10	emergence	0.0086	2	38	[39]	
Chironomus riparius	1st instar, 2 d old	S	Y	tg	96.2	dtw+dw	7.4-8.5	18.1-20.5	165	25 d	NOEC	emergence	0.8	2	19	[14, 30]	P666
Chironomus riparius	1st instar, 2 d old	S	Y	tg	96.2	dtw+dw	7.4-8.5	18.1-20.5	165	25 d	NOEC	time to emergence	0.8	2	20	[14, 30]	
Chironomus riparius	1st instar, 2 d old	S	Y	tg	96.2	dtw+dw	7.4-8.5	18.1-20.5	165	25 d	EC50	emergence	1.6	2	20	[14, 30]	
Pisces																	
Danio rerio	ad, sexually mature	R	N		99	tw		20		21 d	NOEC	mortality	[3]0.0005	3	34	[3]	
Danio rerio	ad, sexually mature	R	N		99	tw		20		21 d	NOEC	behaviour	[3]0.0005	3	33	[3]	
Danio rerio	ad, sexually mature	R	N		99	tw		20		21 d	NOEC	DNA damage	<0.0005	3	35	[3]	
Danio rerio	ad, 6 mo, ♂+ ♀ AB strain	S	Y		98	rec w	7.5±0.5	27±1	66	21 d	NOEC	egg production	0.02	2	18	[7]	
Danio rerio	ad, 6 mo, ♂+ ♀ AB strain	S	Y		98	rec w	7.5±0.5	27±1	66	21 d	NOEC	fertilisation rate	0.02	2	18	[7]	
Danio rerio	ad, 6 mo, ♂+ ♀ AB strain	S	Y		98	rec w	7.5±0.5	27±1	66	21 d	NOEC	GSI males	0.02	2	18	[7]	
Danio rerio	ad, 6 mo, ♂+ ♀ AB strain	S	Y		98	rec w	7.5±0.5	27±1	66	21 d	NOEC	GSI females	≥0.2	2	18	[7]	
Danio rerio	ad, 6 mo, ♂+ ♀ AB strain	S	Y		98	rec w	7.5±0.5	27±1	66	21 d	NOEC	hepatosomatic index	0.02	2	18	[7]	

Species	Species properties	Test type	A	Test compound	Purity [%]	Test	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Ref.	Location in DAR
Danio rerio	ad, 6 mo, ♂+ ♀ AB strain	S	Y		98	rec w	7.5±0.5	27±1	66	21 d	NOEC	VTG females	0.02	2	18	[7]	
Danio rerio	ad, 6 mo, ♂+ ♀ AB strain	S	Y		98	rec w	7.5±0.5	27±1	66	21 d	NOEC	gonad histopath ♂	0.002	2	18	[7]	
Danio rerio	ad, 6 mo, ♂+ ♀ AB strain	S	Y		98	rec w	7.5±0.5	27±1	66	21 d	NOEC	gonad histopath ♀	0.02	2	18	[7]	
Danio rerio	6-8 hours post fertilisation	R	N	a.s.		a.m.		28	23	5 d ELS	EC10	viability	1.068	2	24	[47]	
Danio rerio	6-8 hours post fertilisation	R	N	a.s.		a.m.		28	23	5 d	EC10	hatching	1.068	2	24	[47]	
Danio rerio	6-8 hours post fertilisation	R	N	a.s.		a.m.		28	23	5 d	EC10	malformations	1.068	2	24	[47]	
Oncorhynchus mykiss	2.69 g (t=0)	F	Y	tg	92.6	dtw	7.19-7.51	14.7-15.1	31.1	28 d	NOEC	toxic symptoms	0.16	2	25	[14, 36]	Table B.9.2.3
Oncorhynchus mykiss	2.69 g (t=0)	F	Y	tg	92.6	dtw	7.19-7.51	14.7-15.1	31.1	28 d	LOEC	toxic symptoms	0.32	2	26	[14, 36]	
Oncorhynchus mykiss	2.69 g (t=0)	F	Y	tg	92.6	dtw	7.19-7.51	14.7-15.1	31.1	28 d	NOEC	mortality	0.32	2	29	[14, 36]	
Oncorhynchus mykiss	2.69 g (t=0)	F	Y	tg	92.6	dtw	7.19-7.51	14.7-15.1	31.1	28 d	LOEC	mortality	0.64	2	30	[14, 36]	
Oncorhynchus mykiss	2.69 g (t=0)	F	Y	tg	92.6	dtw	7.19-7.51	14.7-15.1	31.1	28 d	LC50	mortality	0.59	2	30	[14, 36]	
Oncorhynchus mykiss	2.69 g (t=0)	F	Y	tg	92.6	dtw	7.19-7.51	14.7-15.1	31.1	28 d	LOEC	growth	>0.64	2	30	[14, 36]	
Pimephales promelas	eggs	F	Y	tg	92.6	well w	8.1-8.3	25.2-25.8	140-154	33 d	NOEC	growth (length)	0.147	2	5	[14, 52]	Table B.9.2.3
Pimephales promelas	eggs	F	Y	tg	92.6	well w	8.1-8.3	25.2-25.8	140-154	33 d	LOEC	growth (length)	0.193	2	5	[14, 52]	Table B.9.2.3
Pimephales promelas	eggs	F	Y	tg	92.6	well w	8.1-8.3	25.2-25.8	140-154	33 d	NOEC	hatching; fry survival	0.374	2	6	[14, 52]	
Pimephales promelas	eggs	F	Y	tg	92.6	well w	8.1-8.3	25.2-25.8	140-154	33 d	LOEC	hatching; fry survival	0.75	2	6	[14, 52]	
Amphibia																	
Rana temporania	larvae 6 h post fertlisation	R	Y		n.r.	rw	n.r.	15	42	49 d	LOEC	growth	>0.010	2	1	[33]	
Rana temporania	larvae 6 h post fertlisation	R	Y		n.r.	rw	n.r.	15	42	49 d	LOEC	mortality	>0.010	2	1	[33]	
Rana temporania	larvae 6 h post fertlisation	R	Y		n.r.	rw	n.r.	15	42	49 d	LOEC	time to metamorphosis	>0.010	2	1	[33]	
Rana temporania	larvae 6 h post fertlisation	R	Y		n.r.	rw	n.r.	15	42	49 d	NOEC	growth	≥0.010	2	1	[33]	
Rana temporania	larvae 6 h post fertlisation	R	Y		n.r.	rw	n.r.	15	42	49 d	NOEC	mortality	≥0.010	2	1	[33]	
Rana temporania	larvae 6 h post fertlisation	R	Y		n.r.	rw	n.r.	15	42	49 d	NOEC	time to metamorphosis	≥0.010	2	1	[33]	

Notes

1 Acetone used as solvent ≤330 µmol/L; control and solvent control included; 2 test concentrations, 20 replicates per concentration; control replicated 15 times; pH and purity not reported; mean measured concentrations at end of renewal 87±8%.

2 Acetone used as solvent at 0.1% (v/v), solvent control included; measured initial concentrations 108-130% of nominal; mean measured concentrations were 109-130% of nominal; result based on nominal concentrations.

3 Acetone used as solvent at 0.1%; solvent control included; initial concentrations 100-130% of nominal; concentrations at test end 100-130% of nominal; results based on mean measured concentrations.

4 Acetone used as solvent at 0.1%; solvent control included; initial concentrations 100-130% of nominal; concentrations at test end 100-130% of nominal; results based on mean measured concentrations; only 96 h biomass endpoint presented in DAR, value taken over from original study

5 Acetone used as solvent, concentration not reported, control and solvent control included; spiked concentrations were 100-105% of nominal; mean measurements of test concentrations were 104-114% of nominal (n=14 for each concentration);

6 Acetone used as solvent, concentration not reported, control and solvent control included; spiked concentrations were 100-105% of nominal; mean measurements of test concentrations were 104-114% of nominal (n=14 for each concentration); endpoint taken over from original study

7 Acetone used as solvent; 6 concentrations tested; 10 replicates, 1 animal per replicate; renewal (medium, food and toxicant) was applied every 48 h.

8 Acetone used as solvent; 6 concentrations tested; 10 replicates, 1 animal per replicate; all animals died within 48 h; renewal (medium, food and toxicant) was applied every 48 h.

9 Acetone used as solvent; 8 concentrations tested; 10 replicates, 1 animal per replicate; renewal (medium, food and toxicant) was applied every 48 h.

10 Acetone used as solvent; 8 concentrations tested; 10 replicates, 1 animal per replicate; endpoint covers both eggs and live offspring; renewal (medium, food and toxicant) was applied every 48 h.

11 Acetone used as solvent; 9 concentrations tested; 10 replicates, 1 animal per replicate; renewal (medium, food and toxicant) was applied every 48 h.

12 Acetone used as solvent; 9 concentrations tested; 10 replicates, 1 animal per replicate; all animals died within 48 h; renewal (medium, food and toxicant) was applied every 48 h.

13 Acetone used as solvent; 9 concentrations tested; 10 replicates, 1 animal per replicate; development time of eggs to nauplii; renewal (medium, food and toxicant) was applied every 48 h.

14 Acetone used as solvent; 9 concentrations tested; 10 replicates, 1 animal per replicate; endpoint covers both eggs and live offspring; report shows that no effects were observed up to and including the highest test concentration; renewal (medium, food and toxicant) was applied every 48 h.
15 Acetone used as solvent; 9 concentrations tested; 10 replicates, 1 animal per replicate; litter size of a female developing eggs during exposure; renewal (medium, food and toxicant) was applied every 48 h.
16 Acetone used as solvent; 9 concentrations tested; 10 replicates, 1 animal per replicate; result extracted from text; renewal (medium, food and toxicant) was applied every 48 h.
17 Acetone used as solvent; 9 concentrations tested; 10 replicates, 1 animal per replicate; text clarifies that no effects were observed including the highest test concentration ; renewal (medium, food and toxicant) was applied every 48 h.
18 Acetone+5% Tween 80 used as solvent; test acc. to OECD 229 (2012); 14 d pre-exposure for baseline reproduction determination (6 pairs per tank); 21 d exposure at three concentrations, 4 replicates. Blank control and solvent control (0.002% acetone, 0.0001% Tween 80) included. Concentrations measured at d 0, 1, 7, 14, 21. Actual concentrations within ±20% of nominal; results based on nominal. Only three concentrations tested; spacing factor wide (10).
19 Artificial sediment, 4.2% o.c. (measured); 1:9 sediment : water (heights); acclimatised 1 week before adding larvae; test substance added in water phase, 24 h after larvae; aeration during test; solvent: acetone at 0.01% (v/v); solvent control included; organisms fed with Tetramin; 16:8 h light:dark. Measured water phase concentrations 94-118% of nominal at t=0; 44 to 67% at day 25. Sediment concentrations were also measured (not reported here).Result based on initial (t=0) concentration in water phase.
20 Artificial sediment, 4.2% o.c. (measured); 1:9 sediment : water (heights); acclimatised 1 week before adding larvae; test substance added in water phase, 24 h after larvae; aeration during test; solvent: acetone at 0.01% (v/v); solvent control included; organisms fed with Tetramin; 16:8 h light:dark. Measured water phase concentrations 94-118% of nominal at t=0; 44 to 67% at day 25. Sediment concentrations were also measured (not reported here).Result based on initial (t=0) concentration in water phase; endpoint taken over from original study
21 DMF used as solvent at 0.01% (v/v); solvent control included; measured concentrations 98-110% of nominal, results based on nominal concentrations
22 DMF used as solvent at 0.01%; solvent control included; initial concentrations 91-106% of nominal, mean measured concentrations 84-109% of nominal; result based on mean measured concentrations.
23 DMF used as solvent at 0.01%; solvent control included; initial concentrations 91-106% of nominal, mean measured concentrations 84-109% of nominal; result based on mean measured concentrations; endpoint exceed two time the water solubility, therefore Ri=3
24 DMSO used as solvent, 0.4% (v/v); only solvent control included in test set-up; endpoints reported as AC50: 3.6014 µm and AC10: 2.6464 µm
25 Endpoints: fish surfacing, rapidly respiring; loading of control 0.96 g/L; mean measured concentrations 94-115% of nominal; results based on nominal concentrations
26 Endpoints: fish surfacing, rapidly respiring; loading of control 0.96 g/L; mean measured concentrations 94-115% of nominal; results based on nominal concentrations; endpoint taken over from original study
27 EPA guideline; methanol used as solvent at 0.01%; solvent and non solvent control included; 7 test concentrations; 10 replicates per concentration; of which 7 (1 animal per vessel) used for reproduction, growth, survival and 3 (5 animals per vessel) for survival of F0 generation only; results based on mean measured concentrations.
28 EPA guideline; methanol used as solvent at 0.01%; solvent and non solvent control included; 7 test concentrations; 10 replicates per concentration; of which 7 (1 animal per vessel) used for reproduction, growth, survival and 3 (5 animals per vessel) for survival of F0 generation only; results based on mean measured concentrations; endpoint taken over from original study
29 Loading of control 0.96 g/L; mean measured concentrations 94-115% of nominal; results based on nominal concentrations; endpoint based on data from original study and differs from that cited in the DAR
30 Loading of control 0.96 g/L; mean measured concentrations 94-115% of nominal; results based on nominal concentrations; endpoint taken over from original study
31 No concentration effect curve observed, mean measured concentration <80% of nominal, endpoint based on mean measured concentrations 19.8 and 8.1 µg/L; only negative control performed
32 No solvent used, endpoint based on nominal concentrations; test conducted in 96 well microplates (200 µL test solution+20 µL of algae), Ri3 because quality criteria cannot be checked in a microwell plate
33 One concentration tested: 0.5 µg/L; stock: test substance dissolved at 3 mg/L with 48 h stirring, no solvent; 25 fish per aquarium, no replicates, therefore Ri3; control (tap water) included
34 One concentration tested: 0.5 µg/L; stock: test substance dissolved at 3 mg/L with 48 h stirring, no solvent; 25 fish per aquarium, no replicates, therefore Ri3; control (tap water) included. Six fish removed from test aquarium at days 0, 7, 14 and 21
35 One concentration tested: 0.5 µg/L; stock: test substance dissolved at 3 mg/L with 48 h stirring, no solvent; 25 fish per aquarium, no replicates, therefore Ri3; control (tap water) included; endpoint relates to micronucleus occurrence and comet assay in both liver and spermatozoa cells
36 Result expressed in mg active/L
37 Triethylene glycol used as solvent at 0.1 mL/L; 1:40 sand:water system; measured concentrations 36-59%; results based on mean measured concentrations; 5 test concentrations, 4 replicates; no control without solvent included.
38 Triethylene glycol used as solvent at 0.1 mL/L; 1:40 sand:water system; measured concentrations 40-88%; results based on mean measured concentrations; 5 test concentrations, 4 replicates; no control without solvent included.

39 Triethylene glycol used as solvent at 0.1 mL/L; measured concentrations 26-62%; results based on mean measured concentrations; 5 test concentrations, 10 replicates; no control without solvent included.
40 Triethylene glycol used as solvent at 0.1 mL/L; measured concentrations 28-62%; results based on mean measured concentrations; 5 test concentrations, 8 replicates; no control without solvent included.
41 Triethylene glycol used as solvent at 0.1 mL/L;1:40 sand:water system; measured concentrations 36-59%; results based on mean measured concentrations; 5 test concentrations, 8 replicates; no control without solvent included.

Table A2.4. Chronic toxicity of azoxystrobin to saltwater organisms

Species	Species properties	Test	A	Test compound	Purity [%]	Test water	pH	T [°C]	Salinity [‰]	Exp. time	Criteri-on	Test endpoint	Value [mg/L]	Ri	Notes	Ref.	Location in DAR
Algae / Diatomeae																	
Thalassiosira weisflogii		S	Y	SC formulation 'Ortiva'	25	am		20	33	72 h	EC10	growth	1.934	2	1	[54]	
Skeletonema costatum	strain CCAP 1077/IC	S	Y	tg	96.2	am	8.1-8.9	19.9-20.5	31.5	72 h	NOEC	growth rate	0.010	2	2	[14, 60]	p620
Skeletonema costatum	strain CCAP 1077/IC	S	Y	tg	96.2	am	8.1-8.9	19.9-20.5	31.5	72 h	LOEC	growth rate	0.032	2	2	[14, 60]	p620
Rhodomonas lens		S	Y	SC formulation 'Ortiva'	25	am		20	33	72 h	EC10	growth	2.24	2	1	[54]	
Crustacea																	
Americamysis bahia	< 24 h	F	Y	tg	96.2	natural sea water	7.7-8.2	24.1-25.5	15-17	28 d	NOEC	mortality F0	0.00954	2	3	[14, 2]	p617
Americamysis bahia	< 24 h	F	Y	tg	96.2	natural sea water	7.7-8.2	24.1-25.5	15-17	28 d	LOEC	mortality F0	0.016	2	3	[14, 2]	p617

Notes

1 Analysis used to confirm nominal concentration, reported endpoints based on geomean of measured and nominal; test set-up contains negative and solvent control; EC10 calculated from EC20 and EC50

2 Solvent: acetone at 0.1% in test; solvent control included; test duration 120 h, but control did not grow exponentially after 72 h; reported are results for day 0- 3 (72 h); initial concentrations 100-138% of nominal; mean measured concentrations 100-141% of nominal; result based on mean measured concentrations

3 Test animals fed 150 *Artemia* per test animal per day; DMF used as solvent at maximally 0.1% (highest concentration); results based on mean measured concentrations

Annex 3 Commentaren Petit Comité INS

Commentaar petit comité	**Rapportnr :** Azoxystrobine; 14413d**00**
Door: Willie Peijnenburg	**datum: 18-07-2017**

P.	**paragraaf**	**Opmerking lid petit comité**	**Reactie Auteur**
10	3.1.2	In Table 9 worden geomean-waardes afgeleid die betrekking hebben op LOEC en MIC. Ik vraag me af wat het nut van deze waardes is en wat ze nou eigenlijk betekenen en/of voorstellen. Het gaat om een soortement van middelen waarbij ervan uitgegaan lijkt te worden dat je dan een schatting van een EC50-waarde krijgt. Ik betwijfel of deze aanpak enige bodem heeft en op zijn minst is nadere uitleg/justification nodig. Op voorhand ben ik het dan ook niet eens met de statement op Pagina 11 (laatste alinea) dat deze analyse steun levert voor het coveren van de meest gevoelige soort.	Dit stuk tekst en de werkwijze is overgenomen uit MTR afleiding tebuconazole (12194a02, 2012), aangepast met tox-data van azoxystrobine. Het tebuconazole rapport is ook getoetst door petit comité en akkoord bevonden. Tekst aangepast: 'pseudo EC50' values.
12	3.1.3 - voorlaatste zin	Moet hier als samenvattende zin, ook niet expliciet benoemd worden dat studie 5 een NOEC van 2.85 ug/L liet zien? Ook al is de laatste zin van de voorgaande paragraaf van toepassing.	Een NOEC kan uit studie 5 niet worden afgeleid, alleen dat er geen effect is waargenomen bij 2 concentraties. De NOEC kun je pas vaststellen als er wel een concentratie(s) met effect is. De laatste zin is als volgt aangepast: The results of study 5, showing no effect at nominal concentrations of 1 and 2.85 µg/L, are not contradicting this result.

P.	paragraaf	Opmerking lid petit comité	Reactie Auteur
13/14	3.2.2	Hier worden o.a. resultaten van Mosaic berekeningen getoond. Er komen dan twee vragen bij mij op: is het überhaupt justified om unbounded values mee te nemen en ten tweede: wat is de uitkomst van de Mosaic berekeningen als de unbounded values niet meegenomen worden, in vergelijking met de ETX-resultaten? Wellicht helpt dit bij de acceptatie van de Mosaic resultaten? Wat mij betreft zijn de Mosaic resultaten met unboun values niet zonder meer preferred, zoals aangegeven op pagina 14. (Detail: sae = same)	Er is enig verschil tussen uitkomsten van Mosaic en ETX met dezelfde dataset. Voor de acute dataset is de HC5 berekend met Mosaic 0,046 mg/l versus 0,041 mg/L voor ETX. Voor de chronische datset geeft Mosaic 0,00072 mg/L versus 0,00060 mg/L voor ETX. Er is echter geen guidance over welke methode de voorkeur heeft. Het gebruik van zoveel mogelijk betrouwbare data in de normafleiding heeft altijd de voorkeur, dit op voorwaarde dat dit statistisch acceptabel is. Gezien de onzekerheden en het onbreken van voldoende statistische expertise op dit gebied hebben we alsnog de berekeningen met Mosaic niet gebruikt voor de uiteindelijke normen. typo aangepast.
16	3.3.1.2	Figure 4: ik vind dit een wel heel slechte fit door de data. Ik ben geen expert, maar is het toegestaan om een andere verdeling als basis te kiezen? Het probleem is dat de HC5 zo te zien sterk door de gebruikte verdeling beïnvloed wordt.	De fit is inderdaad matig. ETX geeft echter aan dat de fit op bepaalde niveaus acceptabel is. ETX gebruikt alleen een log-normale verdeling. Mosaic gebruikt de log-normale en log-logistische verdeling. De KRW guidance biedt de mogelijkheid om bij specifiek gevoelige soorten een SSD voor alleen die soorten te fitten. Dat is hier echter niet het geval. Zoeken naar 'de beste fittende verdeling' (er zijn veel mogelijkheden) is een optie, maar is tot nu toe niet het uitgangspunt volgens KRW guidance. UIteindelijk is besloten de MOSAIC (fits) niet mee te nemen en wordt niet voldaan aan de voorwaarden voor een SSD als unbound values niet mogen worden opgenomen. De norm is dus uiteindelijk m.b.v. een assessment factor afgeleid.

P.	paragraaf	Opmerking lid petit comité	Reactie Auteur
17	3.3.1.2.	Een assessment factor van 3 wordt voorgesteld. Waarom 3 en niet b.v. 2 of 4? Meer toelichting m.i. nodig.	De toelichting voor het kiezen van een AF van 3 is verder uitgebreid.

Commentaar petit comité	**Rapportnr :** Azoxystrobine; 14413d**00**
Door: Theo Brock	**datum: 17-7-2017**

P.	paragraaf	Opmerking lid petit comité	Reactie Auteur
5	1.2.3.	From a scientific and regulatory point of view it really is required that exposure concentrations in ecotoxicological tests are analytically verfied. If this is done in the dosing solutions then a realstic initial exposure concentration can be expected but it remains uncertain what the dynamics in exposure concentrations in the course of the test were. These dynamics may be influenced by the volume of the test medium in relation to the biomass and activity of the test organisms present. According to OECD guidelines LCx and ECx values from laboratory toxicity tests should be expressed in mean measured exposure concentrations. Only if in the course of time the measured concentrations do not deviate more than 20% from nominal, the nominal concentration can be selected for the toxicity estimate.	It has our preference to decide whether test resuts based on nominal concentrations (where no analysis is performed) can be used in ERL derivation on a case by case basis. It is dependent on substance characteristics and exposure regime. Azoxystrobin is not stongly hydrophobic, hence relatively water soluble, not volatile, does not degrade easily in water (hydrolysis, photolysis). From the footnotes in the toxicity data tables it can be seen that the concentration reduction is limited in the (water only) studies with short duration (up to 7 days) where the concentrations were measured. For these reasons, we have decided to accept studies for which the results are based on nominal concentrations and the test duration was limited or where a renewal of flow through design was employed. Text at the end of section 1.2.3 was extended to better reflect this.
8	Table 5	Please report the reference for the Chironomus riparius data	The reference has been added
14	3.2.3	Please make clear that specific marine taxa apparently are taxa belonging to taxonomic groups that exclusively occur in marine/estuarine environments (e.g. Echinodermata)	This has been clarified.

P.	paragraaf	Opmerking lid petit comité	Reactie Auteur
33	Evaluation criterion 5	The recovery argument is not valid for EQS derivation (only for ERO-RAC derivation). The test duration mimicking chronic exposure was long enough for the onset of maximum effects for sensitive endpoints.	Agreed, text dealing with recovery deleted.
	Annex 2	A column should be added in which it is indicated whether the toxicity estimate is expressed in terms of initial nominal concentration (not measured), verified initial nominal concentration (measured in dosing solution), or mean measured concentration during the test.	This inormation can be inferred from the Y or N (analysed or not) in the column labelled A in combination with the information provided in the footnote. The footnote details in which way the toxicity value is expressed.

Commentaar petit comité	**Rapportnr :** Azoxystrobine; 14413d**00**
Door: Dorien ten Hulscher	**datum: 18 juli 2017**

P.	paragraaf	Opmerking lid petit comité	Reactie Auteur
5	1.2.3: Occasionally, endpoints reported in the DAR exceed the water solubility as reported in Table 2 of this report. According to the WFD guidance, endpoints exceeding the water solubility with more than a factor 2 should not be used for the EQS derivation. This factor could be increased to 3 when the available data on the water solubility has a variation higher than a factor 2. The available endpoints on water solubility of azoxystrobin range from 5.9 to 6.7 mg/L, therefore the highest value is used to set the cut off value at 2 times 6.7 = 13.4 mg/L.	Waarom wordt de hoogste waarde van de oplosbaarheid gebruikt en niet het gemiddelde? De genoemde ‘variation of a factor 2’ slaat niet op de hoogste waarde maar op het gemiddelde. Ik weet niet of dit van invloed is op de selectie van valide studies, maar het is in ieder geval niet correct.	Partially agreed with the comment. The use of highest value is not correct and in case of multiple comparable endpoints the geometric mean of these endpoints should have been used. In this case however, the endpoints are for different pH vales. Therefore the water solubility most appropriate for the pH of the test medium should have been used. The text has been adapted accordingly.

Commentaar petit comité	**Rapportnr :** Azoxystrobine; 14413d**00**
Door: M. Rubach	**datum: 19.07.2017**

P.	**paragraaf**	**Opmerking lid petit comité**	**Reactie Auteur**
General	Document	Please see some textual changes made directly into the document with track changes.	These changes have been applied in the current version of the document.
1.2	1.2	I would be good, if most important abbreviations that occur also later in the text, and their NL equivalents (JG- and MAC-MKN or –MKE) would be explained in an introductory sentence (ie ERL, EQS, MPC). Or at least define them at their first mention or in a short glossary.	A list of abbreviations is inserted in Chapter 6
3.1.1	Tables 5-8 and text above.	Please make it more clear in the Table or in Appendix 2, which endpoints are based on nominal, measured or mean measured concentrations. Generally it is not acceptable to use studies where exposure was not verified appropriately (or the endpoints were not adjusted for rapid disspation). Where exposure concentrations where not verified a clear justification for including the endpoint in the EQS derivation should be added. Please add this information and include it in the consideration for the reduction in the AF later on.	This can be inferred from the Y or N (analysed or not) in the column labelled A in combination with the information provided in the footnote. The footnote details in which way the toxicity value is expressed. The text at the end of section 1.2.3 was extended to describe the rationale for accepting nominal based results for this substance. See also response to the first comment by Theo Brock.
3.1.2.	p.11, pgf 3, sentence starting with: ‘These pseudo EC50 values were plotted with the….’	Please add whether unbound values were included here as well. (In general we agree with the derivation of the pseudo-EC50 and inclusion. It is better to use these data than not using them.)	Sentence added for clarification: Unbounded EC50 values (EC50 >) shown in the data tables were not included in the SSD. Note that the 'pseudo EC50' values are not used in the actual EQS derivation. They are used to help deciding in a semi-quantitative way whether the most sensitive species is included in the data set.

P.	paragraaf	Opmerking lid petit comité	Reactie Auteur
3.1.2	p.11, pgf 3, sentence starts with 'For fungi, on pseudo EC50 per species value ….'	I count 8 data points for funghi in Fig 1, which means every data point from Table 9 was used, even though from the same species…otherwise only 5-6 fungi data points would be available. Also three MICs have the same value of 2.6, so they should not be visible as different data points. Or did I misunderstand something here?	Correct. We have now reduced the 'pseudo EC50' data set to one value per species, resulting in 6 data points instead of 8. The SSD figure has been replaced and the argumentation whether the most sensitive species has been covered is slightly appended.
3.2.1	p.12, 'The available data does not show a difference in sensitivity….'	Was this statistically confirmed?	Info on the statistics was added.
3.2.2.	p.13, 'The dataset also includes unbound data (e.g. EC50 values >) for 12 species,….'	Please provide justification for the inclusion of 12 unbound values.	An explanation has been added to the text in section 3.2.3 on page 14.
3.2.2	p.13 'The goodness of fit …)	Please detail out the good ness of fit measures.	This means goodness of fit to a log normal distribution, this has been added to the text.
3.2.3	p.14 last pgf, 'However, no acute data from specific marine taxa are available…'	What do you mean by specific marine taxa?	Text was slightly adapted and reference to WFD guidance where this issue is detailed, has been added..

P.	paragraaf	Opmerking lid petit comité	Reactie Auteur
3.3.1.2	p.17, 2nd pgf, 'Although there is a poor fit for the SSD as indicated by the statistical tests in ETX and the chron-ic SSDs presented above, the NOEC of the mesocosm and field studies presented in section 3.1.3 is 1 µg/L. In view of the latter,….'	The mesocosm NOEC provides a strong argument and I agree with it in principle (also the AF reduction to 3), but I think it should be strengthened. Potential shortcomings should be addressed here as well (the next sentence is starting with this, but there are other issues to consider). Therefore, please also bring other arguments into consideration, eg 18 species were tested, the quality of the underlying studies (are they real chronic studies?, was exposure verified properly and were the endpoints calculated with the correct concentrations, did they apply different concentration ranges?, were they performed by different authors?), are (in)sensitive taxa over- or underrepresented…). Is a reduction of the AF to 3 then still applicable?	The consideration on the AF applied to the HC5 has been extended.
Annex 1	p.34 2nd pgf, Sentence 'Why this procedure is followed is not explained in the paper.'	Suggest to delete this as this was actually explained. See suggestion in track changes.	The text has been amended
Annex 1	p.35 Statistical analysis	Please detail the methods better.	Methods have been described in more detail.