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## Environmental risk limits for difenoconazole

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This investigation has been performed by order and for the account of Directorate-General for Environmental Protection, Directorate for Soil, Water and Rural Area (BWL), within the framework of the project 'Standard setting for other relevant substances within the WFD'.

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## **Rapport in het kort**

### **Environmental risk limits for difenoconazole**

Dit rapport geeft milieurisicogrenzen voor het fungicide difenoconazool in water. Milieurisicogrenzen zijn de technisch-wetenschappelijke advieswaarden voor de uiteindelijke milieukwaliteitsnormen in Nederland. De milieurisicogrenzen zijn afgeleid volgens de methodiek die is voorgeschreven in de Europese Kaderrichtlijn Water. Hierbij is gebruikgemaakt van de beoordeling in het kader van de Europese toelating van gewasbeschermingsmiddelen (Richtlijn 91/414/EEG), aangevuld met gegevens uit de openbare literatuur.



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# 1 Introduction

## 1.1 Background and scope of the report

In this report, environmental risk limits (ERLs) for surface water are derived for the fungicide difenoconazole. The derivation is performed within the framework of the project ‘Standard setting for other relevant substances within the WFD’, which is closely related to the project ‘International and national environmental quality standards for substances in the Netherlands’ (INS). Difenoconazole is part of a series of 25 pesticides that appeared to have a high environmental impact on the evaluation of the policy document on sustainable crop protection (‘Tussenevaluatie van de nota Duurzame Gewasbescherming’; MNP, 2006) and/or were selected by the Water Boards (‘Unie van Waterschappen’; project ‘Schone Bronnen’; <http://www.schonebronnen.nl/>).

The following ERLs are considered:

- Maximum Permissible Concentration (MPC) – the concentration protecting aquatic ecosystems and humans from effects due to long-term exposure
- Maximum Acceptable Concentration (MAC<sub>eco</sub>) – the concentration protecting aquatic ecosystems from effects due to short-term exposure or concentration peaks.
- Serious Risk Concentration (SRC<sub>eco</sub>) – the concentration at which possibly serious ecotoxicological effects are to be expected.

More specific, the following ERLs can be derived depending on the availability of data and characteristics of the compound:

MPC <sub>eco, water</sub>	MPC for freshwater based on ecotoxicological data (direct exposure)
MPC <sub>sp, water</sub>	MPC for freshwater based on secondary poisoning
MPC <sub>hh food, water</sub>	MPC for fresh and marine water based on human consumption of fishery products
MPC <sub>dw, water</sub>	MPC for surface waters intended for the abstraction of drinking water
MAC <sub>eco, water</sub>	MAC for freshwater based on ecotoxicological data (direct exposure)
SRC <sub>eco, water</sub>	SRC for freshwater based on ecotoxicological data (direct exposure)
MPC <sub>eco, marine</sub>	MPC for marine water based on ecotoxicological data (direct exposure)
MPC <sub>sp, marine</sub>	MPC for marine water based on secondary poisoning
MAC <sub>eco, marine</sub>	MAC for marine water based on ecotoxicological data (direct exposure)

## 1.2 Status of the results

The results presented in this report have been discussed by the members of the scientific advisory group for the INS-project (WK-INS). It should be noted that the Environmental Risk Limits (ERLs) in this report are scientifically derived values, based on (eco)toxicological, fate and physico-chemical data. They serve as advisory values for the Dutch Steering Committee for Substances, which is appointed to set the Environmental Quality Standards (EQSs). ERLs should thus be considered as proposed values that do not have any official status.



## 2 Methods

The methodology for the derivation of ERLs is described in detail by Van Vlaardingen and Verbruggen (2007), further referred to as the 'INS-Guidance'. This guidance is in accordance with the guidance of the Fraunhofer Institute (FHI; Lepper, 2005).

The process of ERL-derivation contains the following steps: data collection, data evaluation and selection, and derivation of the ERLs on the basis of the selected data.

### 2.1 Data collection

In accordance with the WFD, data of existing evaluations were used as a starting point. For pesticides, the evaluation report prepared within the framework of EU Directive 91/414/EC (Draft Assessment Report, DAR) was consulted (EC, 2006; further referred to as DAR). An on-line literature search was performed on TOXLINE (literature from 1985 to 2001) and Current Contents (literature from 1997 to 2007). In addition to this, all potentially relevant references in the RIVM e-tox base and EPA's ECOTOX database were checked.

### 2.2 Data evaluation and selection

For substance identification, physico-chemical properties and environmental behaviour, information from the List of Endpoints of the DAR was used. When needed, additional information was included according to the methods as described in Section 2.1 of the INS-Guidance. Information on human toxicological threshold limits and classification was also primarily taken from the DAR.

Ecotoxicity studies (including bird and mammal studies) were screened for relevant endpoints (i.e. those endpoints that have consequences at the population level of the test species). All ecotoxicity and bioaccumulation tests were then thoroughly evaluated with respect to the validity (scientific reliability) of the study. A detailed description of the evaluation procedure is given in the INS-Guidance (see Section 2.2.2 and 2.3.2). In short, the following reliability indices were assigned:

- Ri 1: Reliable without restriction  
'Studies or data ... generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline ... or in which all parameters described are closely related/comparable to a guideline method.'
- Ri 2: Reliable with restrictions  
'Studies or data ... (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.'
- Ri 3: Not reliable  
'Studies or data ... in which there are interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g., unphysiologic pathways of application) or which were carried out or generated

according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which is not convincing for an expert judgment.’

- Ri 4: Not assignable

‘Studies or data ... which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.).’

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

With respect to the DAR, it was chosen not to re-evaluate the underlying studies. In principle, the endpoints that were accepted in the DAR were also accepted for ERL-derivation with Ri 2, except in cases where the reported information was too poor to decide on the reliability or when there was reasonable doubt on the validity of the tests. This applies especially to DARs prepared in the early 1990s, which do not always meet the current standards of evaluation and reporting.

In some cases, the characteristics of a compound (i.e. fast hydrolysis, strong sorption, low water solubility) put special demands on the way toxicity tests are performed. This implies that in some cases endpoints were not considered reliable, although the test was performed and documented according to accepted guidelines. If specific choices were made for assigning reliability indices, these are outlined in Section 3.3 of this report.

Endpoints with Ri 1 or 2 are accepted as valid, but this does not automatically mean that the endpoint is selected for the derivation of ERLs. The validity scores are assigned on the basis of scientific reliability, but valid endpoints may not be relevant for the purpose of ERL-derivation (e.g. due to inappropriate exposure times or test conditions that are not relevant for the Dutch situation).

After data collection and validation, toxicity data were combined into an aggregated data table with one effect value per species according to Section 2.2.6 of the INS-Guidance. When for a species several effect data were available, the geometric mean of multiple values for the same endpoint was calculated where possible. Subsequently, when several endpoints were available for one species, the lowest of these endpoints (per species) is reported in the aggregated data table.

## 2.3 Derivation of ERLs

For a detailed description of the procedure for derivation of the ERLs, reference is made to the INS-Guidance. With respect to the selection of the final MPC<sub>water</sub> an additional comment should be made:

### 2.3.1 Drinking water

The INS-Guidance includes the MPC for surface waters intended for the abstraction of drinking water (MPC<sub>dw, water</sub>) as one of the MPCs from which the lowest value should be selected as the general MPC<sub>water</sub> (see INS-Guidance, Section 3.1.6 and 3.1.7). According to the proposal for the daughter directive Priority Substances, however, the derivation of the AA-EQS (= MPC) should be based on direct exposure, secondary poisoning, and human exposure due to the consumption of fish. Drinking water was not included in the proposal and is thus not guiding for the general MPC value. The exact way of implementation of the MPC<sub>dw, water</sub> in the Netherlands is at present under discussion within the framework of the “AMvB Kwaliteitseisen en Monitoring Water”. No policy decision has been taken yet, and the MPC<sub>dw, water</sub> is therefore presented as a separate value in this report. The MPC<sub>water</sub> is thus derived considering the individual MPCs based on direct exposure (MPC<sub>eco, water</sub>), secondary poisoning

( $MPC_{sp, water}$ ) or human consumption of fishery products ( $MPC_{hh\ food, water}$ ); the need for derivation of the latter two is dependent on the characteristics of the compound.

Related to this is the inclusion of water treatment for the derivation of the  $MPC_{dw, water}$ . According to the INS-Guidance (see Section 3.1.7), a substance specific removal efficiency related to simple water treatment should be derived in case the  $MPC_{dw, water}$  is lower than the other MPCs. For pesticides, there is no agreement as yet on how the removal fraction should be calculated, and water treatment is therefore not taken into account. In case no A1 value is set in Directive 75/440/EEC, the  $MPC_{dw, water}$  is set to the general Drinking Water Standard of 0.1  $\mu\text{g/L}$  for organic pesticides as specified in Directive 98/83/EC.

### 3 Derivation of environmental risk limits for difenoconazole

#### 3.1 Substance identification, physico-chemical properties, fate and human toxicology

##### 3.1.1 Identity

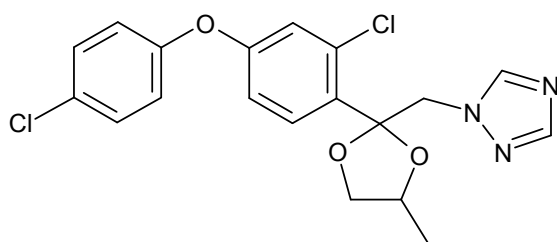


Figure 1. Structural formula of difenoconazole.

Table 1. Identification of difenoconazole.

Parameter	Name or number	Source
Common/trivial/other name	difenoconazole	EC, 2006
Chemical name	1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-4-methyl[1,3]dioxolan-2-ylmethyl]-1H-[1,2,4] triazole	EC, 2006
CAS number	119446-68-3	EC, 2006
EC number	not allocated	
SMILES code	Clc4ccc(Oc1ccc(c(Cl)c1)C2(OCC(O2)C)Cn3ncnc3)	US EPA 2007
Use class	fungicide	EC, 2006
Mode of action	interference with the ergosterol biosynthesis by inhibition of the C-14-demethylation of sterols, which leads to morphological and functional changes of the fungal cell membrane	EC, 2006
Authorised in NL	yes	
Annex 1 listing	no	

### 3.1.2 Physico-chemical properties

Table 2. Physico-chemical properties of difenoconazole.

Parameter	Unit	Value	Remark	Reference
Molecular weight	[g/mol]	406.27		
Water solubility	[mg/L]	15	at a pH of 7.2	EC, 2006
pK <sub>a</sub>	[-]	1.07±0.18	the pK <sub>a</sub> for deprotonation of the triazole moiety of difenoconazole	EC, 2006
log K <sub>OW</sub>	[-]	4.36	at pH 8. In agreement with QSARs	EC, 2006,
	[-]	4.57	ClogP	BioByte, 2006
	[-]	<b>4.30</b>	MlogP	BioByte, 2006
KOWWIN	[-]	5.20		
log K <sub>OC</sub>	[-]	3.58	from soil batch experiments; value finally used for leaching calculations	EC, 2006
Vapour pressure	[Pa]	3.32×10 <sup>-8</sup>	at 25 °C	EC, 2006
Melting point	[°C]	82-83		EC, 2006
Boiling point	[°C]	-	not relevant, decomposes	EC, 2006
Henry's law constant	[Pa.m <sup>3</sup> /mol]	9.0×10 <sup>-7</sup>	at 20 °C	EC, 2006

### 3.1.3 Behaviour in the environment

Table 3. Selected environmental properties of difenoconazole.

Parameter	Unit	Value	Remark	Reference
Hydrolysis half-life (DT <sub>50</sub> )	[d]	> 30	no significant hydrolysis (<10 %) was observed at pH 5, 7 and 9 after 30 days at 25 °C.	EC, 2006
Photolysis half-life (DT <sub>50</sub> )	[d]	> 15	no significant photolysis was observed after 15 days of irradiation	EC, 2006
Readily biodegradable		no		EC, 2006
Water/sediment systems (DT <sub>50, system</sub> )	[d]	315	geometric mean of two systems	EC, 2006
Relevant metabolites			CGA 205375 max. 4.9% in pond system (days 32 and 127), max. 11.6-11.4% in river system (days 90-183)	EC, 2006

### 3.1.4 Bioconcentration and biomagnification

An overview of the bioaccumulation data for difenoconazole is given in Table 4. Detailed bioaccumulation data for difenoconazole are tabulated in Appendix 1.

Table 4. Overview of bioaccumulation data for difenoconazole.

Parameter	Unit	Value	Remark	Reference
BCF (fish)	[L/kg]	330	whole fish	EC, 2006
BMF	[kg/kg]	1	default value for BCF < 2000 L/kg	

### 3.1.5 Human toxicological threshold limits and carcinogenicity

Difenoconazole has a (proposed) R22 classification (EC, 2006). The ADI is  $0.01 \text{ mg.kg}_{\text{bw}}^{-1}\text{d}^{-1}$  (EC, 2006), based on a 2-year combined chronic toxicity/carcinogenicity study in rat with an NOAEL of  $1.0 \text{ mg.kg}_{\text{bw}}^{-1}\text{d}^{-1}$  and an assessment factor of 100. In view of the lack of genotoxicity and the observation of liver adenomas/carcinomas only in mice and only at concentrations at which toxicity was observed, the substance is considered not likely to pose a carcinogenic risk to humans (EC, 2006).

## 3.2 Trigger values

This section reports on the trigger values for ERLwater derivation (as demanded in WFD framework).

Table 5. Difenoconazole: collected properties for comparison to MPC triggers.

Parameter	Value	Unit	Method/Source	Derived at section
Log $K_{\text{p,susp-water}}$	2.58	[-]	$K_{\text{OC}} \times f_{\text{OC,susp}}^1$	$K_{\text{OC}}$ : 3.1.2
BCF	330	[L/kg]		3.1.4
BMF	1	[kg/kg]		3.1.4
Log $K_{\text{OW}}$	4.36	[-]		3.1.2
R-phrases	R22, 50/53			3.1.5
A1 value	1.0	[ $\mu\text{g/L}$ ]	Total pesticides	
DW Standard	0.1	[ $\mu\text{g/L}$ ]	General value for organic pesticides	

<sup>1</sup>  $f_{\text{OC,susp}} = 0.1 \text{ kg}_{\text{OC}}/\text{kg}_{\text{solid}}$  (EC, 2003).

- o difenoconazole has a  $\log K_{\text{p,susp-water}} < 3$ ; derivation of  $\text{MPC}_{\text{sediment}}$  is not triggered.
- o difenoconazole has a  $\log K_{\text{p,susp-water}} < 3$ ; expression of the  $\text{MPC}_{\text{water}}$  as  $\text{MPC}_{\text{susp, water}}$  is not required.
- o difenoconazole has a  $\text{BCF} > 100 \text{ L/kg}$ ; assessment of secondary poisoning is triggered.
- o difenoconazole has a (proposed) R22 classification and a  $\text{BCF} > 100 \text{ L/kg}$ . Therefore, an  $\text{MPC}_{\text{water}}$  for human health via food (fish) consumption ( $\text{MPC}_{\text{hh food, water}}$ ) should be derived.
- o For difenoconazole, no specific A1 value or Drinking Water Standard is available from Council Directives 75/440, EEC and 98/83/EC, respectively. Therefore, the general Drinking Water Standard for organic pesticides applies.

## 3.3 Toxicity data and derivation of ERLs for water

### 3.3.1 $\text{MPC}_{\text{eco, water}}$ and $\text{MPC}_{\text{eco, marine}}$

An overview of the selected freshwater toxicity data for difenoconazole is given in Table 6. Marine toxicity data are given in Table 7. Detailed toxicity data for difenoconazole are tabulated in Appendix 2.

Table 6. Difenoconazole: selected freshwater toxicity data for ERL derivation.

<b>Chronic<sup>a</sup></b>		<b>Acute<sup>a</sup></b>	
<b>Taxonomic group</b>	<b>NOEC/EC10 (µg/L)</b>	<b>Taxonomic group</b>	<b>L(E)C50 (µg/L)</b>
algae	467 <sup>b</sup>	algae	980 <sup>h</sup>
crustacea	10 <sup>c</sup>	crustacea	<b>778<sup>i</sup></b>
insecta	34 <sup>d</sup>	pisces	1300
pisces	59 <sup>e</sup>	pisces	934 <sup>j</sup>
pisces	<b>7.6<sup>f</sup></b>	macrophyta	9900 <sup>k</sup>
macrophyta	2500 <sup>g</sup>		

<sup>a</sup> For detailed information see Appendix 2. Bold values are used for ERL derivation.

<sup>b</sup> geometric mean of EC<sub>10</sub> 590 and 370 µg/L, preferred endpoint growth rate for *Scenedesmus subspicatus* (exposure 72h).

<sup>c</sup> geometric mean of 5.6 and 18 µg/L, parameter reproduction for *Daphnia magna*

<sup>d</sup> geometric mean of 15 and 75 µg/L, parameter emergence for *Chironomus riparius*

<sup>e</sup> geometric mean of 23 and 150 µg/L for *Oncorhynchus mykiss*

<sup>f</sup> most sensitive parameter larval weight for *Pimephales promelas*

<sup>g</sup> in line with algae, the 14-days EC<sub>50</sub> for *Lemna gibba* is as considered acute

<sup>h</sup> geometric mean of 3800 and 960 µg/L, preferred endpoint growth rate for *Scenedesmus subspicatus* (72h exposure).

<sup>i</sup> geometric mean of 770, 430, 830 940, and 1100 µg/L, parameter mortality/immobilisation for *D. magna*

<sup>j</sup> geometric mean of 810, 1100, 650, 1800 and 910 µg/L for *O. mykiss*

<sup>k</sup> in line with algae, the 14-days NOEC for *Lemna gibba* is as considered as chronic

Table 7. difenoconazole: selected marine toxicity data.

<b>Chronic<sup>a</sup></b>		<b>Acute<sup>a</sup></b>	
<b>Taxonomic group</b>	<b>NOEC/EC10 (µg/L)</b>	<b>Taxonomic group</b>	<b>L(E)C50 (µg/L)</b>
		crustacea	150
		fish	950

<sup>a</sup> For detailed information see Appendix 2.

### 3.3.1.1 Treatment of fresh- and saltwater toxicity data

ERLs for freshwater and marine waters should be derived separately. For pesticides, data can only be combined if it is possible to determine with high probability that marine organisms are not more sensitive than freshwater organisms (Lepper, 2005). For difenoconazole, there are only two toxicity data (acute only, base set not complete) and no marine ERLs can be derived.

### 3.3.1.2 Mesocosm and field studies

Not available.

### 3.3.1.3 Derivation of MPC<sub>eco, water</sub> and MPC<sub>eco, marine</sub>

The acute base set is complete. There are long-term NOECs from at least three species representing three trophic levels, and an assessment factor 10 is put on the lowest NOEC of 7.6 µg/L. The MPC<sub>eco, water</sub> is  $7.6 / 10 = 0.76$  µg/L.

The MPC<sub>eco, marine</sub> cannot be derived because not enough data are available.

### 3.3.2 MPC<sub>sp, water</sub> and MPC<sub>sp, marine</sub>

In view of the BCF  $\geq 100$  L/kg, derivation of the MPC<sub>sp, water</sub> and MPC<sub>sp, marine</sub> is triggered. The available toxicity data for mammals and birds are presented in Appendix 3. In Table 8, the MPC<sub>oral</sub> is

derived applying the appropriate assessment factors to the data. No default assessment factors are available for the teratogenicity studies, a factor of 300 is used.

The  $MPC_{oral, min}$  is based on a 2-years NOAEL of 20 mg/kg<sub>diet</sub> (rat) and an assessment factor of 30 (for the choice of  $MPC_{oral, min}$ , see table below and Appendix 3), resulting in an  $MPC_{oral, min}$  of 0.67 mg/kg<sub>diet</sub>.

Table 8. difenoconazole: selection of  $MPC_{oral}$ <sup>a</sup>.

Species	Exposure duration	Endpoint (mg/kg <sub>diet</sub> )	Value	AF	$MPC_{oral}$ (mg/kg <sub>diet</sub> )
mallard duck	18w	NOAEL	625	30	21
bobwhite quail	5d	LC50	4760	3000	1.6
bobwhite quail	20w	NOAEL	100	30	3.3
mouse	90d	NOAEL	200	90	2.2
dog	6m	NOAEL	1000	90	11
mouse	18m	NOAEL	30	30	1.0
rabbit	12d (teratogenicity)	NOAEL	833	300 <sup>b</sup>	2.8
rat	9d (teratogenicity)	NOAEL	400	300 <sup>b</sup>	1.3
rat	28d	NOAEL	1500	300	5.0
rat	90d	NOAEL	250	90	2.8
rat	90d	NOAEL	750	90	8.3
rat	2y	NOAEL	20	30	<b>0.67</b>
rat	2-gen	NOAEL	250	30	8.3

<sup>a</sup> For detailed information see Appendix 3. Bold value is used for ERL derivation.

<sup>b</sup> The assessment factor has been arbitrarily determined to be 300 from a worst case perspective. Both studies are teratology studies in which the a.i. has been applied by gavage during (a part of) gestation. Therefore the duration of exposure is < 28 days.

The  $MPC_{sp, water}$  is  $MPC_{oral, min} / (BCF \times BMF) = 0.67 / (330 \times 1) = 0.0020 \text{ mg/L} = 2.0 \text{ } \mu\text{g/L}$ .

Because toxicity data for marine predators are generally not available, the  $MPC_{oral, min}$  as derived above is used as a representative for the marine environment also. To account for the longer food chains in the marine environment, an additional biomagnification step is introduced ( $BMF_2$ ). This factor is the same as given in Table 4. The  $MPC_{sp, marine}$  is  $0.67 / (330 \times 1 \times 1) = 0.0020 \text{ mg/L} = 2.0 \text{ } \mu\text{g/L}$ .

### 3.3.3 $MPC_{hh \text{ food, water}}$

Derivation of the  $MPC_{hh \text{ food, water}}$  is triggered (Table 5). The  $MPC_{hh, food}$  is calculated from the ADI (0.01 mg/kg bw), a body weight of 70 kg and a daily fish consumption of 115 g, as  $MPC_{hh, food} = 0.01 \times 0.1 \times 70 / 0.115 = 0.61 \text{ mg/kg}$  (Van Vlaardingen en Verbruggen, 2007). Subsequently the  $MPC_{hh \text{ food, water}}$  is calculated according to  $MPC_{hh \text{ food, water}} = 0.61 / (BCF_{fish} \times BMF_1) = 0.61 / (330 \times 1) = 0.0019 \text{ mg/L} = 1.9 \text{ } \mu\text{g/L}$ .

### 3.3.4 $MPC_{dw, water}$

The Drinking Water Standard is 0.1  $\mu\text{g/L}$ . Thus, the  $MPC_{dw, water}$  is also 0.1  $\mu\text{g/L}$ .

### 3.3.5 Selection of the $MPC_{water}$ and $MPC_{marine}$

The lowest MPC value should be selected as the general MPC. The lowest value of the routes included (see Section 2.3.1) is the  $MPC_{eco, water}$ . The  $MPC_{water}$  is 0.76  $\mu\text{g/L}$ .

No  $MPC_{marine}$  can be selected due to the insufficient amount of data.



### 3.3.6 $MAC_{eco}$

#### 3.3.6.1 $MAC_{eco, water}$

Difenoconazole has a potential to bioaccumulate, the mode of action is non-specific and interspecies variation is low. Therefore, an assessment factor of 100 is applied on the lowest short-term  $LC_{50}$  of 778  $\mu\text{g/L}$ , yielding  $MAC_{eco, water}$  of  $778 / 100 = 7.8 \mu\text{g/L}$ .

#### 3.3.6.2 $MAC_{eco, marine}$

No  $MAC_{eco, marine}$  can be derived due to the insufficient amount of data..

### 3.3.7 $SRC_{eco}$

There are more than three NOECs available for at least three trophic levels including algae, *Daphnia* and fish. The  $SRC_{eco}$  is derived as the geometric mean of the freshwater chronic toxicity values, which is 75  $\mu\text{g/L}$ .

## 3.4 Toxicity data and derivation of ERLs for sediment

The  $\log K_{p, \text{susp-water}}$  of difenoconazole is below the trigger value of 3; therefore, ERLs are not derived for sediment.

## 4 Conclusions

In this report, the risk limits Maximum Permissible Concentration (MPC), Maximum Acceptable Concentration for ecosystems ( $MAC_{eco}$ ), and Serious Risk Concentration for ecosystems ( $SRC_{eco}$ ) are derived for difenoconazole in water. No risk limits were derived for the marine compartment because not enough data were available. Derivation of ERLs for sediment was not triggered.

The ERLs that were obtained are summarised in the table below. The MPC values that were set for this compound until now, is also presented in this table for comparison reasons. It should be noted that this is an indicative MPC ('ad-hoc MTR'), derived using a different methodology and based on limited data.

Table 9. Derived MPC,  $MAC_{eco}$ , and SRC values for difenoconazole.

ERL	Unit	MPC	$MAC_{eco}$	$SRC_{eco}$
Water, old <sup>a</sup>	µg/L	0.011		
Water, new <sup>b</sup>	µg/L	0.76	7.8	75
Drinking water <sup>b</sup>	µg/L	0.1 <sup>c</sup>	-	-
Marine	µg/L	n.d. <sup>d</sup>	n.d. <sup>d</sup>	-

<sup>a</sup> indicative MPC ('ad-hoc MTR'), source: Helpdesk Water

[http://www.helpdeskwater.nl/emissiebeheer/normen\\_voor\\_het/zoeksysteem\\_normen/](http://www.helpdeskwater.nl/emissiebeheer/normen_voor_het/zoeksysteem_normen/)

<sup>b</sup> The  $MPC_{dw, water}$  is reported as a separate value from the other  $MPC_{water}$  values ( $MPC_{eco, water}$ ,  $MPC_{sp, water}$  or  $MPC_{hh food, water}$ ). From these other  $MPC_{water}$  values (thus excluding the  $MPC_{dw, water}$ ) the lowest one is selected as the 'overall'  $MPC_{water}$ .

<sup>c</sup> provisional value pending the decision on implementation of the  $MPC_{dw, water}$  (see Section 2.3.1)

<sup>d</sup> n.d. = not derived due to lack of data

## References

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## Appendix 1. Information on bioconcentration

Table A1.1 Bioconcentration data for difenoconazole

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness CaCO <sub>3</sub> [mg/L]	Exp. time [d]	Exp. conc. [mg/l]	BCF type	BCF whole fish [-]	RI	Notes	Reference	
<b>Pisces</b>																	
<i>Lepomis macrochirus</i>	Juveniles; 47 mm; 1.3 g	Y	F				6.7-7.1	16	20-28	28	0.0011	Equi	330	2	1		EC, 2006

### NOTES

- Concentrations are measured using <sup>14</sup>C techniques. A steady state concentration in fish tissues was reached within 3 days of exposure and 97% depuration occurred within 14 days of transfer to clean water.

# Appendix 2. Detailed aquatic toxicity data

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

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness CaCO <sub>3</sub> [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Reference
<b>Algae</b>																
<i>Pseudokirchneriella subcapitata</i>		Y	S	formulation	3.1		7.7-9.0	24-25		72 h	EC50	growth rate	> 2.9	2	1	EC, 2006
<i>Pseudokirchneriella subcapitata</i>		Y	S	formulation	3.1		7.7-9.0	24-25		72 h	EC50	biomass	1.80	2	2	EC, 2006
<i>Scenedesmus subspicatus</i>		Y	S		91.8		7.2-9.3	23		72 h	EC50	growth rate	3.80	2	3	EC, 2006
<i>Scenedesmus subspicatus</i>		Y	S		91.8		7.2-9.3	23		72 h	EC50	biomass	1.20	2	4	EC, 2006
<i>Scenedesmus subspicatus</i>		Y	S		91.8		7.7-8.1	23		72 h	EC50	growth rate		4	5	EC, 2006
<i>Scenedesmus subspicatus</i>		Y	S		91.8		7.7-8.1	24		72 h	EC50	biomass	0.03	2	6	EC, 2006
<i>Scenedesmus subspicatus</i>		N	S	formulation	25		7.5-8.3	23		96 h	EC50	growth rate	2.20	2	7	EC, 2006
<i>Scenedesmus subspicatus</i>		N	S	formulation	25		7.5-8.3	23		96 h	EC50	biomass	1.60	2	8	EC, 2006
<i>Scenedesmus subspicatus</i>		Y	S	formulation	25		7.7-8.1	23		72 h	EC50	growth rate		4	9	EC, 2006
<i>Scenedesmus subspicatus</i>		Y	S	formulation	25		7.7-8.1	23		72 h	EC50	biomass	0.04	2	10	EC, 2006
<i>Scenedesmus subspicatus</i>		Y	S	formulation	25.2		7.9-9.1	22-23	24	72 h	EC50	growth rate	0.96	2	11	EC, 2006
<i>Scenedesmus subspicatus</i>		Y	S	formulation	25.2		7.9-9.1	22-23	24	72 h	EC50	biomass	0.29	2	12	EC, 2006
<b>Crustacea</b>																
<i>Daphnia magna</i>		Y	S		96.1		8.1-8.3	20-22	225-275	48 h	LC50	mortality	0.77	2	13	EC, 2006
<i>Daphnia magna</i>		Y	S	formulation	3.1		7.5-7.6	20	168	48 h	EC50	immobilisation	0.43	2	14	EC, 2006
<i>Daphnia magna</i>	first instar < 24 h old	N	S	formulation	25		7.4-7.5	20		48 h	EC50	immobilisation	0.83	2	15	EC, 2006
<i>Daphnia magna</i>		Y	S	formulation	25		7.9-8.3	20	240	48 h	EC50	immobilisation	0.94	2	16	EC, 2006
<i>Daphnia magna</i>		Y	S	formulation	25.2		7.9-8.0	19-20	250	48 h	EC50	immobilisation	1.10	2	17	EC, 2006
<b>Macrophyta</b>																
<i>Lemna gibba</i>		N	S		96.1			25		14 d	EC50	growth	9.9	2	6,31	EC, 2006
<b>Pisces</b>																
<i>Lepomis macrochirus</i>	juv.: 0.61 g; 29 mm	Y	S	a.s.	96.1		7.0-7.5	22-23	40-45	96 h	LC50	mortality	1.30	2	18	EC, 2006
<i>Oncorhynchus mykiss</i>	juv.: 0.78 g; 44 mm	Y	S	a.s.	96		6.9-7.6	12	46	96 h	LC50	mortality	0.81	2	20	EC, 2006
<i>Oncorhynchus mykiss</i>	juv.: 0.92 g; 45 mm	Y	F	a.s.	96.1		6.6-7.2	11-13	32-33	96 h	LC50	mortality	1.10	2	22	EC, 2006
<i>Oncorhynchus mykiss</i>	juv.: 0.64 g; 41 mm	Y	F	formulation	3.1	dtw	6.6-7.5	14	180	96 h	LC50	mortality	0.7	2	24	EC, 2006
<i>Oncorhynchus mykiss</i>	juvenile	Y	S	formulation	25	dtw	7.9-8.2	17	200-240	96 h	LC50	mortality	0.65	2	26	EC, 2006
<i>Oncorhynchus mykiss</i>	juv.: 0.64 g; 41 mm	Y	S	formulation	25	dtw	7.6-8.3	15	180	96 h	LC50	mortality	1.80	2	27	EC, 2006
<i>Oncorhynchus mykiss</i>	juvenile	Y	S	formulation	25.2		8.5-8.7	13	207	96 h	LC50	mortality	0.91	2	29	EC, 2006

## NOTES

- Formulation contains 30.6 g/L-1. ERC50 exceeds top dose (extrapolated value notifier was 3.3 mg a.s./L). OECD Guideline 201 (1984).
- Formulation contains 30.6 g a.s./L. OECD Guideline 201 (1984). The calculation of an Ebc50 according to this guideline is considered inappropriate.
- OECD Guideline 201 (1984).
- OECD Guideline 201 (1984). The calculation of an Ebc50 according to this guideline is considered inappropriate.
- The notifier only submitted an Ebc50 (area under growth curve), see below. The RMS tried to recalculate an Erc50 based on the raw data but concluded that this was not possible as the growth rate data did not fit into a probit model. Due to this the whole study is considered not useful for ERL derivation. OECD Guideline 201 (1984).
- Not reliable as only an Ebc50 could be calculated and no Erc50. OECD Guideline 201 (1984). The calculation of an Ebc50 according to this guideline is considered inappropriate.
- Formulation contains 250 g a.s./L-OECD Guideline 201 (1984).
- Formulation contains 250 g a.s./L-OECD Guideline 201 (1984). The calculation of an Ebc50 according to this guideline is considered inappropriate.
- Formulation contains 250 g a.s./L. The notifier only submitted an Ebc50 (area under growth curve), see below. The RMS did not try to recalculate an Erc50 based on the raw data as the formulation was considered representative for the proposed formulation. So far, i.e. being unable to recalculate a proper Erc50 based on the raw data, the whole study is considered not useful for ERL derivation. OECD Guideline 201 (1984).
- Formulation contains 250 g a.s./L. No Erc50 available, therefore not useful for ERL derivation. Recalculation as in Grade 1993b is not an option as the RMS does not provide raw data on growth rate in summary DAR. OECD Guideline 201 (1984).

- 11 Formulation contains 252 g a.s./L. OECD Guideline 201 (1984).  
12 Formulation contains 252 g a.s./L. OECD Guideline 201 (1984). The calculation of an Ebc50 according to this guideline is considered inappropriate.  
13 No information on clinical effects, so no acute NOEC can be derived by evaluator. Guideline US EPA FIFRA 72-2.  
14 Formulation with 30.6 g a.s./L. OECD 202.  
15 Formulation with 250 g a.s./L. Study not used for DAR risk assessment due to lack of analytical verification. OECD 202 I (1984).  
16 Formulation with 250 g a.s./L. OECD 202.  
17 Formulation with 252 g a.s./L. OECD Guideline No. 202, 1984. US EPA OPPTS Test Guidelines 850.1010, 1996.  
18 US EPA FIFRA 72-1.  
19 US EPA FIFRA 72-1.  
20 Not used for risk assessment by RMS due to partial analytical verification (only measurements at start test). However, the study is sufficient for ERL derivation in view of relative persistence in water (see DAR, fate and behaviour). RIVM evaluated this study in 1993 as less reliable, though useful for risk assessment. US EPA FIFRA 72-1.  
21 Not used for derivation ERL as validity is 3. US EPA FIFRA 72-1.  
22 US EPA FIFRA 72-1.  
23 No NOEC could be derived. US EPA FIFRA 72-1.  
24 Formulation with 30.6 g a.s./L. LC50 is based on geometric mean of mean measured concentrations. OECD 203 (1992).  
25 Formulation with 30.6 g a.s./L. OECD 203 (1992).  
26 Formulation with 250 g a.s./L. LC50 is based on mean measured concentrations. OECD 203 (1984).  
27 Formulation with 250 g a.s./L. LC50 is based on nominal concentrations. OECD 203 (1984).  
28 Formulation with 250 g a.s./L. LC50 based on mg product/L, recalculated to a.s. via density of 1.0129 kg/L. OECD 203 (1992).  
29 Formulation was used with 252 g a.s./L. LC50 based on mg product/L, recalculated to a.s. via density of 1.0129 kg/L. OECD 203 (1992).  
30 Formulation was used with 252 g a.s./L. LC50 based on mg product/L, recalculated to a.s. via density of 1.0129 kg/L. OECD 203 (1992).  
31 14 days is chronic, but in line with algae, the EC50 is treated as acute, the NOEC as chronic

**Table A2.2. Chronic toxicity of difenoconazole to freshwater organisms.**

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Hardness CaCO3 [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Reference
<b>Algae</b>																
<i>Scenedesmus subspicatus</i>		Y	S		91.8		7.2-9.3	23		72 h	EC10	growth rate	0.59	2	12	EC, 2006
<i>Scenedesmus subspicatus</i>		N	S	formulation	25		7.5-8.3	23		96 h	EC10	growth rate	1.10	2	12	EC, 2006
<i>Scenedesmus subspicatus</i>		Y	S	formulation	25.2		7.9-9.1	22-23	24	72 h	EC10	growth rate	0.37	2	12	EC, 2006
<i>Scenedesmus subspicatus</i>		Y	S		91.8		7.7-8.1	24		72 h	NOEC	growth rate	0.0086	3	1	EC, 2006
<b>Crustacea</b>																
<i>Daphnia magna</i>		Y	F		96.1		8.1-8.3	20	206-275	21 d	NOEC	reproduction/length F0	0.0056	2	2	EC, 2006
<i>Daphnia magna</i>		N	R	formulation	25		7.8-8.2	21		21 d	NOEC	reproduction	0.0180	2	3	EC, 2006
<b>Insecta</b>																
<i>Chironomus riparius</i>	larvae, 2-3 d old	Y	S		91	Elendt M4 medium	7.7	20	240	28 d	NOEC	emergence, development rate	0.0150	2	4	EC, 2006
<i>Chironomus riparius</i>		Y	S	formulation	25.5	Elendt M4 medium	8.4-9.9	20	244	28 d	NOEC	emergence rate	0.0750	2	5	EC, 2006
<b>Macrophyta</b>																
<i>Lemna gibba</i>		N	S		96.1			25		14 d	NOEC	growth	2.5	2	7	EC, 2006
<b>Pisces</b>																
<i>Oncorhynchus mykiss</i>	juv. 1.26 g; 48 mm	Y	F		91.8		7.8-8.3	15-16	150-164	21 d	NOEC	growth, feeding	0.0230	2	8	EC, 2006
<i>Oncorhynchus mykiss</i>	juveniles	Y	R	formulation	25		7.3-8.5	15-17		21 d	NOEC	growth, feeding	0.1500	2	9	EC, 2006
<i>Pimephales promelas</i>	embryos and larvae	Y	F		96.1		6.6-7.2	24	30-31	34	NOEC	larvae weight	0.0076	2	10	EC, 2006
<i>Pimephales promelas</i>	embryos and larvae	Y	F		95		7.0-7.7	25	26-28	68	NOEC	larvae length	0.0087	2	11	EC, 2006

**NOTES**

- The notifier only submitted an EBC50 (area under growth curve), see below. The RMS tried to recalculate an ErC50 based on the raw data but concluded that this was not possible as the growth rate data did not fit into a probit model. Due to this the whole study is considered not useful for ERL derivation. OECD Guideline 201 (1984).  
US EPA FIFRA 72-4.
- The formulation contained 250 mg a.s./L. OECD 202 II (1984).
- The NOEC is based on mean measured concentrations in the water phase. No measurements in the sediment. Sediment-spiked test. ASTM E1706 (1995). Sediment in accordance with OECD 207.
- The NOEC is based on mean measured concentrations in the water phase. No measurements in the sediment. Formulation with 255 g a.s./L. Water-spiked test. BBA/IVA ring-test protocol (1994).  
US EPA FIFRA 122-2.
- US EPA FIFRA 122-2.
- US EPA FIFRA 122-2.
- US EPA FIFRA 122-2.
- Recoveries of a.i. during test: 40-69%. NOEC based on mean measured concentrations.
- Formulation with 250 g a.s./L. OECD 204 (1984). Recoveries of a.i. during test: 86-116%. NOEC based on nominal concentrations.
- US EPA FIFRA 72-4.
- US EPA FIFRA 72-4.
- Recalculated conform RIVM methodology.

**Table A2.1. Acute toxicity of difenoconazole (marine water).**

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	pH	T [°C]	Salinity [%]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Reference
<b>Crustacea</b>																
<i>Myxidopsis bahia</i>	≤ 24 h	Y	F	a.s.	95		7.9-8.1	23-25	31-32	96 h	LC50	mortality	0.15	2	1	EC, 2006
<b>Mollusca</b>																
<i>Crassostrea virginica</i>	mean valve height 40 mm	Y	F	a.s.	95		7.7-7.9	19-20	32-34	96 h	EC50	shell deposition	> 0.30	3	2	EC, 2006
<b>Pisces</b>																
<i>Cyprinodon variegatus</i>	juv: 0.003 g; 6.5 mm	A	S	a.s.	96.1		7.7-8.2	21-22	20	96 h	LC50	mortality	0.82	2	3	EC, 2006
<i>Cyprinodon variegatus</i>	juv: 0.3 g; 28 mm	A	F	a.s.	96		7.6-7.9	22	31-32	96 h	LC50	mortality	1.10	2	4	EC, 2006

**NOTES**

- 1 No information on clinical effects, so no acute NOEC can be derived by evaluator. US EPA FIFRA 72-3.
- 2 NOEC originally reported as 0.21 mg a.s./L, but not acknowledged by RMS in view of high variabilities of clinical effect at submortal concentrations. EC50 by RMS based on < 50% shell deposition at top-dose of 0.3 mg a.s./L. US EPA FIFRA 72-3.
- 3 Brackish test water. US EPA FIFRA 72-3.
- 4 Brackish water. US EPA FIFRA 72-3.



# Appendix 3. Detailed bird and mammal toxicity data

**Table A3.1. Toxicity of difenconazole to birds and mammals.**

Species	Species properties	Purity [%]	Application route	Exp. time	Criterion	Test endpoint	Value [mg/kg <sub>bw</sub> /d]	Value [mg/kg <sub>diet</sub> ]	Ri	Notes	Reference
mallard duck	ducklings	96.1	diet	5d	LC50	mortality	> 5000		2		EC, 2006
mallard duck	34w old	91.1	diet	18w	NOAEL	reproduction and body weight	625		2		EC, 2006
bobwhite	14d old	95.2	diet	5d	LC50	mortality	4760		2		EC, 2006
quail											
bobwhite	28w old	94.3	diet	20w	NOAEL	reproduction and body weight of 1-d old hatching	100		2		EC, 2006
quail											
rat	SPF-Wistar	≥ 95	diet	28d	NOAEL	body weight, carcass weight, organ weight	1500		2		EC, 2006
rat	SPF Wistar (outbred KFM-Han)	94.5	diet	90d	NOAEL	body weight, heart weight, carcass weight, food consumption	250		2		EC, 2006
rat	CRL:CD (SD)® rats	94.5	diet	90d	NOAEL	body weight gain	750		2		EC, 2006
mouse	CD-1® (ICR) mice	94.5	diet	90d	NOAEL	body weight gain	200		2		EC, 2006
dog	purebred beagles	96.1	diet	6m	NOAEL	food consumption	1000		2	1	EC, 2006
rat	Sprague Dawley® CRL: CD rats	94.5 (w 1-20), 95 (w 21-106)	diet	2y	NOAEL	body weight and body weight gain	20		2		EC, 2006
mouse	CD-1® (ICR) mice	94.5 (w 1-20), 95 (w 21-80)	diet	18m	NOAEL	body weight gain (males)	30		2		EC, 2006
dog	purebred beagles	96.1	diet	1y	NOAEL	food consumption	≥ 1500		2		EC, 2006
rat	37-38d old rats	97.4	diet	two generations	NOAEL	body weight (parental animals and offspring)	250		2		EC, 2006
rabbit	New Zealand White rabbits	95.7	by gavage	d 7-19 of presumed gestation	NOAEL	(maternal) body weight, food consumption, abortion, foetal resorption	833	25	2	1	EC, 2006
rat	Cri:COBS®CD® (SD) BR rats		by gavage	d 6 to 15 of presumed gestation	NOAEL	(maternal) body weight gain, food consumption	400	20	2	2	EC, 2006

**NOTES**

- 1 rabbit teratogenicity study by gavage: NOAEL food recalculated by mg/kg<sub>bw</sub>/d times the conversion factor of 33.3
- 2 rat teratogenicity study by gavage: NOAEL food recalculated by mg/kg<sub>bw</sub>/d times the conversion factor of 20

## **Appendix 4. References used in the appendices**

EC. 2006. Draft Assessment report difenoconazole. Updated December 2006. RMS Sweden.



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