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Proposed EQS for Water Framework Directive Annex VIII substances: diazinon

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The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC) is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. This report is the result of research commissioned and funded on behalf of UKTAG by the Scotland & Northern Ireland Forum for Environmental Research (SNIFFER) and the Environment Agency's Science Programme.

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Steve Killeen Head of Science

Use of this report

The development of UK-wide classification methods and environmental standards that aim to meet the requirements of the Water Framework Directive (WFD) is being sponsored by the UK Technical Advisory Group (UKTAG) for WFD on behalf of its members and partners.

This technical document has been developed through a collaborative project, managed and facilitated by the Scotland & Northern Ireland Forum for Environmental Research (SNIFFER), the Environment Agency and the Scottish Environment Protection Agency (SEPA) and has involved the members and partners of UKTAG. It provides background information to support the ongoing development of the standards and classification methods.

Whilst this document is considered to represent the best available scientific information and expert opinion available at the stage of completion of the report, it does not necessarily represent the final or policy positions of UKTAG or any of its partner agencies.

Executive Summary

The UK Technical Advisory Group (UKTAG) has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for diazinon using the methodology described in Annex V of the Directive. There are existing EQSs for diazinon, but the method used to derive them is not considered to comply with the requirements of Annex V and so cannot be used to derive Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for diazinon, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data. Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V.

Where possible, PNECs have been derived for freshwater and saltwater environments, and for long-term/continuous exposure and short-term transient exposure. If they were to be adopted as EQSs, the long-term PNEC would normally be expressed as an annual average concentration and the short-term PNEC as a 95th percentile concentration.

The feasibility of implementing these PNECs as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

Properties and fate in water
Diazinon is a contact organophosphorus insecticide with a wide range of agricultural and veterinary applications. It is hydrolytically stable with a half-life in natural waters of several days, but undergoes microbial degradation. Diazinon is moderately lipophilic (log Kow 3.1–4.0) and so will tend to partition into sediment and biota. Its primary mode of action is through the inhibition of cholinesterases in the nervous system; invertebrates are particularly sensitive.

Availability of data
Laboratory toxicity data are available for nine different freshwater taxonomic groups. The acute toxicity data cover algae, amphibians, annelids, crustaceans, fish, insects, molluscs and planarians. Chronic data are available for algae, crustaceans, fish, insects and rotifers. Laboratory data are supplemented by pond and stream mesocosm studies.

Laboratory and mesocosm experiments confirm the high sensitivity of crustaceans and insects, although fish are also amongst the most sensitive taxa with algae, molluscs, planarians and annelids exhibiting lower sensitivities. There is recent

evidence of endocrine-disrupting effects in fish arising from the disruption of olfactory function at low concentrations of diazinon.

By comparison, few toxicity data are available for marine organisms, represented by just five crustacean and two fish species.

Derivation of PNECs

Long-term PNEC for freshwaters

Reliable chronic data are available for invertebrates and fish. Recent studies have revealed significant reductions in olfactory responses of male Atlantic salmon (*Salmo salar*) following short-term exposure to 0.3 µg l^{-1} diazinon, with a no observed effect concentration (NOEC) of 0.1 µg l^{-1}. Although the exposure period was only 30 minutes, effects on reproductive steroid concentrations, the sensitivity of the olfactory epithelium and sperm volumes were observed, with important long-term implications for reproductive success. These data are supported by similar NOECs for reproduction in the crustaceans *Ceriodaphnia dubia*, *Daphnia magna* and *Gammarus pseudolimnaeus*. The standard assessment factor of 10 applied to the Atlantic salmon NOEC of 0.1 µg l^{-1} is recommended, resulting in a $PNEC_{freshwater\_lt}$ of 0.01 µg l^{-1}.

This is similar to the existing EQS of 0.03 µg l^{-1} for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos) based on a *Daphnia magna* NOEC of 0.15 µg l^{-1}, to which an assessment factor of 5 was applied.

Short-term PNEC for freshwaters

Good quality data are available from acute studies with eight taxa including fish, insects and crustaceans. The most sensitive of the insects and crustaceans are at least an order of magnitude more sensitive than the most sensitive fish species. The lowest reliable effects concentration is a 96-hour LC50 of 0.2 µg l^{-1} to the freshwater shrimp *Gammarus fasciatus*. The specific mode of action of diazinon, coupled with the indications that this species is likely to be among the most sensitive taxa, allows a reduced assessment factor (10) to be applied instead of the default value of 100, resulting in a $PNEC_{freshwater\_st}$ of 0.02 µg l^{-1}.

This is five times lower than the existing EQS of 0.1 µg l^{-1} for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos) generated using a smaller assessment factor (2) applied to the same critical data, as permitted by the method used to derive the EQS.

Long-term PNEC for saltwaters

The limited data suggest similar sensitivities of freshwater and saltwater species, but the greater taxonomic diversity of marine organisms compared with those living in freshwaters introduces greater uncertainty into the prediction of a saltwater PNEC. Nevertheless, in the absence of reliable chronic saltwater toxicity data, a saltwater PNEC may be based on freshwater data. However, an assessment factor of 10 applied to the lowest freshwater chronic NOEC (0.1 µg l^{-1} for olfactory responses in Atlantic salmon) is considered adequate because evidence from acute

tests suggests that long-term NOECs generated for these saltwater taxa would not be lower than those already available. This results in a $PNEC_{saltwater\_lt}$ of 0.01 µg l^{-1}, identical to the $PNEC_{freshwater\_lt}$.

This is similar to the existing EQS of 0.03 µg l^{-1} for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos), which was 'read across' from the freshwater long-term value.

Short-term PNEC for saltwaters

Five taxa are represented among the saltwater acute toxicity dataset, including crustaceans, which are clearly much more sensitive than the other species tested. Acute effect concentrations of 2.5–5.6 µg l^{-1} were reported in reliable studies with the copepod *Acartia tonsa*, the shrimp *Palaemonetes pugio* and the mysid shrimp *Americamysis bahia*.

Although the Annex V guidance does not specifically address short-term PNECs for the protection of marine species, the general guidance on short-term effects was followed. An assessment factor of 10 applied to the *Acartia* 96-hour LC50 of 2.57 µg l^{-1} was recommended, resulting in a $PNEC_{saltwater\_st}$ of 0.26 µg l^{-1}. This assessment factor is justified on the basis that, as a crustacean, *Acartia tonsa* is probably amongst the most sensitive marine species to this insecticide.

This PNEC is slightly higher than the existing EQS of 0.1 µg l^{-1} for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos) based on 'reading across' from the freshwater short-term EQS.

PNECs for sediment and secondary poisoning

Although the lipophilicity of diazinon would result in partition from water to sediment, there are insufficient sediment toxicity data to derive a $PNEC_{sediment}$. For both freshwater and saltwater, PNECs based on the risks of secondary poisoning to mammals and birds (0.06 µg l^{-1}) are higher than those derived for the protection of aquatic life and so do not influence the development of EQSs for diazinon.

Summary of proposed PNECs

Receiving medium/exposure scenario	**Proposed PNEC (µg l^{-1})**	**Existing EQS (µg l^{-1})**
Freshwater/long-term	0.01	0.03
Freshwater/short-term	0.02	0.1
Saltwater/long-term	0.01	0.03
Saltwater/short-term	0.26	0.1
Secondary poisoning	0.06	–

Analysis

The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that non-standard analytical methodologies employing extraction/preconcentration gas chromatography/mass spectrometry (GC-MS) are capable of achieving detection

limits as low as 0.5 ng l^{-1} [and potentially lower using a nitrogen phosphorus detector (NPD)], sufficient to quantify concentrations of diazinon at the most stringent EQS.

Implementation issues

The proposed PNECs are recommended for adoption as EQSs, with the exception of the $PNEC_{saltwater\_st}$. The proposed $PNEC_{saltwater\_st}$ is higher (less stringent) than the existing saltwater short-term EQS. Therefore, to comply with the 'no deterioration' principle, it is recommended that the existing saltwater short-term EQS is retained.

Contents

1. Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)[1] is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. UKTAG has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for diazinon using the methodology described in Annex V of the Directive. There are existing EQSs for diazinon, but the method used to derive them [12] is not considered to comply with the requirements of Annex V and so cannot be used to derive Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for diazinon, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data.[2] Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V. The feasibility of implementing these PNECs as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

This report provides a data sheet for diazinon.

1.1 Properties and fate in water

Diazinon is a contact organophosphorus insecticide with a wide range of agricultural and veterinary applications. It is hydrolytically stable with a half-life in natural waters of several days, but undergoes microbial degradation. Diazinon is moderately lipophilic (log Kow 3.1–4.0) and so will tend to partition into sediment and biota. Its primary mode of action is through the inhibition of cholinesterases in the nervous system; invertebrates are particularly sensitive.

[1] *Official Journal of the European Communities*, **L327**, 1–72 (22/12/2000). Can be downloaded from http://www.eu.int/comm/environment/water/water-framework/index_en.html

[2] Data quality assessment sheets are provided in Annex 1.

2. Results and observations

2.1 Identity of substance

Table 2.1 gives the chemical name and Chemical Abstracts Service (CAS) number for the substance of interest.

Table 2.1 Substance covered by this report

Name	CAS Number
Diazinon	333-41-5

2.2 PNECs proposed for derivation of quality standards

Table 2.2 lists proposed PNECs, obtained using the methodology described in the Technical Guidance Document (TGD) issued by the European Chemicals Bureau (ECB) on risk assessment of chemical substances [11], and existing EQSs obtained from the literature [60].

Section 2.6 summarises the effects data identified from the literature for diazinon. The use of these data to derive the values given in Table 2.2 is explained in Section 3.

Table 2.2 Proposed overall PNECs as basis for quality standard setting

PNEC	TDG deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS[§]
Freshwater short-term	0.02 µg l^{-1} (Section 3.1.1)	-	0.1 µg l^{-1} (MAC)
Freshwater long-term	0.01 µg l^{-1} (Section 3.1.1)	Insufficient data (Section 3.2)	0.03 µg l^{-1} (AA)
Saltwater short-term	0.26 µg l^{-1} (Section 3.1.2)	-	0.1 µg l^{-1} (MAC)
Saltwater long-term	0.01 µg l^{-1} (Section 3.1.2)	Insufficient data (Section 3.2)	0.03 µg l^{-1} (AA)
Freshwater sediment short-term	lack of data (Section 3.4.1)	-	-
Freshwater sediment long-term	lack of data (Section 3.4.1)	Insufficient data (Section 3.4.2)	-
Saltwater sediment short-term	lack of data (Section 3.4.1)	-	-
Saltwater sediment long-term	lack of data (Section 3.4.1)	Insufficient data (Section 3.4.2)	-
Freshwater secondary poisoning	16.7 µg/kg prey ≈ 0.06 µg l^{-1} (Section 3.5)	-	-

PNEC	TDG deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS[§]
Saltwater secondary poisoning	16.7 µg/kg prey ≈ 0.06 µg l^{-1} (Section 3.5)	-	-

[§] Total concentration of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos should not exceed the AA or MAC.
AA = annual average
AF = assessment factor
MAC = maximum allowable concentration
SSD = species sensitivity distribution

2.3 Hazard classification

Table 2.3 gives the R-phrases (Risk-phrases) and labelling for the substance of interest.

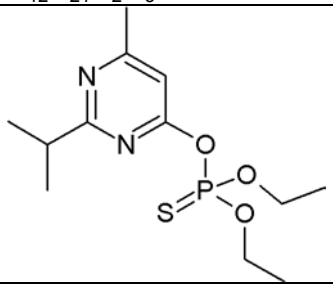
Table 2.3 Hazard classification

R-phrases and labelling	Reference
Xn; R22 N; R50-53	[1]

2.4 Physical and chemical properties

Table 2.4 summarises the physical and chemical properties of the substance of interest.

Table 2.4 Physical and chemical properties of diazinon

Property	Value	Reference
Molecular formula	$C_{12}H_{21}N_2O_3PS$	[8]
Molecular structure		
Molecular weight	304.34326	[8]
Vapour pressure	9.7 mPa at 20°C 9.01×10^{-5} mmHg at 25°C 18.8 mPa at 20°C (Ciba-Geigy) 1.8×10^{-4} mbar	[3] [4] [5] [6]
Henry's Law constant	1.17×10^{-7} atm m^3/mol at 23°C 1.15×10^{-2} Pa m^3/mol; 6.7×10^{-2} Pa m^3/mol	[4] [5]
Solubility in water	40 mg l^{-1} at 20°C 40 mg l^{-1} at 25°C 40 mg l^{-1} at room temperature 60 mg l^{-1} at 20°C	[3, 5] [4] [6] [7]

2.5 Environmental fate and partitioning

Table 2.5 summarises the information obtained from the literature on the environmental fate and partitioning of diazinon.

Table 2.5 Environmental fate and partitioning of diazinon

Property	Value	Reference
Hydrolytic stability (DT50)	Stable in the neutral range. Hydrolysis under acidic and alkaline conditions. DT50 (20°C): pH 3.1: 11.77 hours pH 7.4: 185 days pH 10.4: 6 days.	[3, 5]
Photostability DT50) (aqueous, sunlight, state pH)	–	
Biodegradation	–	
Degradation in water/sediment: DT50 water DT50 whole system	 DT50: 5.2–12.2 days in pond mesocosms DT50: 70–79 hours in natural lake or laboratory water DT50: 7–10 days in pond water with 1% sediment added; 8-15 days in river water with 1% sediment added. 60% of the applied material was mineralised within 7 weeks in both systems.	 [2] [2] [2]
Mineralisation	–	
Bound residue	–	
Distribution in water/sediment systems (active substance)	–	
Distribution in water and sediment systems (metabolites)	–	
Residues relevant to the aquatic environment	–	
Degradation in soil	DT50: ca 11–21 days in laboratory soil DT50 field: min 21 days; max 103 days; estimated average 40 days DT50: 5 days (at 20°C and humidity of 60% field capacity); DT50: 118 days (in sterile soil under same conditions)	[3] [5] [2]
Partition coefficients (log Kow) Koc	3.95 3.81 3.3;[3] 3.81 3.14 3.40 min: 85; max: 1,842	[3] [4] [5] [6] [2] [5]

[3] OECD TG 107: determination of octanol–water partition coefficient.

Property	Value	Reference
Bioaccumulation BCF		
Fish		
Gnathopogon caerulescens (Willow shiner)	274.4 ± 17.7 after 168 hours at 2.4–3.3 µg l^{-1}; disappearance half-life: 9.9 hours.	[4]
Pseudodorasbora parva	152	[4, 6]
Cyprinus auratus	36.6	[4, 6]
Cryprinus carpio	65.1; 120	[4, 6]
Poecilia reticulata	17.5	[4, 6]
Fundulus heteroclitus	10	[4, 6]
Rainbow trout	63	[4]
Loach	26	[4]
Sheepshead minnow	200	[4]
Perch	27	[4]
Anguilla anguilla	muscle 1,600 and liver 800	[4]
Crustaceans		
Procambarus clarkii	4.9	[4]
Shrimp	3	[4, 6]
Other		
Indoplanorbis exustus (snail)	17	[4]
Cipangopaulina malleata (pond snail)	5.9	[6]
Earthworm	8	[4]

DT50 = time taken to degrade by 50%
BCF = bioconcentration factor

Uptake of diazinon by aquatic species is rapid. Relatively low BCFs have been reported for aquatic organisms, ranging from 3 for shrimp to 274 for a fish species (*Gnathopogon caerulescens*); this is consistent with rapid metabolism and loss. Depuration half-lives for fish have been reported to be up to 30 hours (muscle) [7].

Volatilisation and aerial transport of diazinon is of minor importance. Diazinon has a tropospheric half-life of 1.5 hours [7].

2.6 Effects data

A summary of the mode of action for this substance can be found in Section 2.6.5.

Data collation followed a tiered approach.

Freshwater and marine data were collated from existing EQS documents [60, 61]. Further data, published after derivation of the current UK EQS were then retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database.[4]

[4] http://www.epa.gov/ecotox/

Because no data on sediment-dwelling organisms and on mammalian or avian chronic oral toxicity were available in ECOTOX, further databases were searched via the STN portal. Further sources used were:

- AGRITOX database of the French National Institute on Agricultural Research (INRA) [5];
- Hazardous Substances Data Bank (HSDB®) database of the US National Library of Medicine;[5]
- US EPA Integrated Risk Information System (IRIS) database;[6]
- World Health Organization (WHO) *Environmental Health Criteria 198: Diazinon* [2];
- US EPA *Draft Ambient Aquatic Life Water Quality Criteria* [7].

In addition, data were sought from the electronic archives of the UK Pesticide Safety Directorate (PSD).[7]

Toxicity data for diazinon concentrations in sediment (e.g. on a mg/kg sediment basis) were not identified.

2.6.1 Toxicity to freshwater organisms

Single species acute toxicity data are available for eight different taxonomic groups, i.e. algae, crustaceans, fish, amphibians, insects, molluscs, annelids, and planarians. Chronic toxicity data are available for algae, crustaceans, fish, insects and rotifers.

Fish, crustaceans and insects are the most sensitive species for both chronic and acute effects of diazinon. Due to the low number of tests available for other taxa, it is difficult to judge their relative sensitivity to diazinon. However, algae as well as molluscs, planarians and annelid worms appear to be of low sensitivity, whereas amphibians may belong to the more sensitive taxa.

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for diazinon are presented in Figures 2.1 and 2.2. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. These diagrams are not species sensitivity distributions and have not been used to set the diazinon PNECs. The freshwater data for diazinon are highlighted in Tables 2.6 and 2.7.

In addition, two outdoor simulated ecosystem studies of a pond and a stream community are available (Table 2.6):

- Giddings *et al.* 1996 [29] performed a microcosm study with 18 fibreglass tanks (3.2 m in diameter and 1.5 m in depth, sediment and water from natural ponds and each tank stocked with 40 juvenile bluegill sunfish). Eight loading rates with two tanks for each level plus two controls were used. The ponds received three consecutive diazinon applications at 7-day intervals, with single applications ranging from 2 to 500 µg l^{-1} and corresponding time weighted average (TWA) concentrations of 2.4, 4.3, 9.2, 22, 54, 117, 205, and 443 µg l^{-1} (TWA over 70 days).

[5] http://toxnet.nlm.nih.gov/
[6] http://www.epa.gov/iris/index.html
[7] http://www.pesticides.gov.uk/

The most sensitive taxa in the mesocosms were chironomid insects of the families *Pentaneurini*, *Ceratopogonidae* and *Cladocera* (daphnids). The latter were significantly reduced at all treatment levels and for the entire post-treatment period of 70 days, i.e. no observed effect concentration (NOEC) <2.4 µg l^{-1}. Effects on various other zooplankton and macroinvertebrate taxa occurred at diazinon concentrations of 9.2 µg l^{-1} (TWA). Fish biomass was reduced at 22 µg l^{-1} and survival at 54 µg l^{-1}. Dragonflies, some dipteran insects, and plants were not adversely affected by diazinon at the highest concentration tested (443 µg l^{-1} TWA).

- Arthur *et al.* 1983 [17] evaluated the effects of diazinon on macroinvertebrates in three outdoor experimental channels. One channel served as a control and two channels as low and high treatments. The low and high treatment channels were continuously dosed at either 0.3 or 3 µg l^{-1} nominal diazinon concentrations for 12 weeks, which was then increased to 6 and 12 µg l^{-1} for four weeks, and finally the high treatment was increased to 30 µg l^{-1} and the low treatment channel returned to ambient. Only the first 12 week dosing regime achieved nominal diazinon levels (0.3 and 3 µg l^{-1}) as indicated by analytical measurements; the latter two dosing regimes did not reach the intended levels.

  No consistent interchannel differences were observed in total macroinvertebrate abundance or in species diversity indices. *Hyalella* was the most sensitive species, exhibiting substantially higher (3.5–7.8 times) drift rates in the 0.3 µg l^{-1} dosed channel relative to the control channel. *Hyalella* had sharply reduced population levels at diazinon concentrations as low as 5 µg l^{-1}.

  Macroinvertebrate diazinon tolerance from most tolerant to least tolerant was observed as: flatworms, physid snails, isopods and chironomids (most tolerant); leeches and the amphipod *Crangonyx* (less tolerant); and the amphipod *Hyalella*, mayflies, caddisflies and damselflies (sensitive).

Figure 2.1 Cumulative distribution function of freshwater long-term data (µg l^{-1}) for diazinon

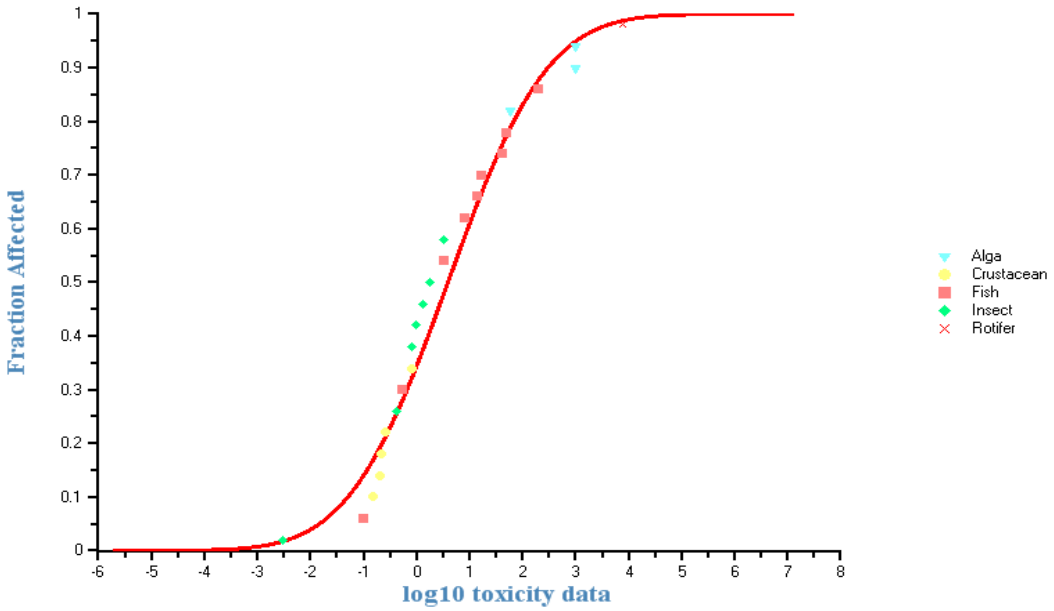


Figure 2.2 Cumulative distribution function of freshwater short-term data (µg l^{-1}) for diazinon

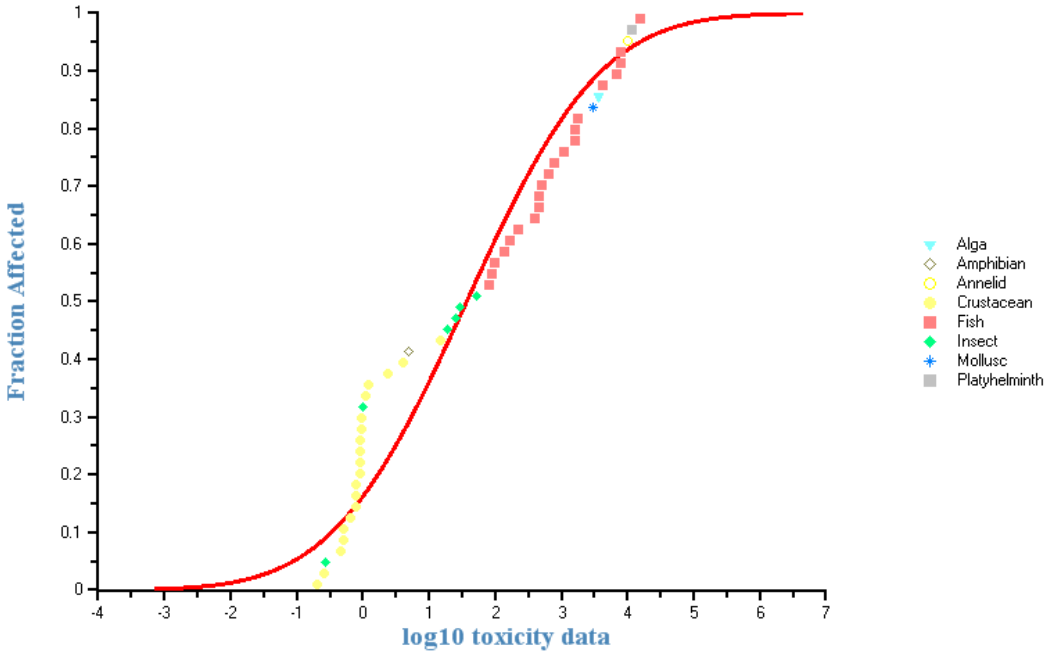


Table 2.6 Long-term aquatic toxicity data for freshwater organisms exposed to diazinon

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (µg l^{-1})[1]	Expo-sure[2]	Toxicant analysis[3]	Comments	Refer-ence
Mixed population (Chlorophyta, Chrysophyta, Cyanophyta)	Algae	ALG	No Effect	NR	14 days	1,000	s	pn	P Study not suitable for EQS setting	[41]
Scenedesmus quadricauda	Green algae	ALG	No Effect	GRO/REPR	10 days	**>1,000**	f	m	P Unbounded NOEC (the highest concentration tested), not suitable for EQS setting	[48]
Selenastrum capricornutum	Algae	ALG	NOEC	NR	7 days	**60**	-	-	-	[38]
Ceriodaphnia dubia	**Cladoceran (<6 hours old)**	**CRU**	**NOEC**	**-**	**7 days**	**0.22**	**-**	**m**	**P**	**[43]**
Daphnia magna	Water flea	CRU	NOEC (LOEC)	REPR (mean brood size)	21 days	<0.15 (0.15)	sr	pn	P; RI 2	[26]
Daphnia magna	**Water flea**	**CRU**	**NOEC (LOEC) (MATC)**	**REPR (mean number of broods, total young per female)**	**21 days**	**0.15 (0.18) (0.164)**	**sr**	**pn**	**P; RI 2**	**[27]**
Daphnia magna	Water flea	CRU	NOEC (LOEC)	REPR (days to 1st reproduction)	21 days	0.22 (0.25)	s	pn	P; RI 2	[27]
Daphnia magna	Water flea	CRU	NOEC	NR	21 days	0.26	-	-	-	[38]
Daphnia magna	Water flea	CRU	NOEC	NR	chronic	0.83	-	-	-	[38]
Gammarus pseudolimneaus	**Scud**	**CRU**	**NOEC (LC50)**	**NR**	**30 days**	**0.2 (0.27)**	**NR**	**NR**	**P**	**[6]**
Brachydanio rerio	Zebra fish	FIS	NOEC	PHY/GRO	8 weeks	**40**	f	m	P ELS	[20]
Jordanella floridae	Flagfish	FIS	MATC	NR	60 days	54	-	-	-	[13]
Jordanella floridae	Flagfish	FIS	NOEC	REPR	120 days	**<14**	f	m	P Life-cycle test	[14]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (µg l^{-1})[1]	Expo-sure[2]	Toxicant analysis[3]	Comments	Refer-ence
Oncorhynchus mykiss	Rainbow trout	FIS	NOEC	GRO	28 days	**>200**	f	m	P Unbounded NOEC (highest concentration tested)	[20]
Pimephales promelas	Fathead minnow	FIS	NOEC	DEV Hatching success of eggs	up to 274 days	**<3.2**	f	m	P; RI 1 Life-cycle test Unbounded NOEC (i.e. lowest concentration tested)	[15]
Pimephales promelas	Fathead minnow	FIS	NOEC	DEV Deformation of spinal cord	167 days	<3.2	f	m	P; RI 1 Life-cycle test Unbounded NOEC (i.e. lowest concentration tested)	[15]
Pimephales promelas	Fathead minnow	FIS	LOEC	NR	sub-chronic (NR)	3.2	-	-	-	[38]
Pimephales promelas	Fathead minnow	FIS	NOEC	NR	sub-chronic (NR)	8	-	-	-	[38]
Pimephales promelas	Fathead Minnow	FIS	NOEC (LOEC) (MATC)	GRO	32 days	16.5 (37.8) (25)	f	m	ELS	[44]
Pimephales promelas	Fathead minnow	FIS	NOEC	MOR	167–274 days	28	f	m	P; RI 1 Life-cycle test	[15]
Pimephales promelas	Fathead minnow	FIS	NOEC (LOEC)	NR	sub-chronic (NR)	50 (90)	-	-	Probably reports results by Jaravinen and Tanner [33]	[38]
Pimephales promelas	Fathead minnow (embryo-larva)	FIS	NOEC (LOEC) (MATC)	GRO	32 days	50 (90) (67)	f	m	P ELS	[33]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (µg l^{-1})[1]	Expo-sure[2]	Toxicant analysis[3]	Comments	Refer-ence
Salmo salar	**Atlantic salmon**	**FIS**	**NOEC**	**PHY** **1. Sensitivity of the olfactory system** 2. Priming effect of female urine on steroid levels in males 3. Effect of female urine on male expressible milt	**4–5 hours**	**0.1** <0.3 <0.3	**f**	**m**	**P; RI 1**	**[39]**
Salvelinus fontinalis	**Brook trout (age 16 months at test start); partial life-cycle**	**FIS**	**NOEC**	**DEV** **Growth and weight of progeny of exposed parents**	**parents 173 days; progeny 122 days**	**<0.55**	**f**	**m**	**P; RI 2** **0.55 µg l^{-1} is referring to exposure of parents. Unbounded (i.e. lowest concentration tested)**	**[15]**
Salvelinus fontinalis	Brook trout (age 16 months at test start)	FIS	NOEC	DEV deformation of spinal cord	173 days	2.4	f	m	P; RI 1	[15]
Salvelinus fontinalis	Brook trout (age 16 months at test start)	FIS	NOEC	MOR	91 days 173 days	2.4 >9.6	f	m	P; RI 1 9.6 µg l^{-1} was highest concentration tested	[15]
Acroneuria lycorias	Stonefly	INS	NOEC (LC50)	NR MOR	30 days	**0.83** (1.25)	NR	NR	P	[6]
Chironomus tentans	**Chironomid**	**INS**	**LOEC**	**DEV/GDVP**	**7 days**	**0.003**	**s**	**NR**	**P; RI 4** **Significant delay in egg hatch, increased duration of the larvae stage, slightly depressed pupation and emergence of adults, and lengthened time from eggs to adults by 33.6%.**	**[40]**
Ephemerelia subvaria	**Mayfly**	**INS**	**NOEC (LC50)**	**NR** **MOR**	**30 days**	**0.42 (1.05)**	**NR**	**NR**	**P**	**[6]**

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (µg l^{-1})[1]	Expo-sure[2]	Toxicant analysis[3]	Comments	Refer-ence
Hydropsyche angustipennis	Caddisfly	INS	LC50	MOR	168 hours	1	s	m	P	[50]
Hydropsyche bettoni	Caddisfly	INS	NOEC (LC50)	NR MOR	30 days	**1.79** (3.54)	NR	NR	P	[6]
Ophiogomphus rupinsulensis	Dragonfly	INS	NOEC	NR	30 days	**1.29**	NR	NR	P	[6]
Pteronarcys dorsata	Stonefly	INS	NOEC	NR	30 days	**3.29**	NR	NR	P	[6]
Brachionus calyciflorus	Rotifer	ROT	NOEC (LOEC)	REPR	2 days	**8000** (13000)	s	NR	P	[47]
Freshwater community	**Mesocosm**	**-**	**NOEC**	**Abundance of cladocerans and some insect taxa**	**70 days**	**<2.4**	**s**	**m**	**P; RI 2**	**[29]**
Stream Community	**Outdoor channel system**	**-**	**-**	**Drift of macro-invertebrates**	**3 weeks**	**0.3 5**	**f**	**m**	**P; RI 2 3.5–7.8 times higher drift than in control. Sharp reduction of *Hyalella azteca* population.**	**[17]**

[1] The lowest NOECs per group are highlighted in bold font.
[2] Exposure: s = static; f = flow-through; sr = static renewal.
[3] Toxicant analysis: m = measured; pn = presumably nominal.
ALG = algae; CRU = crustaceans; FIS = fish; INS = insects; ROT = rotifers
DEV = development; GRO = growth; GDVP = general developmental changes; MOR = mortality; PHY = physiology; REPR = reproduction
ELS = early life stage
LOEC = lowest observed effect concentration
NOEC = no observed effect concentration
MATC = maximum allowable toxicant concentration
LC50 = concentration lethal to 50% of the organisms tested
NR = not reported
P = published data
RI = reliability index (see Annex 1)

Table 2.7 Short-term aquatic toxicity data for freshwater organisms exposed to diazinon

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (µg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments	Reference
Selenastrum capricornutum	Green algae	ALG	EC50	POP/ABND	7 days	**3,700**	-	-	-	[45]
Rana clamitans	Frog (stage 8)	AMP	LC50	MOR	96 hours	**>50**	s	m	P	[32]
Rana clamitans	Frog (stage 8)	AMP	LC50	MOR	16 days	5	s	m	P	[32]
Rana clamitans	Frog (stage 8)	AMP	EC50	DEV/deformities	16 days	14	s	m	P	[32]
Lumbricus variegatus	Oligochaete worm	ANE	L(E)C50	-		**9,980**	-	-	-	[46]
Ceriodaphnia dubia	Water flea	CRU	LC50	MOR	48 hours	0.26	s	m	P; RI 2 ***C. dubia*** **GM 0.49 µg l^{-1}**	[18]
Ceriodaphnia dubia	Water flea	CRU	LC50	MOR	96 hours	0.32	s	m	P	[18]
Ceriodaphnia dubia	Water flea	CRU	LC50	MOR	24 hours	0.37	s	m	P	[18]
Ceriodaphnia dubia	Water flea	CRU	LC50	MOR	48 hours	0.5	s	n	P; RI 2	[16]
Ceriodaphnia dubia	Water flea	CRU	LC50	MOR	48 hours	0.92	s	m	P; RI 2	[21]
Ceriodaphnia dubia	Water flea	CRU	NOEC	MOR	48 hours	0.6	s	m	P	[21]
Ceriodaphnia dubia	Water flea	CRU	LOEC	MOR	48 hours	0.8	s	m	P	[21]
Daphnia magna	Water flea	CRU	50% decrease	PHY/filtration rate	5 hours	0.47	s	pn	P	[27]
Daphnia magna	Water flea	CRU	50% decrease	PHY/ingestion rate	5 hours	0.6	s	pn	P	[27]
Daphnia magna	Water flea	CRU	NOEC	-	48 hours	0.56	-	-	-	[38]
Daphnia magna	Water flea	CRU	NOEC	MOR	48 hours	0.8	s	m	P	[21]
Daphnia magna	Water flea	CRU	LOEC	MOR	48 hours	1.5	s	m	P	[21]
Daphnia magna	Water flea	CRU	EC50	ITX/IMBL	48 hours	0.5	-	-	***D. magna*** **GM 1.03 µg l^{-1}**	[45]
Daphnia magna	Water flea	CRU	EC50	NR	48 hours	0.8	-	-	-	[31]
Daphnia magna	Water flea	CRU	EC50	ITX/IMBL	48 hours	0.96	-	-	-	[45]
Daphnia magna	Water flea	CRU	EC50	-	48 hours	0.96	-	-	-	[38]
Daphnia magna	Water flea	CRU	EC50	ITX/IMBL	48 hours	1.1	-	-	-	[45]
Daphnia magna	Water flea	CRU	EC50	IMBL	48 hours	1.22	s	pn	P	[25]
Daphnia magna	Water flea	CRU	EC50	IMBL	48 hours	1.25	s	pn	P	[25]
Daphnia magna	Water flea	CRU	LC50	MOR	48 hours	2.39	s	m	P	[21]
Daphnia magna	Water flea	CRU	LC50	MOR	48 hours	0.8	s	n	P	[16]
Daphnia magna	Water flea	CRU	LC50	MOR	24 hours	0.9	s	pn	P	[27]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (µg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments	Reference
Daphnia pulex	Water flea	CRU	LC50	MOR	48 hours	0.65	s	n	P ***D. pulex*** **GM 0.78 µg l^{-1}**	[16]
Daphnia pulex	Water flea	CRU	EC50	NR	48 hours	0.8	s	NR	P	[37]
Daphnia pulex	Water flea	CRU	EC50	NR	48 hours	0.9	-	-	-	[38]
Daphnia sp.	Water flea	CRU	LC50	MOR	48 hours	**0.9**	-	-	-	[51]
Gammarus fasciatus	Scud	CRU	LC50	MOR	96 hours	**0.2**	s	m	P	[37]
Hyalella azteca	Scud	CRU	LC50	-	96 hours	**4**	s	pn	P	[23]
Hyalella azteca	Scud	CRU	LC50	MOR	48 hours	15.07	s	m	P	[21]
Hyalella azteca	Scud	CRU	LOEC	MOR	48 hours	11	s	m	P	[21]
Hyalella azteca	Scud	CRU	NOEC	MOR	48 hours	7.5	s	m	P	[21]
Anguilla anguilla	Eel	FIS	LC50	-	96 hours	**80**	ps	pn	P	[28]
Jordanella floridae	Flagfish	FIS	LC50	MOR	96 hours	**1,600**	f	m	P	[15]
Lepomis macrochirus	Bluegill	FIS	LC50	MOR	96 hours	100	-	-	***L. macrochirus*** **GM 221 µg l^{-1}**	[45]
Lepomis macrochirus	Bluegill	FIS	LC50	MOR	96 hours	136	-	-	-	[45]
Lepomis macrochirus	Bluegill	FIS	LC50	MOR	96 hours	168	s	NR	P	[37]
Lepomis macrochirus	Bluegill	FIS	LC50	MOR	96 hours	220	-	-	-	[45]
Lepomis macrochirus	Bluegill	FIS	LC50	MOR	96 hours	460	-	-	-	[45]
Lepomis macrochirus	Bluegill	FIS	LC50	MOR	96 hours	460	f	m	P	[15]
Lepomis macrochirus	Bluegill	FIS	LC50	MOR	96 hours	500	-	-	-	[45]
Oncorhynchus mykiss	Rainbow trout	FIS	LC50	MOR	96 hours	90	s	NR	P ***O. mykiss*** **GM 584 µg l^{-1}**	[37]
Oncorhynchus mykiss	Rainbow trout	FIS	LC50	MOR	96 hours	400	-	-	-	[45]
Oncorhynchus mykiss	Rainbow trout	FIS	LC50	MOR	96 hours	635	-	-	-	[45]
Oncorhynchus mykiss	Rainbow trout	FIS	LC50	MOR	96 hours	1,650	-	-	-	[45]
Oncorhynchus mykiss	Rainbow trout	FIS	LC50	MOR	96 hours	1800	-	-	-	[45]
Pimephales promelas	Fathead minnow (embryo-larva)	FIS	LC50	MOR	96 hours	4,300	s	m	P ***P. promelas*** **GM 6140 µg l^{-1}**	[33]
Pimephales promelas	Fathead minnow (embryo-larva)	FIS	LC50	MOR	96 hours	6,900	f	m	P	[33]
Pimephales promelas	Fathead minnow	FIS	LC50	MOR	96 hours	7,800	f	m	P	[15]
Pimephales promelas	Fathead minnow	FIS	LC50	MOR	96 hours	7800	-	-	-	[45]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (µg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments	Reference
Pimephales promelas	Fathead minnow	FIS	LC50	MOR	48 hours	15940	s	m	P	[21]
Pimephales promelas	Fathead minnow	FIS	LOEC	MOR	48 hours	12,500	s	m	P	[21]
Pimephales promelas	Fathead minnow	FIS	NOEC	MOR	48 hours	6,000	s	m	P	[21]
Poecilia reticulata	Guppy	FIS	LC50	MOR	24 hours	1,100	-	-	-	[45]
Salvelinus fontinalis	Brook trout	FIS	LC50	MOR	96 hours	**770**	f	m	P	[15]
Chironomus tentans	Chironomid	INS	LC50	MOR	7 days	0.27	s	NR	P; RI 4	[40]
Chironomus tentans	Midge	INS	EC50	BEH/SWIM	96 hours	30	s	m	P ***C. tentans*** **GM 27.9 µg l^{-1}**	[19]
Chironomus tentans	Midge	INS	EC50	BEH/SWIM	96 hours	38	s	m	P	[19]
Chironomus tentans	Midge	INS	LC50	MOR	96 hours	19.1	s	m	P	[36]
Chironomus tentans	Midge	INS	LC50	MOR	48 hours	52.47	s	m	P	[21]
Chironomus tentans	Midge	INS	NOEC	MOR	48 hours	30	s	m	P	[21]
Chironomus tentans	Midge	INS	LOEC	MOR	48 hours	37.5	s	m	P	[21]
Hydropsyche angustipennis	Caddisfly	INS	LC50	MOR	168 hours	1	s	m	P	[50]
Hydropsyche angustipennis	Caddisfly	INS	LC50	MOR	96 hours	**1.3**	s	m	P	[50]
Hydropsyche angustipennis	Caddisfly	INS	LC50	MOR	48 hours	2.9	s	m	P	[50]
Pteronarcys californica	Stonefly larvae	INS	LC50	MOR	96 hours	**25**	s	NR	P	[37]
Pomacea paludosa	Apple snail	MOL	L(E)C50	-	24 hours	2950	-	-	-	[22]
Dugesia tigrina	Planaria	PLA	L(E)C50	-	-	11,640	-	-	-	[46]

[1] The lowest L(E)C50s per group are highlighted in bold font. If more than one test per species with the same endpoint and test duration was available, geometric means (GMs) of these results were calculated. The GMs are presented in the 'Comments' column. Test results used to calculate GMs are underlined in the 'Conc.' column.

[2] Exposure: s = static; f = flow-through; ps = presumably static.

[3] Toxicant analysis: m = measured; n = nominal; pn = presumably nominal.

ALG = algae; AMP = amphibians; ANE = annelids; CRU = crustaceans; FIS = fish; INS = insects; MOL = molluscs; PLA = platyhelminthes

ABND = abandoned; BEH = behaviour; DEV = development; ITX = intoxication; IMBL = immobilisation; MOR = mortality; PHY = physiology; POP = population; REPR = reproduction; SWIM = swimming

EC50 = concentration effective against 50% of the organisms tested

LC50 = concentration lethal to 50% of the organisms tested

NOEC = no observed effect concentration

LOEC = lowest observed effect concentration

NR = not reported; P = published data

RI = reliability index (see Annex 1)

2.6.2 Toxicity to saltwater organisms

Single species test toxicity data for marine organisms are available for five different taxonomic groups, i.e. crustaceans, fish, molluscs (bivalves), annelids and echinoderms (sea urchins). Chronic toxicity data are only available for one crustacean and two fish species. Consequently, acute as well as chronic toxicity data are lacking for algae. Results of higher tier mesocosm or field studies with marine aquatic organisms are also unavailable.

Based on the limited data available, crustaceans appear to be the most sensitive taxonomic group with respect to the acute toxicity of diazinon. However, conclusions about the toxicity of other taxonomic groups cannot be drawn due to a lack of data. Results of the available long-term tests with marine crustaceans and fish are within the range of results obtained for freshwater organisms.

Diagrammatic representations of the available saltwater data (cumulative distribution functions) for diazinon are presented in Figures 2.3 and 2.4. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. These diagrams are not species sensitivity distributions and have not been used to set the diazinon PNECs. The saltwater data for diazinon are presented in Tables 2.8 and 2.9.

Figure 2.3 Cumulative distribution function of saltwater long-term data (µg l^{-1}) for diazinon

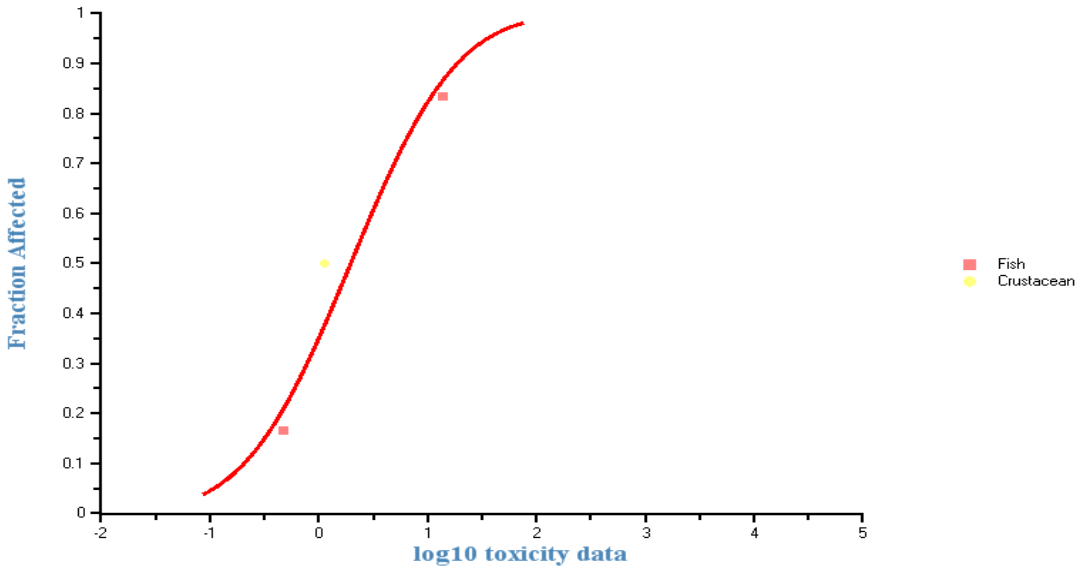


Figure 2.4 Cumulative distribution function of saltwater short-term data (µg l^{-1}) for diazinon

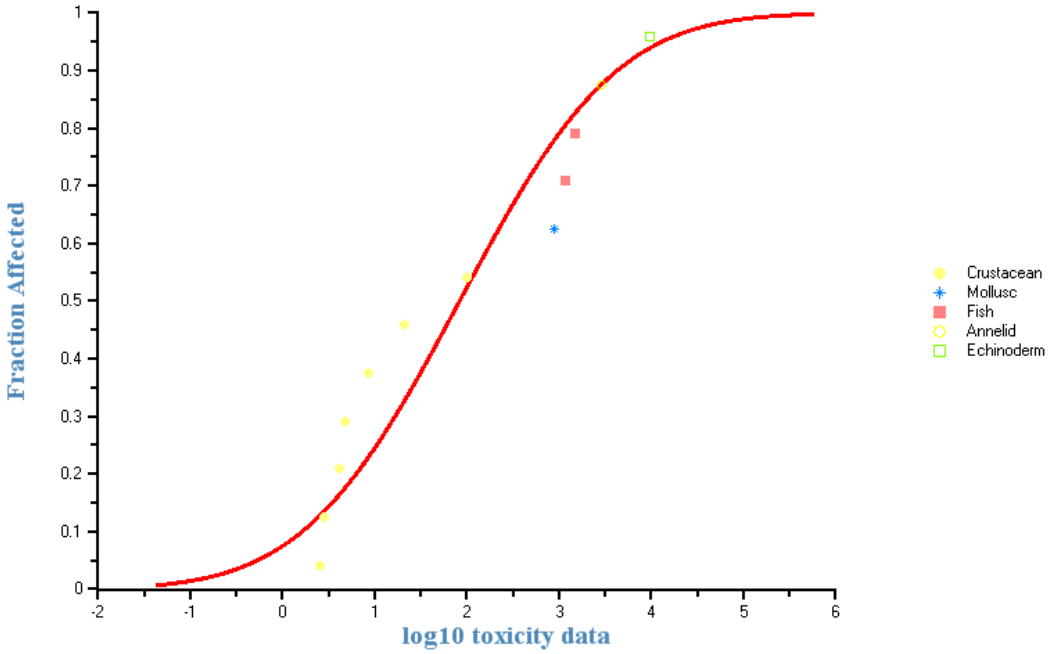


Table 2.8 Long-term aquatic toxicity data for saltwater organisms exposed to diazinon

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (µg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments	Reference
Americamysis bahia	Mysid shrimp (juvenile)	CRU	NOEC (LOEC) (MATC)	MOR/GRO/REPR	28 days	**1.15** (3.27) (1.94)	f	m	P; RI 2	[42]
Cyprinodon variegatus	Sheepshead minnow (juvenile)	FIS	NOEC LOEC	REPR/fecundity	128 days	**<0.47** **0.47**	f	m	P; RI 2	[30]
Platichthys flesus	Flounder	FIS	LOEC	NR	chronic	14	-	-	-	[38]

[1] The lowest NOECs per group are highlighted in bold font.
[2] Exposure: f = flow-through.
[3] Toxicant analysis: m = measured.
CRU = crustaceans; FIS = fish
GRO = growth; MOR = mortality; REPR = reproduction
ELS = early life stage
NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
MATC = maximum allowable toxicant concentration
NR = not reported
P = published data
RI = reliability index (see Annex 1)

Table 2.9 Short-term aquatic toxicity data for saltwater organisms exposed to diazinon

Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration	Conc. (µg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments	Refer-ence
Acartia tonsa	Copepod (adult)	CRU	LC50	MOR	96 hours	**2.57**	s	m	P; RI 2	[34]
Americamysis bahia	Mysid shrimp (juvenile)	CRU	LC50	MOR	96 hours	4.82	f	m	P; ***A. bahia*** **GM 5.56 µg l^{-1}**	[42]
Americamysis bahia (≈ Mysidopsis bahia)	Mysid shrimp	CRU	LC50	MOR	96 hours	8.5	s	m	P	[24]
Americamysis bahia	Mysid shrimp	CRU	LC50	MOR	96 hours	4.2	-	-	-	[45]
Palaemonetes pugio	Grass shrimp (larval)	CRU	L(E)C50	-	-	**2.8**	-	-	-	[49]
Penaeus duorarum	Pink shrimp (larval)	CRU	LC50	MOR	96 hours	**21**	s	m	P	[24]
Penaeus japonicus	Kurma shrimp	CRU	-	MOR	24 hours	100	s	pn	P 100% of prawns exposed to 100 µg l^{-1} diazinon(the only concentration tested) were dead after 6 hours.	[35]
Cyprinodon variegatus	Sheepshead minnow (juvenile)	FIS	LC50	MOR	96 hours	**1,470**	f	m	P; RI 2	[30]
Menidia menidia	Inland silverside (juvenile)	FIS	L(E)C50	-	-	**1,170**	-	-	-	[49]
Crassostrea virginica	American or Virginia oyster	MOL	EC50	ITX/IMBL	96 hours	**880**	-	-	-	[45]
Neanthes arenaceodentata	Annelid worm (juvenile)	ANE	L(E)C50	-	-	**>2,880**	-	-	-	[49]
Arbacia punctulata	Sea urchin (larval)	ECD	L(E)C50	-	-	**>9,600**	-	-	-	[49]

[1] The lowest L(E)C50s per group are highlighted in bold font. If more than one test per species with the same endpoint and test duration was available, geometric means (GMs) of these results were calculated. The GMs are presented in the 'Comments' column. Test results used to calculate GMs are underlined in the 'Conc.' column. [2] Exposure: s = static; f = flow-through. [3] Toxicant analysis: m = measured; pn = presumably nominal.
ANE = annelids; CRU = crustaceans; ECD = echinoderms; FIS = fish; MOL = molluscs
ITX = intoxication; IMBL = immobilisation; MOR = mortality
EC50 = concentration effective against 50% of the organisms tested; LC50 = concentration lethal to 50% of the organisms tested
P = published data
RI = reliability index (see Annex 1)

2.6.3 Toxicity to sediment-dwelling organisms

Toxicity data for diazinon concentrations in sediment (e.g. mg/kg sediment) were not found.

2.6.4 Endocrine-disrupting effects

A number of studies have been published on the effects of diazinon on the endocrine system of fish. Of particular importance are effects on reproductive steroid levels due to disruption of the olfactory function at low levels of diazinon.

Moore and Waring [39] used electrophysiological recordings to investigate the effects of short-term exposures (30 minutes) to diazinon on Atlantic salmon (*Salmo salar*) olfactory responses to prostaglandin. At a nominal concentration of 1 µg l^{-1}, olfactory responses were significantly reduced compared with controls. The effect was also dose-dependent, with a 10-fold decrease in the sensitivity of olfactory epithelium after exposure to 2 µg l^{-1} diazinon. The NOEC for this endpoint was 0.1 µg l^{-1}.

The authors then investigated the effects of diazinon on reproductive steroid production and sperm volume following priming with ovulated female salmon urine [39]. After a 120-hour exposure to diazinon, fish were exposed to female salmon urine for a further 3 hours. Fish were then anaesthetised, sperm and blood plasma collected and an analysis of sperm volume and steroid levels performed. Results from the treated individuals were compared with those of control fish (not exposed to female urine) and primed fish (exposed to female salmon urine). Concentrations as low as 0.3 and 0.8 µg l^{-1} diazinon caused a significant reduction in plasma 17,20β-dihydroxy-4-pregnen-3-one and gonadotrophin II (GtH-II) levels, respectively, compared with primed controls. Diazinon also affected plasma testosterone and 11-ketotestosterone levels. However, there was no obvious dose response for these endpoints. In addition to the steroid levels, the volume (mg/g body weight) of expressible sperm (milt) was also significantly reduced at the 0.3 µg l^{-1} level compared with primed fish.

The study demonstrates that the effect of diazinon on olfactory function in fish results in reduced levels of reproductive steroids and volume of sperm. Given the importance of odorants and pheromones in the reproduction of fish, this finding could have serious implications for long-term reproductive success in these fish. This study was well documented and of high quality. Chemical analysis of exposure concentrations (stored and tested up to 2 weeks after the test) indicate considerable degradation (up to 80 per cent) of the test concentrations. Consequently, all effects were based on nominal concentrations. However, this study would still be regarded as reliable and suitable for PNEC derivation.

Studies on the effects of diazinon on homing and anti-predator behaviour in Chinook salmon (*Oncorhynchus tshawytscha*) at low diazinon concentrations [68] support the findings of Moore and Waring [39]. In fish exposed for 2 hours to diazinon, olfactory-mediated alarm responses were significantly inhibited at a diazinon concentration of 1 µg l^{-1}. Hatchery fish exposed to 10 µg l^{-1} diazinon in a 24-hour static exposure and then released into the wild 2 km below the hatchery showed a significant impairment in homing behaviour. Only 15 per cent returned to the hatchery compared with 40 per cent of released fish in the control group.

Maxwell and Dutta [69] exposed adult bluegills (*Lepomis macrochirus*) to a single dose (60 µg l^{-1}) of diazinon for a period of 24 hours to 2 weeks, measuring the levels of estradiol in blood serum and the histopathology of ovarian tissue. Estradiol levels were significantly reduced in all exposures, although a direct relationship between exposure time and response could not be identified. The exposures to diazinon also resulted in severe damage to fish oocytes.

Bisson and Hontela [70] investigated the effects of diazinon *in vitro* on the adrenocortical cells of rainbow trout. Cortisol secretion and cell viability were measured in response to diazinon exposure. An EC50 of 233 µM (70.8 mg l^{-1}) and an LC50 of 305 µM (92 mg l^{-1}) diazinon were reported for adrenocorticotropic (ACTH) stimulated cortisol production and cell viability, respectively. The authors suggest the effects on cortisol were probably due to cytotoxicity rather than endocrine effects, as the dose responses for the two endpoints were almost identical.

2.6.5 Mode of action of diazinon

Diazinon is a contact organophosphorus insecticide with a wide range of insecticidal activity. It has been used from the early 1950s, mainly formulated as wettable powders and emulsifiable concentrates. It is also available in mixed formulations with other insecticides [2].

A primary mode of toxic action of organophosphorus insecticides is inhibition of cholinesterases present in the nervous system. The actual toxicant may be the oxygenated homologue of diazinon, diazoxon. Insect enzymes efficiently convert the P═S bond into a P═O bond, thus producing the toxic oxygen homologue. Crustaceans are likely to have a similar ability. Insects and crustaceans probably differ from vertebrates by having a less efficient de-esterification process for the removal of the oxygen homologue from their system, making them more sensitive to diazinon [7].

The fate of diazinon in the aquatic environment is thought to be regulated by two main processes: chemical hydrolysis and microbial degradation. Both processes are influenced by the pH, temperature and organic content of the water (see Section 2.5). Photodegradation of diazinon in water is unlikely under environmental conditions [10]. An important factor regulating the rate of microbial decomposition of diazinon is adaptation of microbes to the chemical [7].

Biological processes appear to be the main factor in the degradation of diazinon. In samples of pond and river water each containing 1 per cent of sediment, diazinon was degraded with a DT50 of 7–10 days in the pond system and 8–15 days in the river water (Table 2.5). Mineralization accounted for ≥60 per cent of the applied material within 7 weeks in both systems [2]. 2-Isopropyl-6-methylpyrimidin-4-ol (IMHP) was a major degradation product when low concentrations of diazinon were studied in compost, soil and water. Although IMHP is potentially leachable, it is less toxic than diazinon. Diazinon-O-analogue (or diazoxon), a much more potent inhibitor of acetylcholine esterase (AChE) than diazinon, can be produced by aqueous chlorine in wastewater treatment plants.

A Food and Agricultural Organization (FAO) expert panel concluded in 1996 that, due to lack of information, it was not possible to assess the significance of the occurrence of diazinon metabolites in water/sediment systems [9], whereas the Australian Pesticides

and Veterinary Medicines Authority (APVMA) considers diazinon as readily degradable in aquatic environments [10].

Diazinon, on prolonged storage, may become more toxic due to transformation products, e.g. sulfotepp (*S*,*S*-TEPP) and monosulfotepp (*O*,*S*-TEPP). In the presence of a small quantity of water (of the order of 0.2–2.0 per cent), diazinon decomposes to give the highly toxic degradation products *S*,*S*-TEPP and *O*,*S*-TEPP; these degradation products are 300- and 2,500-fold, respectively, more toxic than diazinon [10]. However, a screening programme conducted in 1994 throughout Australia to determine if toxic levels of breakdown products of diazinon were present in formulations revealed that, of the 169 samples evaluated, only eight contained the breakdown products *O*,*S*-TEPP and *S*,*S*-TEPP. These were directly correlated with the presence of water in the container [7].

In excess water, diazinon is hydrolysed to give nontoxic byproducts. Thus, water-based formulations containing diazinon do not represent a risk in the same way as source material and hydrocarbon-based/emulsifiable concentrate (EC) formulations [10]. Improvements in the manufacture of diazinon since 1979 have significantly reduced the content of highly toxic impurities such as tetraethyl pyrophosphate (TEPP). As a result of these progressive improvements, the acute oral LD50 of technical grade diazinon has increased, e.g. from 250 to 1,250 mg/kg in the rat [2].

In mammals, diazinon is reported to be almost completely absorbed from the intestinal tract and easily absorbed dermally. Elimination is rapid in (mainly) the urine and faeces. In mammals, metabolism was reported to progress primarily via hydrolysis of the ester linkage, yielding 4-hydroxy-2-isopropyl-6-methylpyrimidine (metabolite B1 or G-27550) followed by oxidation of the isopropyl group to give primary and tertiary alcohols, of which the latter may become conjugated. Another primary route is oxidation to diazoxon, which may be hydrolysed to metabolite B1 or oxidised at the isopropyl group before hydrolysis. Other less important routes include oxidation of the methyl group. Unchanged diazinon was not a major residue in tissues of rats, though low residues of diazinon and diazoxon were reported, especially in fat [9].

Metabolism in plants progresses, as in animals, primarily by hydrolysis of the ester linkage, yielding metabolite B1 (G-27550), followed by oxidation of the isopropyl group to primary and tertiary alcohols and/or oxidation of the methyl group to the alcohol. Diazoxon is not reported as a significant plant metabolite, though low levels have been found in mammals [9].

3. Calculation of PNECs as a basis for the derivation of quality standards

3.1 Derivation of PNECs by the TGD deterministic approach (AF method)

3.1.1 PNECs for freshwaters

PNEC accounting for the annual average concentration
Among the taxonomic groups (algae, crustaceans, fish, insects and rotifers) for which long-term toxicity test results are available, fish, crustaceans and insects are the most sensitive species. Hence, the 'base set' of toxicity data (i.e. tests with algae, crustaceans, and fish) required by the TGD [11] is available and the assessment factor method can be applied.

In comparison with other organisms, algae are of low sensitivity to diazinon with no-effect concentrations of 60 and >1,000 μg l^{-1} reported for *Selenastrum capricornutum* and *Scenedesmus quadricauda* after 7 and 10 day exposures, respectively [38, 48]. Given the mode of action of diazinon (i.e. cholinesterase inhibition), the lower sensitivity of algae is not unexpected.

The lowest concentration reported to cause significant effects in crustaceans is 3 ng l^{-1} diazinon. When reared at that concentration, the midge *Chironomus tentans* showed a significant delay in egg hatch, increased duration of the larvae stage, slightly depressed pupation and emergence of adults, and lengthened time from eggs to adults. These details are mentioned in a summary report on a 1977 PhD thesis from the University of Guelph (Canada) [40]. Unfortunately, it was not possible to obtain the original thesis to check these data. Compared with more recent studies with *C. tentans* (see Table 2.7) the 3 ng l^{-1} result may be the result of a unit error by a factor of 100 in the summary report (i.e. the reported 3 ng l^{-1} LOEC may be 0.3 μg l^{-1}). Recently obtained LC50s for *C. tentans* [19, 21, 36] are in the range 2–50 μg l^{-1} compared with the LC50 of 0.27 μg l^{-1} reported in the summary report of the PhD thesis. As the reliability of the 3 ng l^{-1} LOEC is not assignable, this data point is excluded from PNEC derivation.

The next lowest long-term test result is a NOEC of <0.15 μg l^{-1} diazinon for the reduction of mean brood size in a 21-day test with *Daphnia magna* (see Table 2.6). The NOEC for reduced number of young per female was 0.15 μg l^{-1} (LOEC 0.18 μg l^{-1}) [26]. This study is considered reliable with restrictions (see Annex 1), as it does not mention chemical analysis. Other NOECs of 21-day studies with *Daphnia magna* are only slightly higher (0.26 and 0.83 μg l^{-1}), as is the NOEC obtained for the crustacean *Ceriodaphnia dubia* (0.22 μg l^{-1}) (Table 2.6). The only long-term crustacean data point that could be located

that was based on measured diazinon concentrations was the 7-day NOEC of 0.22 µg l^{-1} *Ceriodaphnia dubia* [43].

The lowest data point for fish was a reported NOEC of 0.1 µg l^{-1} for effects on fish olfactory function in Atlantic salmon exposed to diazinon for 30 minutes [39] (see Section 2.6.4). The significance of this effect was seen when free-swimming fish experienced a significant reduction in reproductive steroid concentrations and reduced sperm volumes when exposed to 0.3 µg l^{-1} diazinon for 120 hours [39]. This was the lowest concentration tested. Although this study is based on short-term exposures, the endpoints are likely to have long-term implications for the reproduction of salmon. This was a well-documented study with measured exposure concentrations, although effects are based on nominal values. This study is considered reliable for PNEC derivation.

The next lowest effect observed in fish is the LOEC of 0.55 µg l^{-1} for significantly reduced growth of progeny of *Salvelinus fontinalis* in uncontaminated water. The parental generation was reared in water contaminated with diazinon at concentrations as low as 0.55 µg l^{-1} (the lowest concentration tested) [15]. However, this result needs to be considered with care because the effect also occurred when parental fish were reared at test concentrations up to 9.6 µg l^{-1} diazinon (highest concentration tested). No dose–response relationship was visible, i.e. the growth retardation in progeny was not statistically different when the parents were exposed to the highest or the lowest diazinon concentration tested.

The lowest fish NOECs for parameters that show a dose–response relationship were reported in the same high quality study [15]. These NOECs refer to the deformation of the spinal cord and increased mortality in *Salvelinus fontinalis* (NOEC 2.4 µg l^{-1}) and to reduced fecundity in *Pimephales promelas* (NOEC <3.2 µg l^{-1}).

The lowest reported value for crustaceans was the 21-day NOEC of 0.15 µg l^{-1} for reduced number of young per female in *Daphnia magna* [27]. However, this value was based on nominal concentrations and so it has been used in a supporting capacity only. The only long-term crustacean data point that could be located that was based on measured diazinon concentrations was the 7-day NOEC of 0.22 µg l^{-1} in *Ceriodaphnia dubia* [43].

Consequently, it is recommended that the $PNEC_{freshwater\_lt}$ is derived on the basis of the NOEC of 0.1 µg l^{-1} for effects on fish olfactory function in Atlantic salmon [39] with the standard assessment factor of 10 (which is equivalent to the long toxicity-exposure-ratio trigger of 10 used in pesticide risk assessment):

$PNEC_{freshwater\_lt}$ = 0.1 µg l^{-1}/AF (10) = 0.01 µg l^{-1} diazinon

Metabolites of diazinon are apparently not of significant environmental relevance (see Section 2.6.5).

PNEC accounting for transient concentration peaks
Short-term toxicity data are available for eight different taxonomic groups, i.e. algae, crustaceans, fish, amphibians, annelids, molluscs, insects and planarians. Crustaceans and insects are the most sensitive organisms, being one to two orders of magnitude more sensitive than fish to acute effects of diazinon.

As with long-term exposures, algae are one of the least sensitive taxa to the effects of diazinon, with a 7-day EC50 (population growth) of 3,700 µg l^{-1} reported for *Selenastrum capricornutm* [45].

The lowest short-term value available for crustaceans is a 96-hour LC50 of 0.2 µg l^{-1} for the crustacean *Gammarus fasciatus*. This value is taken from a report which compiled the results of quality-assessed acute tests conducted at the Columbia National Fisheries Research Laboratory in 1965–1984 [37], and judged acceptable (by the authors) according to good laboratory practices. Test techniques were generally those of the American Society for Testing and Materials [71] and the Committee on Methods for Toxicity Tests with Aquatic Organisms [65]. Therefore, it is deemed reasonable to consider this test result reliable.

The next lowest test result is the *Ceriodaphnia dubia* geometric mean (GM) 48-hour LC50 of 0.49 µg l^{-1}. This GM is calculated on the basis of three 48-hour LC50s of 0.26, 0.5 and 0.92 µg l^{-1}, obtained by Bailey *et al.* [18], Ankley *et al.* [16], and Burkepile *et al.* [21], respectively. These studies are of good quality with measured exposure concentrations reported for two of the three values [18, 21]. The GM EC50s of other cladoceran species such as *Daphnia magna*, *Daphnia pulex* and *Daphnia* sp. are also in the range of 0.8–1.0 µg l^{-1} (see Table 2.7).

Fish appear to be less sensitive than crustaceans, with GMs from the 96-hour LC50 values of bluegills, rainbow trout and fathead minnow calculated as 221, 584 and 6,140 µg l^{-1}, respectively (Table 2.7).

It is recommended that a PNEC to account for short-term effects is calculated from the *Gammarus fasciatus* 96-hour LC50 of 0.2 µg l^{-1} and using the guidance given in the TGD on effects assessment for intermittent releases (Section 3.3.2 of Part II of [11]). As diazinon is a pesticide with a specific mode of action (cholinesterase inhibition) and crustaceans belong to the most sensitive organisms, only a reduced assessment factor of 10 (instead of 100) is necessary in order to extrapolate from the 50 per cent acute effect level to the short-term no-effect level.

$PNEC_{freshwater\_st}$ = 0.2 µg l^{-1}/AF (10) = 0.02 µg l^{-1} diazinon

PNEC based on outdoor simulated ecosystem studies
The two available outdoor simulated ecosystem studies [17, 29] (see Section 2.6.1) cannot be used alone for the derivation of PNECs since, in both studies, effects were observed at the lowest concentrations tested (i.e. they report unbounded LOECs). However, the results of the studies can be used to check the appropriateness of PNECs derived on the basis of laboratory information.

The ecological significance of a 3.5 to 8 times increased drift rate of a species at an average concentration of 0.3 µg l^{-1} diazinon in a watercourse as observed by Arthur *et al.* [17] may be disputable. The consequences of such an event can only be assessed in relation to information on the species' population density and reproduction strategy. However, the event shows that effects in complex natural systems may be seen at very low concentrations.

In the study by Giddings *et al.* [29], the lowest tested concentration of 2.4 µg l^{-1} diazinon caused a severe and long-lasting reduction in different cladoceran species, a group in the community structure of eutrophic lakes. In the control microcosms, the cladoceran densities fluctuated between 20 and 1,000 individuals per litre, but these animals were often absent in samples from the treated microcosms. When they were present, their densities were typically below 10 individuals per litre [29].

Based on the available field data the proposed long-term PNEC for freshwaters (0.01 µg l^{-1}) should be protective of effects in the field.

3.1.2 PNECs for saltwaters

The effects database for marine species is very small (three long-term tests for fish and crustaceans and 12 short-term toxicity tests with five crustaceans, two fish and one each with annelid, mollusc and echinoderm species. The toxicity data for marine taxa do not obviously differ from the range of values for freshwater organisms (see Tables 2.6–2.9). However, the marine database is too small to draw firm conclusions on possible differences. The sensitivity of marine crustaceans towards the effects of diazinon seems to be approximately a factor of 10 lower than the sensitivity of freshwater crustaceans. However, copepods are not adequately represented in the marine database (there is only one short-term test with *Acartia tonsa*) and, in the marine environment, these organisms occupy a similar ecological niche and function to cladocerans in freshwaters. In freshwaters, cladocerans (*Daphnia, Ceriodaphnia*, etc.) are the most sensitive organisms to diazinon.

As there are no obvious differences in the sensitivity of freshwater or saltwater species from the same taxonomic groups, freshwater data can be used for the marine effect assessment. Because diazinon acts by a specific mode of action (i.e. inhibition of cholinesterase activity), it is unlikely that marine taxonomic groups are significantly more sensitive towards diazinon than crustaceans. However, there may be marine crustacean species that are as sensitive towards diazinon as the most sensitive freshwater species.

PNEC accounting for the annual average concentration

Long-term single species toxicity data for marine organisms are only available for a crustacean species and two fish species (Table 2.8). The short-term data do not include effect data for marine algal species. However, the assessment factor method, according to the TGD provisions on marine effects assessment [11], can be applied as the required minimum data set of at least short-term toxicity data for algae, crustaceans and fish (the so-called ‘base set’ of primary producer, primary consumer and secondary consumer organisms) is available when the freshwater database is also taken into account. The information on algae in the freshwater database (Tables 2.6 and 2.7) shows that this group is not particularly sensitive. This is not surprising because diazinon acts specifically by inhibition of cholinesterase activity in the nervous system, a target lacking in plants. Therefore, it can be assumed that plants are not relevant for the environmental assessment of diazinon.

The second relevant study is a life-cycle study with the marine crustacean *Americamysis bahia* with a NOEC of 1.15 µg l^{-1} [42]. This study is of high quality and considered valid.

The lowest NOEC available in the saltwater database is unbounded (<0.47 µg l^{-1}, the lowest concentration tested) and refers to reduced fecundity of *Cyprinodon variegatus* in

a partial life-cycle study (LOEC = 69 per cent of egg production per female at 0.47 µg l^{-1} diazinon compared with the control) [30]. This study is considered of high quality and valid for PNEC derivation. The only other long-term data point available for saltwater fish was a chronic LOEC of 14 µg l^{-1} reported in flounder (*Platichthys flesus*) [38]. The limited data indicate that fish are of lower sensitivity than invertebrates to diazinon.

The lowest available long-term data point for saltwater organisms was the *Cyprinodon variegatus* NOEC of <0.47 µg l^{-1}. In freshwater fish, however, a NOEC of 0.1 µg l^{-1} for effects on fish olfactory function in Atlantic salmon was reported [39]. This value would normally be divided by an assessment factor of 100 according to the TGD provisions for marine effects assessment, which are applicable when three long-term tests for freshwater or saltwater species representing three trophic levels are available. However, this standard assessment factor can be reduced to 10 if short-term tests on marine species (e.g. molluscs and echinoderms) are available and the studies indicate that these species do not belong to the most sensitive group. It would also be determined, with high probability, that long-term NOECs generated for these marine groups would not be lower than those already obtained.

Short-term tests with marine molluscs, annelids and echinoderms indicate that these species belong to the least sensitive groups (see Table 2.9). It seems unlikely that long-term tests with these organisms could result in lower chronic toxicity data than obtained for crustaceans and fish. Consequently, it is recommended that the $PNEC_{saltwater\_lt}$ is derived on the basis of the NOEC of 0.1 µg l^{-1} for effects on fish olfactory function in Atlantic salmon [39] with an assessment factor of 10:

$PNEC_{saltwater\_lt}$ = 0.1 µg l^{-1}/AF (10) = 0.01 µg l^{-1} diazinon

PNEC accounting for transient concentration peaks
Short-term toxicity data are available for five different marine taxonomic groups, i.e. crustaceans, fish, annelids, molluscs and echinoderms. Crustaceans are by far the most sensitive group, being one to two orders of magnitude more sensitive to diazinon than fish, annelids and echinoderms.

No short-term saltwater algal data could be located.

The lowest acute value available for a marine invertebrate species is the 96-hour LC50 of 2.57 µg l^{-1} for the crustacean *Acartia tonsa*. This value is taken from a report of good quality [34] and is considered valid for PNEC derivation. The next lowest LC50s are 2.8 µg l^{-1} for the shrimp *Palaemonetes pugio* and the geometric mean LC50 of 5.56 µg l^{-1} for the shrimp species *Americamysis bahia* (Table 2.9). This value is generated from two good quality studies with measured exposure concentrations.

Fish appear to be less sensitive than invertebrates. The lowest good quality short-term data point was a 96-hour LC50 of 1470 µg l^{-1} in sheepshead minnow (*Cyprinodon variegates*) [30].

The TGD does not provide specific guidance for assessment of acute effects of intermittent releases to marine water bodies. Therefore, a PNEC for short-term effects can be calculated on the basis of general guidance given in the TGD on effects assessment for intermittent releases (Section 3.3.2 of Part II of [11]). Diazinon is a

pesticide with a specific mode of action (cholinesterase inhibition) and crustaceans belong to the most sensitive group of organisms. Therefore, use of the *Acartia tonsa* 96-hour LC50 of 2.57 µg l^{-1} with a reduced assessment factor of 10 (instead of 100) is recommended in order to extrapolate from the 50 per cent acute effect level to the short-term no-effect level.

$PNEC_{saltwater\_st}$ = 2.57 µg l^{-1}/AF (10) = 0.26 µg l^{-1} diazinon

3.2 Derivation of PNECs by the TGD probabilistic approach (SSD method)

The minimum number of long-term toxicity data (at least 10 NOECs from eight taxonomic groups) is not available. Therefore, the SSD approach cannot be used for PNEC derivation.

3.3 Derivation of existing EQSs

The 2000 report [60] on proposed EQSs for organophosphate sheep dip chemicals (chlorfenvinphos, coumaphos, diazinon, fenchlorphos and propetamphos) in water reviewed additional critical data and information that had become available since the previous report in 1993 [61]. The 1993 report also covered flumethrin.

For the long-term protection of the most sensitive freshwater species, the lowest chronic NOECs were in the range 150–200 ng l^{-1}. Because field data also indicated that adverse effects could occur at concentrations in excess of 50 ng l^{-1}, an assessment factor of 5 was applied to the lowest chronic NOEC value of 0.15 µg l^{-1} obtained for *Daphnia magna* following 21 days exposure to diazinon. This resulted in an EQS of 30 ng l^{-1} expressed as an annual average concentration.

The short-term freshwater standard was also based on invertebrate data, with *Daphnia* and *Gammarus* sp. being the most sensitive species. Most of the laboratory acute LC50 values for these two species were around 1 µg l^{-1}, but the lowest value reported was a 96-hour LC50 of 0.2 µg l^{-1} for *Gammarus faciatus*. Taking into account both of these values, an EQS of 100 ng l^{-1} expressed as a maximum allowable concentration was proposed. This was derived by applying an assessment factor of 10 to the ‘general‘ LC50 value of 1 µg l^{-1}, or by using a lower factor of 2 applied to the LC50 of 0.2 µg l^{-1} for *Gammarus faciatus*.

Data for saltwater invertebrates did not indicate that they were more sensitive than the freshwater organisms. Marine data for fish did show two species to be more sensitive than any freshwater fish; however, the effects concentrations reported were still well within the acute toxicity range found for freshwater invertebrates. Due to limited saltwater toxicity data and because the freshwater dataset was extensive and supported by field data, the EQSs derived for the protection of freshwater life were proposed as tentative values for the protection of saltwater organisms until further data were available.

The organophosphate chemicals considered in the report were found to share a common toxic action and the combined effects of diazinon, chlorfenvinphos and propetamphos

were reported to be additive. Therefore, the proposed standards were applied as 'total' organophosphorus concentrations, i.e. when one or more of the five organophosphorus compounds were detected in a watercourse, the total concentration should not exceed those standards specified above.

3.4 Derivation of PNECs for sediment

3.4.1 PNEC derivation by the TGD deterministic approach

Because the log Kow of diazinon is >3 (Table 2.5), the derivation of PNECs for the protection of benthic organisms is required. However, experimental toxicity data for diazinon in sediment are not available. Consequently, no sediment PNEC can currently be derived.

3.4.2 PNEC derivation by the TGD probabilistic approach

No experimental sediment toxicity data are available. Consequently, the SSD approach cannot be used for $PNEC_{sediment}$ derivation.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

The acute oral, dermal and inhalation toxicity of diazinon to mammals is low (acute oral LD50 1,250 mg/kg body weight in the rat [2]), but diazinon toxicity to birds ranges from moderate to highly toxic (1.63 mg/kg for mallard duck and 85 mg/kg for brown headed cowbird [10]).

Short-term and long-term studies in mice, rats, rabbits, dogs and monkeys have shown that the only effect of concern is dose-related inhibition of acetyl cholinesterase activity [2]. Reproductive and developmental studies have revealed no evidence of embryotoxic or teratogenic potential. There was no effect on reproductive performance at dose levels that were nontoxic to the parent animals. Mutagenicity studies with various endpoints *in vivo* and *in vitro* gave no evidence of a mutagenic potential. There is no evidence of carcinogenicity in rats or mice. Diazinon does not cause delayed neuropathy in hens. In the dog and guinea-pig, diazinon has been reported to cause acute pancreatitis, but this is considered to be a species-specific effect [2].

The following mammalian no observed adverse effect levels (NOAELs) were established [2]:

Rat (two-year feeding study):	0.06 mg/kg body weight per day	≈ 1.5 mg/kg diet
Dog (one-year feeding study):	0.015 mg/kg body weight per day	≈ 0.5 mg/kg diet
Rhesus monkey (two-year study):	0.5 mg/kg body weight per day	
Human volunteers (36-day study):	0.025 mg/kg body weight per day	

With regard to avian toxicity, dietary LC50s are reported to range from 32 to 1,450 mg/kg [10]. Two reliable studies gave LC50s of 32 mg/kg for mallard duck and 38 ppm for brown headed cowbird. In chronic reproduction studies over 20 weeks, the NOEC levels

were 8.3 mg/kg for mallard and 32 ppm for bobwhite quail. For the bobwhite, the NOEC is the highest concentration tested while, for the mallard, the maximum acceptable toxicant concentration (MATC) was 8.3–16.3 mg/kg and the LOEC 16.3 mg/kg. Endpoints were related to reduced number of hatchlings and survivors after 14 days.

Table 3.1 Mammalian and avian oral toxicity data for the assessment of non-compartment specific effects relevant for the food chain (secondary poisoning)

Study and result	Details
Long-term toxicity to mammals	
Kirchner 1991 [72] Mann 1993 [73] Cited in WHO 1998 [2] **Rat, NOAEL 0.06 mg/kg bw/day = ≈ 1.5 mg/kg diet**	Rat (Sprague-Dawley), dietary exposure at 0, 0.1, 1.5, 125 and 250 mg/kg for 99 weeks. Serum cholinesterase activities were reduced at ≥1.5 mg/kg diet. Red blood cell and brain cholinesterase activities were inhibited in groups fed on a diet containing 125 or 250 mg/kg. No treatment-related changes were seen during pathology or histology examination [72]. As the dietary concentration of 1.5 mg diazinon/kg (equivalent to a mean intake of 0.06 mg/kg bw per day) inhibited serum cholinesterase only, this dose level was considered to be the NOAEL [73].
Rudzki *et al.* 1991 [74] Cited in WHO 1998 [2] **Dog, NOAEL 0.015 mg/kg bw/day = ≈ 0.5 mg/kg diet**	Beagle dogs received diazinon for 52 weeks at dietary concentrations of 0, 0.1, 0.5, 150 and 300 mg/kg. Significantly decreased cholinesterase activities were noted at ≥0.5 mg/kg diet. Serum cholinesterase activity was inhibited at 150 mg/kg diet at all sampling intervals and on several occasions at 0.5 mg/kg diet. Red blood cell and brain cholinesterase activities were reduced at ≥150 mg/kg diet. In addition, a slight reduction in the mean serum amylase activity was noted in both sexes fed a diet of ≥150 mg/kg. The dietary diazinon concentration of 0.5 mg/kg (equivalent to a mean daily diazinon intake of 0.015 mg/kg) inhibited serum cholinesterase activity only, and was considered to be the NOAEL based on inhibition of brain and erythrocyte cholinesterase.

Study and result	Details
Cockrell *et al.* 1966 [75] Cited in WHO 1998 [2] **Rhesus monkey,** **NOAEL = 0.5 mg/kg bw/day**	Rhesus monkeys received initial daily doses of 0, 0.1, 1.0 and 10.0 mg diazinon/kg bw administered by gastric intubation. After 34 days, the doses were lowered to 0.05, 0.5 and 5.0 mg/kg and, after 106 weeks of treatment, the study was terminated. The daily dose of 0.5 mg diazinon/kg inhibited plasma and (occasionally) erythrocyte cholinesterase activity. The toxicologically relevant brain cholinesterase activity remained unaffected. Therefore, this dose level was considered to be the NOAEL, based on inhibition of erythrocyte cholinesterase.
Effects on Reproduction of mammals	
Giknis 1989 [76] Cited in WHO 1998 [2] **Rat (parents & pups)** **NOAEL = 10 mg/kg diet**	Diazinon (94.9% pure) was administered in the feed to groups of 30 male and 30 female Sprague-Dawley rats for 10 weeks prior to mating, throughout mating of the F_0 animals, and during two generations up to weaning and sacrifice of the F_2 pups. The dietary concentrations used were 0, 10, 100 and 500 mg/kg. The body weight increase was reduced at 500 mg/kg in the F_0 females, and at 100 and 500 mg/kg for the F_1 animals of both sexes. Decreases in pup survival and corresponding decreases in pup weight were observed in both generations at 500 mg/kg and in the F_1 pups at 100 mg/kg. The NOAEL was 10 mg/kg diet for pups and parental animals.
Embryotoxicity and teratogenicity	
Fritz 1974 [77] Cited in WHO 1998 [2] **Rat, NOAEL = 50 mg/kg bw/day**	Diazinon was administered orally by gavage to groups of 28–30 pregnant Sprague-Dawley-derived rats on days 6–15 of gestation at dose levels of 0, 15, 50 and 100 mg/kg body weight. On day 21 of gestation, all dams were killed and the foetuses delivered by caesarean section. The dams of the 100 mg/kg group reacted to the treatment with a marked decrease in food consumption and a body weight loss in the early administration phase. The dams of the 15 and 50 mg/kg group showed no reaction. The parameters of reproduction showed no treatment-related intergroup differences. The foetal body weights were similar in all groups and the examination of the offspring did not reveal any teratogenic effects of the treatment. The NOAEL was 50 mg/kg bw.

Study and result	Details
Hoberman *et al.* 1979 [78] Cited in WHO 1998 [2]	Repeated administration of diazinon (40, 50 or 60 mg/kg bw/day) to rats on days 7–19 of gestation reduced the growth of the dams but had no effect on the number of resorptions or corpora lutea, on litter size, or on foetal weight. The cholinesterase activity of the foetal brain was reduced. A dosage of 75 mg/kg bw/day was fatal to dams in 4–5 days.
Effects on reproduction of birds	
Marselas *et al.* 1989 [79] Marselas *et al.* 1989 [80] Cited in WHO 1998 [2] Mallard duck Bobwhite Quail	A laboratory reproduction study was conducted with mallard in which birds were allowed to build their own nests and incubate eggs [79]. Birds were fed diets containing 0, 5, 10 and 20 mg diazinon/kg diet (20 pairs per dose level). The exposure to 5 and 10 mg/kg did not result in any overt signs of toxicity or effects on reproductive performance. At 20 mg/kg, there was an increase in the number of hens that did not incubate and, although egg production was not affected, there was some reduction in the number of hatchlings and 14-day-old survivors. A companion study [80] with bobwhite quail was conducted at dietary concentrations of 10, 20 and 40 mg/kg. Exposures did not result in effects on reproductive performance and no mortalities or overt signs of toxicity were observed.

3.5.2 PNECs for secondary poisoning of predators

The BCF of diazinon frequently appears to remain below 100. However, some reported whole body BCF values in fish are higher (reported up to 274; see Section 2.5). Hence, the trigger of BCF >100 is met and the derivation of PNECs for secondary poisoning (secpois) of predators is required.

The reported oral NOAELs for chronic effects are much lower for mammals than for birds. The lowest dietary NOEC, based on the lowest mammalian NOAEL for inhibition of brain AChE in a one year study with dogs is 0.5 mg diazinon/kg diet (see Table 3.1).

The appropriate assessment factor to derive a PNEC based on a chronic $NOEC_{food}$ from a mammalian study is 30 (Table 23 of [11]).

$PNEC_{secpois\_biota}$ = $NOEC_{food}$ (0.5 mg/kg)/AF 30 = 16.7 µg/kg prey (wet weight)

Reported BCF values for fish are frequently below 100 but range up to 274. For other organisms such as crustaceans and molluscs, the few available data indicate BCF values below 10. Information on biomagnification of diazinon is not available, but due to the normally rapid metabolisation of the compound the occurrence of this effect is unlikely.

The corresponding safe concentration in water (preventing bioaccumulation in prey to levels >$PNEC_{secpois\_biota}$) can, therefore, be calculated as follows:

$PNEC_{secpois\_water} = PNEC_{secpois\_biota}/BCF$

If the highest reported BCF of 274 is used for the calculation, this would result in a corresponding water concentration of:

$PNEC_{secpois\_water} = 16.7/274 = 0.06$ µg l^{-1} diazinon

This concentration is higher than the proposed long-term PNECs for the protection of pelagic communities in both inland and marine water bodies. Therefore, if quality standards are set on the basis of these PNECs, the protection of predators from secondary poisoning will be included and the derivation of additional quality standards for secondary poisoning is unnecessary.

4. Analysis and monitoring

Numerous methods for the determination of diazinon in environmental media have been published by the US EPA.

Methods 622, 614, and 1657 and preparation Methods 3510/3520 in conjunction with analytical Method 8140 [54–59] can be used for surface water and industrial and municipal wastewaters. All of the methods employ liquid–liquid extraction, extract volume reduction, and gas chromatography (GC) in conjunction with selective detection, e.g. flame photometric detection (FPD), thermionic detection or mass spectrometry (MS).

Reported limits of detection (LODs) range from a high of approximately 6 µg l^{-1} (Method 8140 applied to water) down to 12 ng l^{-1} (Method 614) [54, 58]. In most cases, recovery depends upon the particular matrix. Methods are also available for soils, sludges, sediments, and solid wastes. Sample preparation typically involves liquid–liquid extraction in a separating funnel, Soxhlet extractor or with sonication.

More complex samples (some waters and most soils, sediments, sludges or solid wastes) need to be subjected to a clean-up method before analysis. The use of Florisil®, gel permeation chromatography (GPC) and solid-phase extraction (SPE) are common approaches. Diazinon is determined by GC/FPD [54, 59]. Although not specific for diazinon, some general interferences are noted in the US EPA methods.

A range of non-standard methods is available to determine diazinon in water samples. Methods typically employ some form of liquid–liquid extraction or the use of SPE (usually C18-silica) for isolation of diazinon residues. Detection methods vary from high performance liquid chromatography/ultra violet (HPLC-UV) absorbance detection with an LOD of 0.5 µg l^{-1} [67] to using GC in conjunction with chemical ionisation ion trap MS that gives a LOD of 0.5 ng l^{-1} in water [68]. Preconcentration of diazinon from drinking water onto CI8-silica or polystyrene–divinylbenzene co-polymer with a subsequent backflush onto an HPLC column (UV detection) provided a LOD of 30 µg l^{-1} [53]. Improved LODs may be achieved by preconcentrating diazinon from drinking and river water onto C18-SPE disks and eluting the adsorbed compounds directly into a GC pre-column. Detection by nitrogen phosphorus detection (NPD) gives a LOD of 20 pg l^{-1} [64].

The determination of diazinon in environmental matrices other than water generally involves:

- homogenisation with an organic solvent (polar or nonpolar);
- isolation of the residues from this initial extract;
- usually some additional clean-up prior to the analysis of the extract by GC.

Detection limits on such methods are reported to be of the order of 2 µg kg^{-1} for soils and sediments. The most common non-MS modes of detection exploit the presence of phosphorus or sulphur (FPD), or phosphorus or nitrogen (NPD). Standardised methods are available from the *Official Methods of Analysis of the Association of Official Analytical Chemists* [52], which are based on the extraction of crops with ethyl acetate and co-

distillation clean-up prior to GC/thermionic detection (Method 968.24). The use of a Florisil column chromatography clean-up followed by GC/FPD (Method 970.53) and extraction of the sample with acetonitrile is also possible. The residue is then partitioned into petroleum ether followed by Florisil clean-up and GC/KCl thermionic detection (Method 970.52). Detection is based on combinations of gas, thin-layer and paper chromatography. Several methods employ the homogenisation of the plant material with aqueous acetonitrile [63, 66] or other polar organic solvents such as acetone/methanol mixtures [62]. Reported LODs for diazinon using such methods are typically 10–50 $\mu g\ kg^{-1}$.

For water, proposed PNECs derived for diazinon range from 10 to 260 ng l^{-1}. To provide adequate precision and accuracy, the data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it can be seen from the literature that analytical methodologies (non-standard) employing extraction/preconcentration GC-MS are capable of achieving detection limits as low as 0.5 ng l^{-1} (and potentially lower using NPD detection), suggesting that current methods offer adequate performance to analyse diazinon.

5. Conclusions

5.1 Availability of data

Laboratory toxicity data are available for nine different freshwater taxonomic groups. The acute toxicity data cover algae, amphibians, annelids, crustaceans, fish, insects, molluscs and planarians. Chronic data are available for algae, crustaceans, fish, insects and rotifers. Laboratory data are supplemented by pond and stream mesocosm studies.

Laboratory and mesocosm experiments confirm the high sensitivity of crustaceans and insects, although fish are also amongst the most sensitive taxa with algae, molluscs, planarians and annelids exhibiting lower sensitivities. There is recent evidence of endocrine-disrupting effects in fish arising from the disruption of olfactory function at low concentrations of diazinon.

By comparison, few toxicity data are available for marine organisms, represented by just five crustacean and two fish species.

5.2 Derivation of PNECs

The proposed PNECs are described below and summarised in Table 5.1.

5.2.1 Long-term PNEC for freshwaters

Reliable chronic data are available for invertebrates and fish. Recent studies have revealed significant reductions in olfactory responses of male Atlantic salmon (*Salmo salar*) following short-term exposure to 0.3 µg l^{-1} diazinon, with a NOEC of 0.1 µg l^{-1}. Although the exposure period was only 30 minutes, effects on reproductive steroid concentrations, the sensitivity of the olfactory epithelium and sperm volumes were observed, with important long-term implications for reproductive success. These data are supported by similar NOECs for reproduction in the crustaceans *Ceriodaphnia dubia*, *Daphnia magna* and *Gammarus pseudolimnaeus*. The standard assessment factor of 10 applied to the Atlantic salmon NOEC of 0.1 µg l^{-1} is recommended, resulting in a $PNEC_{freshwater\_lt}$ of 0.01 µg l^{-1}.

This is similar to the existing EQS of 0.03 µg l^{-1} for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos) based on a *Daphnia magna* NOEC of 0.15 µg l^{-1}, to which an assessment factor of 5 was applied.

5.2.2 Short-term PNEC for freshwaters

Good quality data are available from acute studies with eight taxa including fish, insects and crustaceans. The most sensitive of the insects and crustaceans are at least an order of magnitude more sensitive than the most sensitive fish species. The lowest reliable effects concentration is a 96-hour LC50 of 0.2 µg l^{-1} to the freshwater shrimp *Gammarus fasciatus*. The specific mode of action of diazinon, coupled with the indications that this species is likely to be among the most sensitive taxa, allows a reduced assessment

factor (10) to be applied instead of the default value of 100, resulting in a $PNEC_{freshwater\_st}$ of 0.02 µg l^{-1}.

This is five times lower than the existing EQS of 0.1 µg l^{-1} for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos) generated using a smaller assessment factor (2) applied to the same critical data, as permitted by the method used to derive the EQS.

5.2.3 Long-term PNEC for saltwaters

The limited data suggest similar sensitivities of freshwater and saltwater species, but the greater taxonomic diversity of marine organisms compared with those living in freshwaters introduces greater uncertainty into the prediction of a saltwater PNEC. Nevertheless, in the absence of reliable chronic saltwater toxicity data, a saltwater PNEC may be based on freshwater data. However, an assessment factor of 10 applied to the lowest freshwater chronic NOEC (0.1 µg l^{-1} for olfactory responses in Atlantic salmon) is considered adequate because evidence from acute tests suggests that long-term NOECs generated for these saltwater taxa would not be lower than those already available. This results in a $PNEC_{saltwater\_lt}$ of 0.01 µg l^{-1}, identical to the $PNEC_{freshwater\_lt}$.

This is similar to the existing EQS of 0.03 µg l^{-1} for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos), which was 'read across' from the freshwater long-term value.

5.2.4 Short-term PNEC for saltwaters

Five taxa are represented among the saltwater acute toxicity dataset, including crustaceans, which are clearly much more sensitive than the other species tested. Acute effect concentrations of 2.5–5.6 µg l^{-1} were reported in reliable studies with the copepod *Acartia tonsa*, the shrimp *Palaemonetes pugio* and the mysid shrimp *Americamysis bahia*.

Although the Annex V guidance does not specifically address short-term PNECs for the protection of marine species, the general guidance on short-term effects was followed. An assessment factor of 10 applied to the *Acartia* 96-hour LC50 of 2.57 µg l^{-1} was recommended, resulting in a $PNEC_{saltwater\_st}$ of 0.26 µg l^{-1}. This assessment factor is justified on the basis that, as a crustacean, *Acartia tonsa* is probably amongst the most sensitive marine species to this insecticide.

This PNEC is slightly higher than the existing EQS of 0.1 µg l^{-1} for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos) based on 'reading across' from the freshwater short-term EQS.

5.2.5 PNECs for sediment and secondary poisoning

Although the lipophilicity of diazinon would result in partition from water to sediment, there are insufficient sediment toxicity data to derive a $PNEC_{sediment}$. For both freshwater and saltwater, PNECs based on the risks of secondary poisoning to mammals and birds (0.06 µg l^{-1}) are higher than those derived for the protection of aquatic life and so do not influence the development of EQSs for diazinon.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1})	Existing EQS (µg l^{-1})
Freshwater/long-term	0.01	0.03
Freshwater/short-term	0.02	0.1
Saltwater/long-term	0.01	0.03
Saltwater/short-term	0.26	0.1
Secondary poisoning	0.06	–

5.3 Analysis

The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that non-standard analytical methodologies employing extraction/preconcentration GC-MS are capable of achieving detection limits as low as 0.5 ng l^{-1} (and potentially lower using NPD), sufficient to quantify concentrations of diazinon at the most stringent EQS.

5.4 Implementation issues

The proposed PNECs are recommended for adoption as EQSs, with the exception of the $PNEC_{saltwater\_st}$. The proposed $PNEC_{saltwater\_st}$ is higher (less stringent) than the existing saltwater short-term EQS. Therefore, to comply with the 'no deterioration' principle, it is recommended that the existing saltwater short-term EQS is retained.

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79. Marselas G, Beavers J B, Smith G J and Jaber M J, 1989 *Diazinon: a one generation reproduction study with the mallard (*Anas platyrhynchos*) using parental incubation.* Easton, MA: Wildlife International Ltd. Unpublished report submitted to WHO by Ciba-Geigy Ltd, Basel.

80. Marselas G, Beavers J B, Smith G J and Jaber M J, 1989 *Diazinon: a one generation reproduction study with the Northern bobwhite (*Colinus virginainus*) using parental incubation.* Easton, MA: Wildlife International Ltd. Unpublished report submitted to WHO by Ciba-Geigy Ltd, Basel.

List of abbreviations

AA	annual average
AChE	acetylcholine esterase
AF	assessment factor
ANOVA	analysis of variance
ASTM	American Society for Testing and Materials
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstracts Service
CL	confidence interval
DT50	time taken to degrade by 50%
EC50	concentration effective against 50% of the organisms tested
ECB	European Chemicals Bureau
EQS	Environmental Quality Standard
FPD	flame photometric detection
GC	gas chromatography
GC-MS	gas chromatography/mass spectrometry
GLP	Good Laboratory Practice (OECD)
GM	geometric mean
LC50	concentration lethal to 50% of the organisms tested
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOEC	lowest observed effect concentration
lt	long term
MAC	maximum allowable concentration
MATC	maximum allowable toxicant concentration
NA	not applicable
ND	no data
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NPD	nitrogen phosphorus detection
NR	not reported
OECD	Organisation for Economic Co-operation and Development
PNEC	predicted no-effect concentration

secpois	secondary poisoning
SEPA	Scottish Environment Protection Agency
SNIFFER	Scotland & Northern Ireland Forum for Environmental Research
SPE	solid-phase extraction
SSD	species sensitivity distribution
st	short term
TDI	tolerable daily intake
TGD	Technical Guidance Document
TWA	time weighted average
UKTAG	UK Technical Advisory Group
US EPA	US Environmental Protection Agency
WFD	Water Framework Directive
WHO	World Health Organization

ANNEX 1 Data quality assessment sheets

Identified and ordered by reference number (see References & Bibliography).

Data relevant for PNEC derivation were quality assessed in accordance with the so-called Klimisch Criteria (Table A1).

Table A1 Klimisch Criteria*

Code	Category	Description
1	Reliable without restrictions	Refers to studies/data carried out or generated according to internationally accepted testing-guidelines (preferably GLP**) or in which the test parameters documented are based on a specific (national) testing guideline (preferably GLP), or in which all parameters described are closely related/comparable to a guideline method.
2	Reliable with restrictions	Studies or data (mostly not performed according to GLP) in which the test parameters documented do not comply totally with the specific testing guideline, but are sufficient to accept the data or in which investigations are described that cannot be subsumed under a testing guideline, but which are nevertheless well-documented and scientifically acceptable.
3	Not reliable	Studies/data in which there are interferences between the measuring system and the test substance, or in which organisms/test systems were used that are not relevant in relation to exposure, 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 assessment.
4	Not assignable	Studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature.

* Klimisch H-J, Andreae M and Tillmann U, 1997 *A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.* Regulatory Toxicology and Pharmacology, **25**, 1–5.
** OECD Principles of Good Laboratory Practice (GLP). See:
http://www.oecd.org/department/0,2688,en_2649_34381_1_1_1_1_1,00.html

Reference number	15

Information on the test species	
Test species used	*Salvelinus fontinalis*
Source of the test organisms	Commercial hatchery
Holding conditions prior to test	Yearling brook trout obtained from hatchery were acclimated for 4 months to laboratory conditions prior to test. Lake Superior water (9–15°C depending on month, 45 mg l^{-1} total hardness, pH 7.3). Feeding twice daily according to hatchery schedule with good quality frozen trout food.
Life stage of the test species used	16 months old at initiation of exposure

Information on the test design	
Methodology used	Carried out to a standardised methodology, the test procedure was described
Form of the test substance	Technical grade diazinon (92.5% purity)
Source of the test substance	not stated
Type and source of the exposure medium	Lake Superior water (total hardness 45 mg l^{-1}, pH 7.3, 9–15°C, depending on month)
Test concentrations used	0 (control), 0.55, 1.1, 2.4, 4.8 and 9.6 µg l^{-1} (averaged measured concentrations for exposure of parental brook trout) 0 (control), 0.8, 1.4, 2.7, 5.6 and 11.1 µg l^{-1} (averaged measured concentrations for exposure of eggs following spawning of parental fish).
Number of replicates per concentration	2
Number of organisms per replicate	12
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through system
Measurement of exposure concentrations	Yes. Analysis at least once a week. GC with ECD
Measurement of water quality parameters	pH, dissolved oxygen and temperature, hardness, alkalinity, acidity and conductance
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated

Study conducted to GLP	Not stated. But study conducted according to the 'Recommended Bioassay Procedures established by the Committee on Aquatic Bioassays and the Director of the National Water Quality Laboratory'. The detailed guideline for brook trout is appended to the report.
Overall comment on quality	Quality of experiment good, but the LOEC of 0.55 µg l^{-1} for growth inhibition of progeny of parents exposed to diazinon can only be used with caution. A clear dose–response relationship for that observation is lacking. Use of statistics (ANOVA and Dunnett's test) for determination of significant differences between treatments.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

ANOVA = analysis of variance

Reference number	15

Information on the test species	
Test species used	*Pimephales promelas*
Source of the test organisms	Environmental Research Laboratory Duluth stock
Holding conditions prior to test	As in the test system. Test started with 4–5 days old larvae.
Life stage of the test species used	Larvae, 4–5 days old

Information on the test design	
Methodology used	Carried out to a standardised methodology, the test procedure was described
Form of the test substance	Technical grade diazinon (92.5% purity)
Source of the test substance	not stated
Type and source of the exposure medium	Lake Superior water (total hardness 42–47 mg l^{-1}, pH 7.2–7.8, 24–26°C)
Test concentrations used	0 (control), 3.2, 6.9, 13.5, 28.0 and 60.3 µg l^{-1} (averaged measured concentrations).
Number of replicates per concentration	2
Number of organisms per replicate	50
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through system
Measurement of exposure concentrations	Yes. Analysis at least once a week. GC with ECD
Measurement of water quality parameters	pH, dissolved oxygen and temperature, hardness, alkalinity, acidity and conductance
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated. But study conducted according to the 'Recommended Bioassay Procedures established by the Committee on Aquatic Bioassays and the Director of the National Water Quality Laboratory'. The detailed guideline for brook trout is appended to the report.
Overall comment on quality	Quality of experiment good. Use of statistics (ANOVA and Dunnett's test) for determination of significant differences between treatments.

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	1

Reference number	16

Information on the test species	
Test species used	*Ceriodaphnia dubia*
Source of the test organisms	US EPA laboratory culture
Holding conditions prior to test	not reported
Life stage of the test species used	all organisms ≤48 hours old at test initiation

Information on the test design	
Methodology used	Acute toxicity test according to US EPA procedures
Form of the test substance	diazinon, 95-99% purity
Source of the test substance	ChemServices, PA
Type and source of the exposure medium	10% mineral water (Perrier, France) diluted in high purity water from a Millipore system
Test concentrations used	Five test levels plus control
Number of replicates per concentration	2
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static. Feeding not reported.
Measurement of exposure concentrations	no
Measurement of water quality parameters	pH, hardness, dissolved oxygen and conductivity were measured but not reported in the paper
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated.
Overall comment on quality	Good–moderate, concentration levels not measured. LC50 determination with trimmed Spearman–Karber.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	17

Information on the test species	
Test species used	Community of benthic stream invertebrates.
Source of the test organisms	not reported
Holding conditions prior to test	regional outdoor conditions, details not reported
Life stage of the test species used	NA

Information on the test design	
Methodology used	Microcosm with three outdoor channel systems, of which the upper 122 m were used for testing. For description of study area, readers are referred to a reference.
Form of the test substance	Technical grade diazinon (92.5% purity) in aqueous solution (30 mg l^{-1}), applied at 2 treatment levels at a time.
Source of the test substance	Ciba Geigy, Greensboro, NC.
Type and source of the exposure medium	pond water, pond sediment
Test concentrations used	Treatment A: nominal concentrations 0 (control), 0.3 and 3 µg l^{-1}. Treatment B: 0, 6 and 12 µg l^{-1}. Treatment C: 0, 0 and 30 µg l^{-1}.
Number of replicates per concentration	0
Number of organisms per replicate	NA
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through. No feeding.
Measurement of exposure concentrations	Weekly in the channels, three times a week in the storage tanks of stock solutions. Analysis by gas chromatography.
Measurement of water quality parameters	Temperature, dissolved oxygen, pH, conductivity, alkalinity and hardness
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated.
Overall comment on quality	Moderate to good. But it could not be revealed whether dosings during treatments B and C did not result in the calculated concentrations. Further, there was no difference in responses between the 0.3 and 3.0 µg l^{-1} levels during treatment A. No consistent dose dependency of observed effects.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	18

Information on the test species	
Test species used	*Ceriodaphnia dubia*
Source of the test organisms	Laboratory culture
Holding conditions prior to test	Maintained at 25°C in moderately hard water according to US EPA guidelines (cited). Fed with algae (*Selenastrum capricornutum*) and blended trout chow.
Life stage of the test species used	neonates <24 hours old

Information on the test design	
Methodology used	Acute toxicity test according to US EPA procedures
Form of the test substance	diazinon 99.0% pure (lot 36-6C)
Source of the test substance	AccuStandard (New Haven, CT)
Type and source of the exposure medium	Appropriate salts added to reverse-osmosis treated well water to obtain moderately hard water according to US EPA standards (guideline cited).
Test concentrations used	Five test levels plus control
Number of replicates per concentration	4
Number of organisms per replicate	5
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static. No feeding.
Measurement of exposure concentrations	Stock solutions as well as initial concentrations of each test were verified analytically.
Measurement of water quality parameters	pH, hardness, dissolved oxygen and conductivity were measured but not reported in the paper
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated.
Overall comment on quality	Good, concentration levels not measured. LC50 determination with trimmed Spearman–Karber.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	21

Information on the test species	
Test species used	*Ceriodaphnia dubia*
Source of the test organisms	University of Mississippi Aquatic Toxicology Laboratory, laboratory culture
Holding conditions prior to test	According to cited references
Life stage of the test species used	<48 hours at test initiation

Information on the test design	
Methodology used	Acute toxicity test
Form of the test substance	granular Ortho Fire Ant Repellant™ (5% a.i.) dissolved in de-ionised water
Source of the test substance	
Type and source of the exposure medium	Filtered spring water of the University of Mississippi Field Station, hardness and alkalinity adjusted to 60–80 mg l^{-1} as $CaCO_3$
Test concentrations used	mean measured concentrations (µg l^{-1}): 0, 1.19, 8
Number of replicates per concentration	10
Number of organisms per replicate	1
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static. Feeding.
Measurement of exposure concentrations	Measured by plate immunoassay
Measurement of water quality parameters	Yes, according to APHA 1992* guidelines
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated.
Overall comment on quality	Good, concentration measured. LC50 determination with trimmed Spearman–Karber.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

* American Public Health Association (APHA), 1992 *Standard methods for the examination of water and wastewater*. Washington, DC: APHA.

Reference number	27

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Continuous laboratory cultures
Holding conditions prior to test	Culture in dechlorinated tap water (total hardness 250 mg l^{-1} as $CaCO_3$, pH 7.9 $\pm$ 0.2, 22 $\pm$ 1°C, 12/12 h photoperiod). Density <50 animals per litre. Renewal of culture medium three times per week. Daily feeding of daphnids and libitum with the alga *Nanochloris oculata*.
Life stage of the test species used	neonates $\leq$24 hours old at initiation of experiment, development to adults, reproduction

Information on the test design	
Methodology used	Not carried out to a standardised methodology, but the test procedure was described
Form of the test substance	Technical grade diazinon (92% purity)
Source of the test substance	Cequisa
Type and source of the exposure medium	Dechlorinated tap water (total hardness 250 mg l^{-1} as $CaCO_3$, pH 7.9 $\pm$ 0.2, 22 $\pm$ 1°C)
Test concentrations used	0 (control), 0.15, 0.18, 0.22, 0.25 and 0.3 µg l^{-1} diazinon and a solvent control with acetone (1 µl l^{-1}).
Number of replicates per concentration	3
Number of organisms per replicate	15
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static exposure of 15 animals in 50 ml test solution per replicate for 21 days. Renewal of test solutions every 2nd day. Daphnia were fed daily with *Nanochloris oculata* at a density of 5 x 10^5 cells/ml.
Measurement of exposure concentrations	Not indicated. Presumably not.
Measurement of water quality parameters	pH, dissolved oxygen and temperature measured and recorded on each renewal day
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good (but reference to nominal concentrations). Determination of NOECs and LOECs based on statistics (ANOVA and Duncan's multiple range test)

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	29

Information on the test species	
Test species used	Pelagic and benthic communities of natural ponds (sediment form one pond, water from another). Addition of 40 juvenile bluegill sunfish per test system.
Source of the test organisms	natural ponds, source of fish not mentioned
Holding conditions prior to test	regional outdoor conditions, details not reported
Life stage of the test species used	

Information on the test design	
Methodology used	Microcosm study with 18 fibreglass tanks of 3.2 m diameter and 1.5 m depth. Stocked with sediment and water from nearby natural ponds.
Form of the test substance	Technical grade diazinon (88% active ingredient) in aqueous solution, applied at eight treatment levels applied three times at 7 day intervals.
Source of the test substance	not reported
Type and source of the exposure medium	pond water, pond sediment
Test concentrations used	nominal concentrations ranging from 0 (control), 2–500 µg l^{-1}. Measured time weighted average (70 days) concentrations were 0, 2.4, 4.3, 9.2, 22, 54, 117, 205 and 443 µg l^{-1}:
Number of replicates per concentration	2
Number of organisms per replicate	40 bluegill sunfish plus benthic and pelagic communities
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static, addition of well water to replace losses by evaporation. No feeding of communities in tanks
Measurement of exposure concentrations	By gas chromatography
Measurement of water quality parameters	Temperature, dissolved oxygen, pH, conductivity, alkalinity and hardness
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated.
Overall comment on quality	Good.

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference number	30

Information on the test species	
Test species used	*Cyprinodon variegatus*
Source of the test organisms	juvenile fish were collected near Gulf Breeze, FL
Holding conditions prior to test	Adaptation to ambient salinity and 30°C for a minimum of 17 days prior to testing
Life stage of the test species used	Begin of exposure with juveniles of in average 22 mm length and 0.38 g in weight.

Information on the test design	
Methodology used	Toxicity test , partial life-cycle)
Form of the test substance	Diazinon, triethylene glycol used as carrier
Source of the test substance	not reported
Type and source of the exposure medium	filtered salt water from Santa Rosa Sound, FL
Test concentrations used	0 (control), 0.47, 0.98, 1.8, 3.5 and 6.5 µg l^{-1} average measured concentrations
Number of replicates per concentration	1
Number of organisms per replicate	22
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through. Feeding.
Measurement of exposure concentrations	Weekly analysis of water
Measurement of water quality parameters	Salinity, dissolved oxygen, temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated.
Overall comment on quality	Good. Concentration levels measured. Use of statistics to determine effect levels (probability analysis, ANOVA, etc.)

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	34

Information on the test species	
Test species used	*Acartia tonsa*
Source of the test organisms	Chesapeake Bay at Solomons, Maryland
Holding conditions prior to test	Cultivation in natural sea water at an adjusted salinity level of 20‰. 17 ± 1°C and 14-hour photoperiod with fluorescent illumination. Daily feeding with algal cells. Culture in 2.3-litre glass vessels. Replacement of medium once a week.
Life stage of the test species used	adult copepods

Information on the test design	
Methodology used	Acute toxicity test
Form of the test substance	97.6% solution technical grade diazinon
Source of the test substance	Not reported
Type and source of the exposure medium	Natural sea water at an adjusted salinity level of 20‰
Test concentrations used	0 (control), 0.04, 0.2, 0.4, 0.8, 1.6, 3.2 and 8.0 µg l^{-1}
Number of replicates per concentration	4
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static. Feeding.
Measurement of exposure concentrations	Not reported
Measurement of water quality parameters	Yes, at begin and end of tests
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated.
Overall comment on quality	Good, but concentration levels not measured. LC50 determination according to Litchfield and Wilcoxon

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference number	39

Information on the test species	
Test species used	*Salmo salar*
Source of the test organisms	Hatchery purchased
Holding conditions prior to test	Tap water
Life stage of the test species used	Mature male Atlantic salmon parr (98–176 mm length)

Information on the test design	
Methodology used	Methodology well described
Form of the test substance	Diazinon
Source of the test substance	Not reported
Type and source of the exposure medium	Dechlorinated water
Test concentrations used	0.8–45 $\mu g\ l^{-1}$
Number of replicates per concentration	Replicated, but number not reported
Number of organisms per replicate	1
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	At end of study (test solutions stored in cold and dark and tested after 7 and 14 days)
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated.
Overall comment on quality	Well-documented study that appears to be well carried out

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference number	40

Information on the test species	
Test species used	*Chironomus tentans*
Source of the test organisms	Not reported
Holding conditions prior to test	Not reported
Life stage of the test species used	Larvae

Information on the test design	
Methodology used	Reference is a summary report. Methodology not described.
Form of the test substance	Not reported
Source of the test substance	Not reported
Type and source of the exposure medium	Not reported
Test concentrations used	Not reported
Number of replicates per concentration	Not reported
Number of organisms per replicate	Not reported
Nature of test system (static, semi-static or flow-through, duration, feeding)	Not reported
Measurement of exposure concentrations	Not reported
Measurement of water quality parameters	Not reported
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Reference is summary report. Original document (PhD thesis) could not be retrieved. No statement on data quality possible.

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference number	42

Information on the test species	
Test species used	*Americamysis bahia*
Source of the test organisms	Santa Rosa Sound near Pensacola, FL
Holding conditions prior to test	1) Flowing sea water in aquaria or fibreglass tanks or 2) static recirculating method with biological filters fitted into the aquaria. Larval *Artemia salina* fed *ad libitum* in all culture devices.
Life stage of the test species used	a) Juveniles <48 hours old (96-hour LC50 test) b) Life-cycle test for a total of 28 days (covered mostly two life-cycles of the organism)

Information on the test design	
Methodology used	Toxicity test in flow-through systems according to published and cited test-protocols (US EPA report)
Form of the test substance	Diazinon, triethylene glycol used as carrier
Source of the test substance	not reported
Type and source of the exposure medium	flowing salt water
Test concentrations used	Five levels plus control
Number of replicates per concentration	not reported
Number of organisms per replicate	not reported.
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through. Feeding.
Measurement of exposure concentrations	At least four times during a life-cycle test. At least twice in a 96-hour test. Gas-liquid chromatography with ECD detector.
Measurement of water quality parameters	According to test protocol
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated. US EPA test protocol used
Overall comment on quality	Moderate to good. Short publication in a book dealing with test of 11 pesticides. Information on test details rather scarce but reference is made to a published EPA test protocol. Concentration levels measured. Use of statistics to determine effect levels (probability analysis, ANOVA, etc.)

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**2**

ANNEX 2 Data sheets: water column data

Ordered and identified by reference numbers as listed in References & Bibliography.

Reference	**6**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	1. Stonefly 2. Mayfly 3. Scud 4. Caddisfly 5. Dragonfly 6. Stonefly
Organism (scientific name)	1. *Acroneuria lycorias* 2. *Ephemerelia subvaria* 3. *Gammarus pseudolimneaus* 4. *Hydropsyche bettoni* 5. *Ophiogomphus rupinsulensis* 6. *Pteronarcys dorsata*
Life stage (e.g. egg, embryo, ELS, adult)	Not reported
Exposure regime (e.g. static, renewal, etc.)	Not reported
Test method	Not reported
Analysis (measured or nominal)	Not reported
Temperature	Not reported
Hardness	Not reported
pH	Not reported
Salinity	Not reported
Exposure duration	30 days
Endpoint (e.g. NOEC, EC50)	LC50; NOEC
Effect (e.g. reproduction, survival, growth)	LC50: mortality? NOEC: not reported
Concentration	1. LC50: 1.25 µg l^{-1} 1. NOEC: 0.83 µg l^{-1} 2. LC50: 1.05 µg l^{-1} 2. NOEC: 0.42 µg l^{-1} 3. LC50: 0.27 µg l^{-1} 3. NOEC: 0.2 µg l^{-1} 4. LC50: 3.54 µg l^{-1} 4. NOEC: 1.79 µg l^{-1} 5. LC50: 2.2 µg l^{-1} 5. NOEC: 1.29 µg l^{-1} 6. LC50: 4.6 µg l^{-1} 6. NOEC: 3.29 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	No supplementary information on experimental design and data analysis available
Comments	Further data quality assessment not possible. Data source cited in the reference is Bell, H L, unpublished data, National Water Quality Laboratory, Duluth, MN.

Reference	**14**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Flagfish
Organism (scientific name)	*Jordanella floridae*
Life stage (e.g. egg, embryo, ELS, adult)	Life-cycle exposure of 1-day-old flagfish to diazinon at average concentrations of 0, 14, 26, 54, 88 and 240 µg l^{-1}. Two replicates per test level.
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	
Analysis (measured or nominal)	Measured concentrations (weekly by gas chromatograph)
Temperature	25.5–26.5°C
Hardness	Not reported
pH	Not reported
Salinity	Not reported
Exposure duration	Total 120 days (0–120 days development of parental generation, 61–120 days spawning success and development of progeny)
Endpoint (e.g. NOEC, EC50)	NOEC
Effect (e.g. reproduction, survival, growth)	Average end of hatch Average weight of progeny (35 days post hatch)
Concentration	LOEC 14 µg l^{-1} (lowest concentration tested) ≈ NOEC <14 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

Reference	**15**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Brook trout
Organism (scientific name)	*Salvelinus fontinalis*
Life stage (e.g. egg, embryo, ELS, adult)	Yearling brook trouts after 4 month acclimatisation to lab conditions (i.e. 16 months old at initiation of exposure)
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	Chronic toxicity test
Analysis (measured or nominal)	Analysis at least once a week
Temperature	9–15°C, depending on month
Hardness	45
pH	7.3
Salinity	Lake Superior water
Exposure duration	173 days (parents) 122 days (progeny)
Endpoint (e.g. NOEC, EC50)	NOEC
Effect (e.g. reproduction, survival, growth)	Survival, growth (length, weight), scoliosis/lordosis of spinal cord
Concentration	NOEC (growth, parents): 2.4 µg l^{-1} (at day 91, no significant difference at day 173) NOEC (survival, parents): 4.8 µg l^{-1} NOEC (increase in length per day, progeny): after 122 days exposure, the growth rate and average weight of progeny was significantly lower if parents were exposed to diazinon (0.55 µg l^{-1} or more), but no clear dose–response relationship. Progeny of non-exposed control parents showed no growth inhibition up to the highest concentration tested. NOEC (scoliosis, parents): 2.4 µg l^{-1} (no scoliosis observed in any group of progeny exposed up to 11.1 µg l^{-1} for 122 days)
Initial quality assessment (e.g. good, moderate, poor)	Quality of experiment good but the LOEC of 0.55 µg l^{-1} for growth inhibition of progeny of parents exposed to diazinon can only be used with caution. A clear dose–response relationship for that observation is lacking.
Comments	NOEC (growth and weight of progeny) unbounded. Absence of a clear dose–response relationship.

Reference	**15**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Fathead minnow
Organism (scientific name)	*Pimephales promelas*
Life stage (e.g. egg, embryo, ELS, adult)	5 day old larvae at begin of exposure
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	Chronic toxicity test
Analysis (measured or nominal)	Analysis at least once a week
Temperature	24–26°C
Hardness	42–47
pH	7.2–7.8
Salinity	Lake Superior water
Exposure duration	Up to 274 days (parents) 30–60 days (progeny)
Endpoint (e.g. NOEC, EC50)	NOEC
Effect (e.g. reproduction, survival, growth)	Survival, length, scoliosis of spinal cord, hatching success
Concentration	NOEC (growth after 167–274 days, parents): 28 µg l^{-1} NOEC (scoliosis after 167 days, parents): <3.2 µg l^{-1} (lowest conc. tested) NOEC (hatching success): <3.2 µg l^{-1} (lowest concentration tested)
Initial quality assessment (e.g. good, moderate, poor)	Quality of experiment good but results can only be used with caution
Comments	NOEC (scoliosis, hatching) unbounded.

Reference	**15**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Fathead minnow Bluegill Flagfish Brook trout
Organism (scientific name)	*Pimephales promelas* *Lepomis macrochirus* *Jordanella floridae* *Salvelinus fontinalis*
Life stage (e.g. egg, embryo, ELS, adult)	*Pimephales*: 13, 15, 20 weeks (tests 1, 2 and 3, respectively) *Lepomis*: 1 year (both tests) *Jordanella*: (6 and 7 weeks) (tests 1 and 2, respectively) *Salvelinus*: 1 year (all three tests)
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	96-hour acute toxicity tests
Analysis (measured or nominal)	Analysis 3–6 times during each 96-hour test
Temperature	25°C (*Pimephales, Lepomis, Joranella*) 12°C (*Salvelinus*)
Hardness	42–47
pH	7.2–7.8
Salinity	Lake Superior water
Exposure duration	96 hour
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	mortality
Concentration	LC50 *Pimephales*: 7.8 mg l^{-1} (6.6, 6.8 and 10.0 mg l^{-1}) LC50 *Lepomis*: 0.46 mg l^{-1} (0.44 and 0.48 mg l^{-1}) LC50 *Jordanella*: 1.6 mg l^{-1} (1.5 and 1.8 mg l^{-1}) LC50 *Salvelinus*: 0.77 mg l^{-1} (0.45, 0.80 and 1.05 mg l^{-1})
Initial quality assessment (e.g. good, moderate, poor)	Quality of experiments good but results can only be used with caution.
Comments	

Reference	**16**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Water flea
Organism (scientific name)	*Ceriodaphnia dubia* *Daphnia magna* *Daphnia pulex*
Life stage (e.g. egg, embryo, ELS, adult)	All organisms ≤48-hourours at test initiation
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	48-hour acute toxicity test
Analysis (measured or nominal)	Nominal
Temperature	25°C
Hardness	Not reported
pH	Not reported
Salinity	Test water: 10% mineral water (Perrier, France) diluted in high purity water from a Millipore system
Exposure duration	48 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	*Ceriodaphnia dubia*, 48-hour LC50: 0.5 µg l^{-1} *Daphnia magna*, 48-hour LC50: 0.8 µg l^{-1} *Daphnia pulex*, 48-hour LC50: 0.65 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Moderate
Comments	Report focuses on the combined effects of piperonyl butoxide and various organophosphate insecticides.

Reference	**17**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Various benthic stream invertebrates
Organism (scientific name)	
Life stage (e.g. egg, embryo, ELS, adult)	Various
Exposure regime (e.g. static, renewal, etc.)	Flow-though 0.8 m³/min, continuous dosing: Control (0 µg l^{-1}); Dosing regime A: 0.3 and 3 µg l^{-1}; Dosing regime B: 6 and 12 µg l^{-1}; Dosing regime C: 30 µg l^{-1} in the 'high dosing' channel of B. Termination of low dosing.
Test method	Outdoor channel system, each channel 520 metres long. Upper 122 m of three channels used for experiment
Analysis (measured or nominal)	Measurement of diazinon concentrations in channels throughout the study by gas chromatography
Temperature	Monitored
Hardness	Measured weekly
pH	Monitored
Salinity	Freshwater
Exposure duration	Total 18 weeks (12 weeks regime A, 4 weeks regime B, and 2 weeks C
Endpoint (e.g. NOEC, EC50)	
Effect (e.g. reproduction, survival, growth)	Increase in drift of certain groups/species of invertebrates. Changes in population densities of macrozoobenthos
Concentration	At 0.3 µg l^{-1} at two observation intervals, increased drift of macrozoobenthos (3.5–7.8 times) compared with the control, but no consistent difference to higher dose (3 µg l^{-1}). At 5 µg l^{-1}, sharp decrease of *Hyallella atzteca* population density.
Initial quality assessment (e.g. good, moderate, poor)	Good but no consistent dose–response relationship
Comments	

Reference	**18**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Water flea
Organism (scientific name)	*Ceriodaphnia dubia*
Life stage (e.g. egg, embryo, ELS, adult)	All organisms <24 hours at test initiation
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	48–96-hour acute toxicity test
Analysis (measured or nominal)	Stock solutions as well as initial concentrations of each test were verified analytically.
Temperature	25 ± 1°C
Hardness	'Moderately hard' artificial water prepared according to a cited reference. 'Natural water' from two different locations
pH	pH, hardness, dissolved oxygen and conductivity were measured but not reported in the paper
Salinity	Test water: 10% mineral water (Perrier, France) diluted in high purity water from a Millipore system
Exposure duration	48–96 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	48-hour LC50 in artificial 'moderately hard' water: 0.26–0.58 µg l^{-1} 48-hour LC50 in 'sump 104' natural water: 0.48 µg l^{-1} 48-hour LC50 in 'Mosher Slough' natural water: 0.52 µg l^{-1} ≈ LC50 in 'natural water': 0.5 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	Report focuses on the combined effects of chlorpyrifos and diazinon.

Reference	**19**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Midge Fathead minnow
Organism (scientific name)	*Chironomus tentans*
Life stage (e.g. egg, embryo, ELS, adult)	4th instar larvae (head capsule width 0.63–0.71 mm, body length ≥ 10 mm)
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	Test according to US EPA standard operating procedure for laboratory cultures of *C. tentans*. Silica sand used as sediment.
Analysis (measured or nominal)	Test concentrations verified by analysis
Temperature	20°C
Hardness	
pH	7.3–7.8
Salinity	
Exposure duration	96 hour
Endpoint (e.g. NOEC, EC50)	EC50
Effect (e.g. reproduction, survival, growth)	Ability to perform normal swimming motion when pinched with a forceps
Concentration	Series 1: 30 (95% CI 24–36) µg l^{-1} Series 2: 38 (95% CI 27–74) µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

Reference	**20**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Zebra fish
Organism (scientific name)	*Brachydanio rerio*
Life stage (e.g. egg, embryo, ELS, adult)	fertilised eggs to 6 weeks after fertilisation
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	Early life stage test
Analysis (measured or nominal)	Concentrations in test aquaria measured, verified once a week (recovery rates 83–87% of nominal concentration.)
Temperature	26 $\pm$ 1°C
Hardness	360 mg l^{-1} expressed as $CaCO_3$
pH	7.4
Salinity	Freshwater
Exposure duration	6 weeks
Endpoint (e.g. NOEC, EC50)	NOEC
Effect (e.g. reproduction, survival, growth)	Hatching, survival, growth (weight)
Concentration	0.04 mg l^{-1} (weight)
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	Focus is on design of the ELS and comparison of susceptibility of rainbow trout with zebra fish.

Reference	**20**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Rainbow trout
Organism (scientific name)	*Oncorhynchus mykiss*
Life stage (e.g. egg, embryo, ELS, adult)	Fingerlings
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	28-day fish test
Analysis (measured or nominal)	Concentrations in test aquaria measured, verified once a week (recovery rates 83–87% of nominal conc.)
Temperature	15–17°C
Hardness	360 mg l^{-1} expressed as $CaCO_3$
pH	7.4
Salinity	Freshwater
Exposure duration	28 days
Endpoint (e.g. NOEC, EC50)	NOEC
Effect (e.g. reproduction, survival, growth)	Growth (weight)
Concentration	0.2 mg l^{-1} (weight): highest concentration in test
Initial quality assessment (e.g. good, moderate, poor)	Moderate
Comments	NOEC unbounded. Therefore, not suitable for EQS setting. Focus is on design of the ELS and comparison of susceptibility of rainbow trout with zebra fish.

Reference	**21**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Water flea Water flea Midge Fathead minnow
Organism (scientific name)	*Ceriodaphnia dubia* *Daphnia magna* *Hyalella azteca* *Chironomus tentans* *Pimephales promelas*
Life stage (e.g. egg, embryo, ELS, adult)	*C. dubia* (<48 hour) *D. magna* (<48 hour) *H. azteca* (425–1000 µm) *C. tentans* (13–15 days) *P. promelas* (<24 hour)
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	Acute toxicity test
Analysis (measured or nominal)	Measured by plate immunoassay
Temperature	20 ± 1°C
Hardness	60–80 mg l^{-1} $CaCO_3$
pH	Measured but not reported
Salinity	Spring water of the University of Mississippi Field Station
Exposure duration	48 hours
Endpoint (e.g. NOEC, EC50)	48-hour LC50, 48-hour NOEC, 48-hour LOEC
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	All figures µg l^{-1} *C. dubia:* LC50 0.92, NOEC 0.6, LOEC 0.8 *D. magna*: LC50 2.39, NOEC 0.8, LOEC 1.5 *H. azteca*: LC50 15.07, NOEC 7.5, LOEC 11.0 *C. tentans*: LC50 52.47, NOEC 30, LOEC 37.5 *P. promelas*: LC50 15940, NOEC 6000, LOEC 12500
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

Reference	**23**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Scud
Organism (scientific name)	*Hyalella azteca*
Life stage (e.g. egg, embryo, ELS, adult)	Different age classes: 0–2, 2–4, 6–8, 8–10, 12–14, 161–18, 20–22 and 24–26 days
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	All culture, test and analytical procedures were performed with strict adherence to approved QA/QC guidelines of the Duluth US EPA laboratory.
Analysis (measured or nominal)	Nominal
Temperature	25°C
Hardness	40 mg l^{-1} as $CaCO_3$
pH	7.4–8.1
Salinity	Filtered Lake Superior water
Exposure duration	96 hour
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	The toxicity of diazinon was similar for animals ranging in age from 0–2 to 24–26 days at test initiation. The age class with the lowest LC50 was the 12–14 day old (4 µg l^{-1}), while the 0–2 day old age class appeared to be the least sensitive (6.3 µg l^{-1}). Overall 96-hour LC50 4 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Good but reference to nominal toxicant concentrations.
Comments	

Reference	**24**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Pink shrimp
Organism (scientific name)	*Penaeus duorarum* *Mysidopsis bahia*
Life stage (e.g. egg, embryo, ELS, adult)	*M. bahia*: ≤ 24-hour old post release juveniles *P. duorarum*: 3–5 day old post larvae
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	
Analysis (measured or nominal)	Stock solutions measured by gas chromatography prior to dosing
Temperature	25°C
Hardness	Not reported but mentioned that all water quality parameters remained within limits set by ASTM.
pH	
Salinity	Sand and 1 µm fibre-filtered natural sea water adjusted to 25 ‰ salinity with deionised water
Exposure duration	96 hour
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	*P. duorarum*: 21 (95% CI: 19-24) µg l^{-1} *M. bahia*: 8.5 (95% CI: 8.2–8.9) µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

Reference	**25**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	a) Water flea b) Bluegill c) Fathead minnow
Organism (scientific name)	a) *Daphnia magna* b) *Lepomis macrochirus* c) *Pimephales promelas*
Life stage (e.g. egg, embryo, ELS, adult)	a) Less than 24 hours old b, c) Less than one year old
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	Acute toxicity test
Analysis (measured or nominal)	Not reported (presumably nominal)
Temperature	20 $\pm$ 1°C
Hardness	192 mg l^{-1}
pH	8.2
Salinity	Well water
Exposure duration	a) 48 hours b, c) 96 hours
Endpoint (e.g. NOEC, EC50)	a) EC50 b, c) LC50
Effect (e.g. reproduction, survival, growth)	a) Swimming ability (immobilization) b, c) Mortality
Concentration	a) 0.00122 mg l^{-1} (dust formulation); 0.00125 mg l^{-1} (EC formulation) b) 0.17 mg l^{-1} (dust formulation); 0.53 mg l^{-1} (EC formulation) a) 5.6–10.0 mg l^{-1} (dust formulation); 3.2–5.8 mg l^{-1} (EC formulation)
Initial quality assessment (e.g. good, moderate, poor)	Moderate (nominal concentrations)
Comments	

Reference	**26**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Water flea
Organism (scientific name)	*Daphnia magna*
Life stage (e.g. egg, embryo, ELS, adult)	Neonates <24 hours at initiation of experiment
Exposure regime (e.g. static, renewal, etc.)	Static; exposure to 0, 0.23, 0.45, 0.690 and 0.90 µg l^{-1} diazinon
Test method	24-hour daphnia acute toxicity test according to EEC standard method (EEC 1984) 5-hour feeding study
Analysis (measured or nominal)	Not reported but presumably nominal
Temperature	22 $\pm$ 1°C
Hardness	Not reported
pH	Not reported
Salinity	Dechlorinated tap water
Exposure duration	24 hours 5 hours
Endpoint (e.g. NOEC, EC50)	LC50 EC50 (decrease in filtration and ingestion rate)
Effect (e.g. reproduction, survival, growth)	Mortality ; filtration rate; ingestion rate
Concentration	LC50 (24 hours): 0.9 µg l^{-1} EC50 (5 hours, filtration): 0.47 µg l^{-1} EC50 (5 hours, ingestion): 0.6 µg l^{-1} NOEC (5 hours, filtration and ingestion Rates): 0.23 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Moderate (reference to nominal concentrations), only 24-hour LC50
Comments	

EEC, 1984 Method C.2 of Commission Directive 92/69/EEC (Annex V of Council Directive 67/548/EEC).

Reference	**27**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Water flea
Organism (scientific name)	*Daphnia magna*
Life stage (e.g. egg, embryo, ELS, adult)	Neonates ≤24 hours at initiation of experiment, development to adults, reproduction
Exposure regime (e.g. static, renewal, etc.)	Static renewal every 2 days; exposure to 0, 0.15, 0.18, 0.22, 0.25 and 0.30 µg l^{-1} diazinon
Test method	21-day daphnia reproduction test
Analysis (measured or nominal)	Not reported but presumably nominal
Temperature	22 ± 1°C
Hardness	250 mg l^{-1} $CaCO_3$
pH	7.9 ± 0.2
Salinity	Freshwater
Exposure duration	21 days
Endpoint (e.g. NOEC, EC50)	NOEC
Effect (e.g. reproduction, survival, growth)	Total young per female; mean brood size, mean number of broods, mean time to first reproduction; intrinsic rate of increase 'r'; length of adults
Concentration	NOEC (mean brood size and mean length of adults): <0.15 µg l^{-1} NOEC (total young per female, mean number of broods, mean longevity): 0.15 µg l^{-1} NOEC (mean number of days to first reproduction): 0.22 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Good (but reference to nominal concentrations). Determination of NOECs and LOECs based on statistics (ANOVA and Duncan's multiple range test)
Comments	

ANOVA = analysis of variance

Reference	**28**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Eel
Organism (scientific name)	*Anguilla anguilla*
Life stage (e.g. egg, embryo, ELS, adult)	Juvenile eel, weight 20–30 g, length 16–20 cm
Exposure regime (e.g. static, renewal, etc.)	Not explicitly reported but presumably static
Test method	Acute fish test
Analysis (measured or nominal)	Not reported (presumably nominal)
Temperature	20°C
Hardness	250 mg l^{-1} $CaCO_3$
pH	7.9 $\pm$ 0.2
Salinity	
Exposure duration	24, 48, 72, 96 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	24-hour LC50 0.16 mg l^{-1} (95% CI 0.10 – 0.23 mg l^{-1}) 48-hour LC50 0.11 mg l^{-1} (95% CI 0.08 – 0.14 mg l^{-1}) 72-hour LC50 0.09 mg l^{-1} (95% CI 0.07 – 0.11 mg l^{-1}) 96-hour LC50 0.08 mg l^{-1} (95% CI 0.06 – 0.1 mg l^{-1})
Initial quality assessment (e.g. good, moderate, poor)	Moderate (no verification of toxicant concentrations by analysis)
Comments	

Reference	**29**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Outdoor pond mesocosms
Organism (scientific name)	
Life stage (e.g. egg, embryo, ELS, adult)	
Exposure regime (e.g. static, renewal, etc.)	Static, three consecutive toxicant applications at 7-day intervals. Nominal application rates 2–500 µg l^{-1} (eight treatment levels), resulting in 70-day time weighted average (TWA) concentrations of: 2.4, 4.3, 9.2, 22, 54, 117, 205 and 443 µg l^{-1}.
Test method	Outdoor mesocosm test.
Analysis (measured or nominal)	Measured toxicant concentrations (water, sediment)
Temperature	12–28°C (depth averaged), depending on season
Hardness	150 mg l^{-1} $CaCO_3$ in spring, 70 mg l^{-1} in summer
pH	Usually 8.5–9.0 (depth averaged); extremes: 7.8–9.9
Salinity	
Exposure duration	
Endpoint (e.g. NOEC, EC50)	
Effect (e.g. reproduction, survival, growth)	Phytoplankton: pigment content Macrophytes: growth (weight) Zooplankton: Identification and counting (structure and abundance) Macroinvertebrates: Identification and counting
Concentration	Mesocosm NOEC 4.3 µg l^{-1}, LOEC 9.2 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Good, but conclusions on mesocosm NOEC/LOEC by authors cannot be generalised for EQS setting.
Comments	Data indicate significant and lasting impacts on the abundance of cladocerans and some insect taxa at the lowest treatment level (2.4 µg l^{-1} TWA). Although in the investigated mesocosms these organisms were not ecologically significant under functional aspects (because of their rather low abundance), they are in functional terms very significant in many other natural water bodies. Besides, the EQS does not aim only at the sustainable function of ecosystems but on the preservation of community structures as well. So, generalising the observed effects on cladocerans and some insect taxa and also taking alterations of the community structure into account, the ecosystem NOEC is <2.4 µg l^{-1}.

Reference	**30**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Sheepshead minnow
Organism (scientific name)	*Cyprinodon variegatus*
Life stage (e.g. egg, embryo, ELS, adult)	a) 96-hour acute toxicity test: juveniles, 14 mm standard length b) Partial life-cycle test: initiation of test with juvenile fish, 17–25 mm standard length, average weight 0.38 g
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	a) 96-hour acute toxicity test b) Partial life-cycle test
Analysis (measured or nominal)	a) Measured b) Measured weekly
Temperature	30 ± 2°C
Hardness	Not reported
pH	Not reported
Salinity	20–25‰, filtered natural sea water
Exposure duration	96 hours (acute toxicity test) 128 days exposure, 32 days depuration (partial LC)
Endpoint (e.g. NOEC, EC50)	Acute toxicity test: LC50 Partial life-cycle test: MATC, NOEC
Effect (e.g. reproduction, survival, growth)	Acute toxicity test: mortality Partial LC: reduced fecundity
Concentration	Acute toxicity test: 96-hour LC50 1,470 µg l^{-1} NOEC. MATC <0.47 µg l^{-1} (lowest concentration tested)
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	Dose–response relationship of reduced fecundity (no. eggs per female) is weak. No effects on fertility, mortality and growth of progeny. Consider similarities in the test of brook trouts by Allison and Hermanutz 1977 [15].

Reference	**32**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	Technical diazinon
Organism (common name)	Green frog
Organism (scientific name)	*Rana calamitans*
Life stage (e.g. egg, embryo, ELS, adult)	Eggs–larvae
Exposure regime (e.g. static, renewal, etc.)	1. Static 2. Static renewal
Test method	
Analysis (measured or nominal)	measured
Temperature	18.1 ± 1.1°C
Hardness	not reported
pH	7.35–7.43
Salinity	pond water from a reference site
Exposure duration	1. 96 hours 2. Discontinuous exposure for 96 hours, then replacement of test solutions with unspiked pond water for 7.5 days. During this time, embryos hatched and began feeding in uncontaminated conditions. After 7.5 days, again for 96 hour, re-introduction of fresh test solutions at the same exposure level as in the first 96-hour exposure period. Frequency and characterisation of deformities were calculated at hatching (day 8 of test). Survival, hatching success, and growth were estimated upon termination of the test (day 16).
Endpoint (e.g. NOEC, EC50)	1. LC50 2. EC50, LC50
Effect (e.g. reproduction, survival, growth)	1. Mortality 2. Mortality, hatching success, deformities
Concentration	96-hour LC50 >50 µg l^{-1} (highest concentration tested) 16-day LC50 = 5 µg l^{-1} 16-day EC50 (deformities) 14 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Toxicity data published in the report are quality assessed. The data base used to prepare the report was compiled from the results of tests conducted at the Columbia National Fisheries Research Laboratory in 1965–1984, and judged acceptable (by the authors) according to good laboratory practices. Test techniques were generally those of the American Society for Testing and Materials [71] and the Committee on Methods for Toxicity Tests with Aquatic Organisms [65].
Comments	

Reference	**33**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Fathead minnow
Organism (scientific name)	*Pimephales promelas*
Life stage (e.g. egg, embryo, ELS, adult)	Newly hatched larvae
Exposure regime (e.g. static, renewal, etc.)	Static Flow-through
Test method	
Analysis (measured or nominal)	Toxicant concentrations were determined weekly by gas chromatography
Temperature	23.5–26°C
Hardness	Test water was sand-filtered Lake Superior water sterilised with ultraviolet light. Mean total hardness 45.8 mg l^{-1}
pH	7.4–7.8
Salinity	Freshwater
Exposure duration	96 hours 32 days
Endpoint (e.g. NOEC, EC50)	
Effect (e.g. reproduction, survival, growth)	Mortality, growth (weight)
Concentration	Static 96-hour LC50 4.3 mg l^{-1}, flow-through LC50 6.9 mg l^{-1} 32-day NOEC growth 50 µg l^{-1} (LOEC 90 µg l^{-1}); 32-day NOEC survival 140 µg l^{-1} (LOEC 290 µg l^{-1})
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

Reference	**34**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Copepod
Organism (scientific name)	*Acartia tonsa*
Life stage (e.g. egg, embryo, ELS, adult)	Adult copepodes
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	
Analysis (measured or nominal)	Measured
Temperature	17 $\pm$ 1°C
Hardness	Not reported
pH	Not reported
Salinity	Natural sea water diluted to 20‰ salinity with glass-redistilled water
Exposure duration	96 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	2.57 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

Reference	**35**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Kuruma prawn
Organism (scientific name)	*Penaeus japonicus*
Life stage (e.g. egg, embryo, ELS, adult)	Juveniles, 5 g body weight
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	
Analysis (measured or nominal)	Not reported (but presumably exposure to nominal diazinon concentration)
Temperature	26°C
Hardness	Not reported
pH	Not reported
Salinity	ca. 18‰
Exposure duration	24 hours
Endpoint (e.g. NOEC, EC50)	100% of prawns exposed to 100 µg l^{-1} diazinon (the only test concentration) dead after 6 hours
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	100 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Results not suitable to derive EQS. Objective of test was to determine to what extent piperonyl butoxide may reduce toxicity of several organophosphate pesticides.
Comments	

Reference	**36**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Midge
Organism (scientific name)	*Chironomus tentans*
Life stage (e.g. egg, embryo, ELS, adult)	Organism stock cultured according to standard operating protocols of the US EPA (1993). 4th instar larvae used for bioassays (head capsule widths between 0.63 and 0.71 mm and body length >1 cm)
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	Acute 96-hour toxicity test. Five concentrations plus control per test, three replicates per concentration, 10 larvae per test vessel (1-litre beaker). 20 g fine silica sand per beaker as sediment.
Analysis (measured or nominal)	Yes (according to Belden and Lydy 2000)
Temperature	21 $\pm$ 1°C
Hardness	Not reported
pH	Not reported
Salinity	Not reported
Exposure duration	96 hours
Endpoint (e.g. NOEC, EC50)	EC50
Effect (e.g. reproduction, survival, growth)	Inability of organism to perform typical s-shaped swimming motion
Concentration	19.1 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

US Environmental Protection Agency (US EPA), 1993 *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms* (4th edn.). EPA/600/4-90/027F. Washington, DC: US EPA, Office of Research and Development.
Belden J B and Lydy M J, 2000 *Impact of atrazine on organophosphate insecticide toxicity*. Environmental Toxicology and Chemistry, **19**, 2266–2274.

Reference	**37**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	
Organism (scientific name)	1. *Daphnia pulex* 2. *Gammarus fasciatus* 3. *Lepomis macrochirus* 4. *Oncorhynchus mykiss* 5. *Pteronarcys californica*
Life stage (e.g. egg, embryo, ELS, adult)	1. 1st instar 2. Mature 3. Juveniles, weight per individual ca. 1 g 4. Juveniles, weight per individual ca. 1.2 g 5. 2nd year class
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	
Analysis (measured or nominal)	Measured diazinon and the impurities in the test substances
Temperature	1. 15°C 2. 21°C 3. 18°C 4. 13°C 5. 15°C
Hardness	44 mg l^{-1}
pH	7.1
Salinity	Freshwater
Exposure duration	1. 48 hours 2–5. 96 hours
Endpoint (e.g. NOEC, EC50)	1. EC50 2–5. LC50
Effect (e.g. reproduction, survival, growth)	1. Not reported 2–5. Mortality
Concentration	1. 0.8 µg l^{-1} (CI 0.6–1.1 µg l^{-1}) 2. 0.2 µg l^{-1} (CI 0.15–0.28 µg l^{-1}) 3. 168 µg l^{-1} (CI 120–220 µg l^{-1}) 4. 90 µg l^{-1} 5. 25 µg l^{-1} (CI 20–30 µg l^{-1})
Initial quality assessment (e.g. good, moderate, poor)	Toxicity data published in the report are quality assessed. The database used to prepare the report was compiled from the results of tests conducted at the Columbia National Fisheries Research Laboratory in 1965–1984, and judged acceptable (by the authors) according to good laboratory practices. Test techniques were generally those of the American Society for Testing and Materials [71] and the Committee on Methods for Toxicity Tests with Aquatic Organisms [65].
Comments	

Reference	**39**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Atlantic salmon
Organism (scientific name)	*Salmo salar*
Life stage (e.g. egg, embryo, ELS, adult)	Mature male Atlantic salmon parr (98–176 mm length)
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	Measurement of trans-epithelial voltage gradients from the surface of the olfactory epithelium of anaesthetised fish. 1. The responses of the olfactory epithelium to a 10^{-9} M concentration of prostaglandin F2a (PGF2a) were recorded after perfusion of the olfactory rosette with different concentrations of diazinon (0, 0.1, 1, 2, 5, 10, 20 µg l^{-1}). 2. Dose–response studies of the olfactory epithelium were carried out using serial dilutions of PGF2a (10^{-12} – 10^{-7} M) and diazinon concentrations of 0, 0.1, 1, 5 and 10 µg l^{-1}). 3. Exposure of salmon parr to the urine from ovulated female salmon elevated plasma levels of 17,20ß-dihydroxy-4-pregnen-3-one significantly. However, exposure to nominal concentrations of diazinon ranging from 0.3 to 45 µg l^{-1} abolished this priming effect. Similar results were obtained for plasma levels of gonadotrophin II (GtH II), although slightly higher nominal concentrations of diazinon were required (0.8–45 µg l^{-1}) to abolish the priming effect of females salmon urine on the plasma levels of GtH II. 4. The levels of expressible milt were elevated significantly in male parr 3 hours after exposure to the urine from ovulated female salmon. Exposure of mature male parr to all concentrations of diazinon (0.3–3.45 µg l^{-1}) depressed this priming effect significantly and there was no significant difference in the level of expressible milt when compared to fish not exposed to female urine
Analysis (measured or nominal)	Measurement of diazinon but results expressed on the basis of nominal concentrations. Measured concentrations usually much lower than nominal ones.
Temperature	7.5–10.7°C
Hardness	405 mg l^{-1} as $CaCO_3$
pH	7.5
Salinity	Freshwater

Exposure duration	4–5 hours during experiments
Endpoint (e.g. NOEC, EC50)	NOEC
Effect (e.g. reproduction, survival, growth)	1, 2. Sensitivity of the olfactory system towards PGF2a. Significant effects at nominal 1 µg l^{-1}. NOEC 0.1 µg l^{-1} 3. Abolishment of priming effect of urine of ovulated female salmon on plasma levels of 17,20ß-dihydroxy-4-pregnen-3-one and GtH II. NOECs <0.3 and <0.8 µg l^{-1}, respectively 4. Abolishment of priming effect of urine of ovulated female salmon on expressible milt. NOEC <0.3 µg l^{-1}
Concentration	NOECs <0.3 to 1.0 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Quality of experimental design and description good.
Comments	Ecological relevance of the observed effects unclear.

Reference	**40**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Midge Scud
Organism (scientific name)	*Chironomus tentans* *Gammarus lacustris limnaeus*
Life stage (e.g. egg, embryo, ELS, adult)	Larvae (*C. tentans*)
Exposure regime (e.g. static, renewal, etc.)	Not reported (*C. tentans*) Flow-through (*G. lacustris*)
Test method	
Analysis (measured or nominal)	Not reported
Temperature	Not reported
Hardness	Not reported
pH	Not reported
Salinity	Not reported
Exposure duration	7 days (*C. tentans*) 'long-term continuous exposure' (*G. lacustris*)
Endpoint (e.g. NOEC, EC50)	LC50, LOEC (*C. tentans*) LOEC? (*G. lacustris*)
Effect (e.g. reproduction, survival, growth)	*C. tentans* LC50: mortality, LOEC: significant delay in egg hatch, increased duration of the larvae stage, slightly depressed pupation and emergence of adults, and lengthened time from eggs to adults by 33.6%. *G. lacustris* LOEC: Increase in activity
Concentration	*C. tentans* LC50: 0.27 µg l^{-1}; LOEC: 0.003 µg l^{-1} *G. lacustris* LOEC: 3 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Abstract of a PhD thesis. No quality assessment possible.
Comments	

Reference	**41**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Mixed population of algae
Organism (scientific name)	*Chlorophyta, Chrysophyta, Cyanophyta*
Life stage (e.g. egg, embryo, ELS, adult)	
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	
Analysis (measured or nominal)	Not reported, presumably nominal
Temperature	21 $\pm$ 2°C
Hardness	Not reported
pH	Not reported
Salinity	Lake Houston (Texas) water
Exposure duration	Up to 14 days
Endpoint (e.g. NOEC, EC50)	
Effect (e.g. reproduction, survival, growth)	Algal counts, Warburg tests, ATP determination, carbon analyses, biomass accumulation, chlorophyll a determination
Concentration	Preliminary testing indicated that in concentrations <1 mg l^{-1} microbial populations were little affected and, between 1 and 5 mg l^{-1}, consistent effects were observed. A standard dosage of 5 mg l^{-1} was chosen for this study.
Initial quality assessment (e.g. good, moderate, poor)	No classical toxicity test.
Comments	Study not suitable for EQS setting

Reference	**42**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Mysid shrimp
Organism (scientific name)	*Americamysis bahia*
Life stage (e.g. egg, embryo, ELS, adult)	a) Juveniles <48 hours old (96-hour LC50 test) b) Life-cycle test
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	a) 96-hour acute toxicity test b) Life-cycle test, testing protocols published (Nimmo *et al.* 1978)
Analysis (measured or nominal)	a) Analysis of toxicant concentrations twice during 96-hour test. b) Analysis of toxicant concentrations at least four times during a 28-day test (results showed variance of <20% for each concentration).
Temperature	22–25°C
Hardness	Not reported but according to cited test protocol
pH	Not reported but according to cited test protocol
Salinity	Not reported but according to cited test protocol
Exposure duration	a) 96 hours b) 28 days (1–2 life-cycles)
Endpoint (e.g. NOEC, EC50)	a) LC50 b) MATC
Effect (e.g. reproduction, survival, growth)	a) Mortality b) Reproductive success, delay in release of young, growth, abnormal behaviour, feeding activity, mortality
Concentration	a) LC50 4.82 µg l^{-1} (95% CL 4.11–5.87) Adults more sensitive than juveniles b) MATC >1.15 <3.27 (i.e. MATC = 1.94 µg l^{-1}, NOEC = 1.15 µg l^{-1}, LOEC = 3.27 µg l^{-1}).
Initial quality assessment (e.g. good, moderate, poor)	moderate to good
Comments	

Nimmo D R, Hamaker T L and Sommers C A, 1978 *Culturing the mysid (*Mysidopsis bahia*) in flowing sea water or a static system.* In Bioassay Procedures for the Ocean Disposal Permit Program. EPA-600/9-78-010. pp. 59–60. ERL,GB X107. Gulf Breeze, FL: US Environmental Protection Agency, Environmental Research Laboratory.

Nimmo D R, Hamaker T L and Sommers C A, 1978 *Entire life cycle toxicity test using mysids (*Mysidopsis bahia*) in flowing water*. In Bioassay Procedures for the Ocean Disposal Permit Program. EPA-600/9-78-010. pp. 64–68. ERL,GB X108. Gulf Breeze, FL: US Environmental Protection Agency, Environmental Research Laboratory.

Reference	**43**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Fathead minnow
Organism (scientific name)	*Pimephales promelas*
Life stage (e.g. egg, embryo, ELS, adult)	Early life stages, starting with embryos ≤24 hours old
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	ELS
Analysis (measured or nominal)	Measured (at least twice a week)
Temperature	Not reported
Hardness	44–49 mg l^{-1} as $CaCO_3$
pH	Not reported
Salinity	Carbon-filtered, UV-sterilised Lake Superior water
Exposure duration	32 days
Endpoint (e.g. NOEC, EC50)	Chronic Value (CV), NOEC, LOEC
Effect (e.g. reproduction, survival, growth)	Growth (dry weight) was the most sensitive parameter
Concentration	CV (MATC):24.97 µg l^{-1}; NOEC: 16.5 µg l^{-1}; LOEC 37.8 µg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

Reference	**47**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Rotifer
Organism (scientific name)	*Brachionus calyciflorus*
Life stage (e.g. egg, embryo, ELS, adult)	Cysts to adults
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	Life-cycle test
Analysis (measured or nominal)	Not reported
Temperature	25°C
Hardness	Synthetic freshwater
pH	7.5
Salinity	Synthetic freshwater
Exposure duration	2 days
Endpoint (e.g. NOEC, EC50)	NOEC
Effect (e.g. reproduction, survival, growth)	Intrinsic rate of increase, i.e. growth potential of the population
Concentration	8 mg l^{-1}
Initial quality assessment (e.g. good, moderate, poor)	Moderate
Comments	Focus is on design of the rotifer life-cycle test.

Reference	**48**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Green algae
Organism (scientific name)	*Scenedesmus quadricaudaa*
Life stage (e.g. egg, embryo, ELS, adult)	All life stages
Exposure regime (e.g. static, renewal, etc.)	Static conditions
Test method	Algae test (life-cycle)
Analysis (measured or nominal)	Not reported (presumably nominal)measured
Temperature	21 ± 1°C
Hardness	Not reported
pH	Not reported
Salinity	Subcultures for bioassays were established in Chu's culture media no. 10 (reference given) with added micronutrient solution (reference given) to provide trace elements. This mixture furnished a nutrient level approximating that of eutrophic lakes.
Exposure duration	Up to 10 days. Carbon assimilation and cell number determined at beginning of exposure and then every 2 days until exponential growth of algal cells terminated. At that time dry weight biomass was determined. Exposure at 2 (presumably nominal) concentrations, 0.1 and 1.0 mg l^{-1} diazinon.
Endpoint (e.g. NOEC, EC50)	NOEC
Effect (e.g. reproduction, survival, growth)	Cell number, biomass, ^{14}C uptake
Concentration	No effect on either parameter at any exposure concentration within the 10-day test interval.
Initial quality assessment (e.g. good, moderate, poor)	Moderate (nominal concentrations, only two test concentrations)
Comments	

Reference	**50**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Caddisfly
Organism (scientific name)	*Hydrospyche angustipennis*
Life stage (e.g. egg, embryo, ELS, adult)	1st instar larvae (12 days old at test initiation)
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	Static acute single species test
Analysis (measured or nominal)	Analysis of toxicant 1 hour after begin and 1 hour before end of exposure
Temperature	20°C
Hardness	210 mg l^{-1} $CaCO_3$
pH	8.2
Salinity	'Dutch Standard Water' – standardised synthetic analogue of common Dutch surface water
Exposure duration	48, 96 and 168 hours
Endpoint (e.g. NOEC, EC50)	LC50 (Ecx)
Effect (e.g. reproduction, survival, growth)	Survival (effects on larval development, gut content and growth)
Concentration	48-hour LC50: 2.9 (2.2–3.9) µg l^{-1} 96-hour LC50: 1.3 (1.2–1.5) µg l^{-1} 168-hour LC50: 1.0 (0.8–1.1) µg l^{-1} No effects up to highest exposure concentration (8 µg l^{-1}) observed on larval development, gut content and growth.
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

Reference	**50**
CAS number	333-41-5
Chemical	Diazinon
Chemical species	
Organism (common name)	Caddisfly
Organism (scientific name)	*Hydrospyche angustipennis*
Life stage (e.g. egg, embryo, ELS, adult)	5th instar larvae
Exposure regime (e.g. static, renewal, etc.)	Renewal each 24 hours
Test method	
Analysis (measured or nominal)	Analysis of toxicant 1 hour after beginning of exposure/renewal and 1 hour before end.
Temperature	20°C
Hardness	210 mg l^{-1} $CaCO_3$
pH	8.2
Salinity	'Dutch Standard Water' – standardised synthetic analogue of common Dutch surface water
Exposure duration	48-hour
Endpoint (e.g. NOEC, EC50)	ECx
Effect (e.g. reproduction, survival, growth)	Behaviour of larvae (activity), mean length of ventilation period
Concentration	Significant increase of mean length of ventilation period at highest exposure concentration (40 µg l^{-1}) Significant increase in activity of larvae at 3.4 µg l^{-1}. However, no consistent dose-response relationship observed – activity of larvae at other exposure concentrations (up to 40 µg l^{-1}) not different from control.
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

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