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## Proposed EQS for Water Framework Directive Annex VIII substances: dimethoate

Science Report – HOEP670085/SR17



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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 Environment Agency.

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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 project managed by the Environment Agency and has involved members and partners of UKTAG. It provides background information to support the ongoing development of the standards and classification methods.

While this report is considered to represent the best available scientific information and expert opinion available at the time of its completion, 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 dimethoate using the methodology described in Annex V of the Directive. There are existing EQSs for dimethoate, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for dimethoate, 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**

Dimethoate is an organophosphate insecticide used to kill mites and insects systemically and on contact. Dimethoate is not expected to persist in the water column due to biodegradation (based on a half-life of 8 days for degradation in river water). The substance is also not expected to persist in soil when released to the terrestrial compartment due to rapid degradation (77 per cent loss over 14 days). The low log K<sub>oc</sub> values of 1.26–1.56 indicate that dimethoate will not strongly sorb to soils, but would be subject to leaching. Given typical bioconcentration factor (BCF) values of <6, dimethoate is unlikely to accumulate in fish.

## **Availability of data**

Long-term laboratory data are available for eight different freshwater taxonomic groups, i.e. algae, amphibians, crustaceans, fish, hydroids, insects, macrophytes and molluscs. Freshwater short-term toxicity data are available for eight taxonomic groups, i.e. algae, amphibians, annelids, crustaceans, fish, insects, molluscs and protozoa. Freshwater invertebrates and fish are more sensitive to than algae to both technical grade dimethoate and various dimethoate formulations. For marine organisms, single species short-term toxicity data are available for five different taxonomic groups (algae, crustaceans, fish, macrophytes and molluscs). However, no long-term toxicity data are available for saltwater taxa. Laboratory data are supplemented by freshwater mesocosm data, which confirm the greater sensitivity of crustaceans to dimethoate.

Dimethoate has been shown to disrupt reproductive function in mammalian species. Although the pathogenesis of dimethoate-induced reproductive toxicity remains to be determined, a reduction in serum testosterone levels is thought to play an important role in this process. Data for snails indicate there may be endocrine-mediated effects in egg production and development, but this hypothesis needs to be substantiated.

## Derivation of PNECs

### Long-term PNEC for freshwaters

The lowest valid long-term (lt) toxicity value for freshwater invertebrates is a 21-day no observed effect concentration (NOEC) of 24 µg active ingredient (a.i.) l<sup>-1</sup> for effects of the Roxion formulation on the growth of the water flea *Daphnia magna*. Reliable long-term NOECs are available for algae, invertebrates and fish; thus, based on the EU Technical Guidance Document (TGD) methodology, an assessment factor of 10 could be applied to the lowest valid toxicity value. However, the use of this factor would result in a PNEC<sub>freshwater\_lt</sub> higher than certain short-term toxicity data (which have been found to be in the range 2.0–7.8 µg l<sup>-1</sup>) – although it is accepted that the reliability of these short-term data [which are from studies that did not meet OECD principles of Good Laboratory Practice (GLP)], is questionable (see below). This issue has been addressed by the application of larger precautionary assessment factor of 50, which provides further protection for freshwater organisms from the long-term effects of dimethoate. This results in a PNEC<sub>freshwater\_lt</sub> of 0.48 µg l<sup>-1</sup>.

This value is similar to the existing EQS of 1.0 µg l<sup>-1</sup>. This was derived by applying assessment factors of 100 and 10 to the most reliable acute and chronic data respectively; for aquatic crustaceans a 'safe' concentration in the range 10–60 µg l<sup>-1</sup> was suggested. However, the available data indicated that insects were one or two orders of magnitude more sensitive than crustaceans. Therefore, a lower EQS was proposed for the protection of freshwater life; this was expressed as an annual average because of the large safety margin between this value and the more reliable acute toxicity data.

### Short-term PNEC for freshwaters

Reliable short-term (st) data are available for algal, invertebrate and fish species. The lowest valid short-term toxicity value for freshwater invertebrates is a 48-hour EC50 of 2,000 µg a.i. l<sup>-1</sup> for the effects of technical grade dimethoate on the immobilisation of the water flea *Daphnia magna*. Lower short-term toxicity values have been reported in non-GLP studies, but these are considered to be unreliable due to the absence of measured concentration data and, in some instances, because of the collection of organisms from the field. Given the issues with the reliability of the data from the non-GLP studies, a larger precautionary assessment factor of 500 applied to the lowest valid toxicity value has been adopted, resulting in a PNEC<sub>freshwater\_st</sub> of 4.0 µg l<sup>-1</sup>.

There is no current short-term EQS for freshwaters.

### Long-term PNEC for saltwaters

No long-term single species toxicity data for marine organisms are available. The absence of long-term data means that it is not possible to generate a PNEC<sub>saltwater\_lt</sub> based on the saltwater data alone, and it is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation. Reliable long-term NOECs are available for algae, invertebrates and fish; therefore, based on the methodology outlined in the TGD, an assessment factor of 10 could be applied to the lowest valid toxicity value. However, the application of this factor to the a 21-day NOEC of 24 µg a.i. l<sup>-1</sup> for effects of the Roxion formulation on the growth of the water flea *Daphnia magna*, would result in a PNEC<sub>freshwater\_lt</sub> higher than certain short-term toxicity data (which have been found to be in the range 2.0–7.8 µg l<sup>-1</sup>) – although it is accepted that the reliability of these short-term data, which are from studies that did not meet OECD principles of Good Laboratory Practice (GLP), is questionable. This issue has been addressed by the application of larger precautionary assessment factor of 50, which would provide further protection for saltwater organisms from the long-term effects of dimethoate. This results in a PNEC<sub>saltwater\_lt</sub> of 0.48 µg l<sup>-1</sup>.

There is no current long-term EQS for saltwaters.

### Short-term PNEC for saltwaters

Single species short-term toxicity data for marine organisms are available for five different taxonomic groups, i.e. algae, crustaceans, fish, macrophytes and molluscs. However, all these data were deemed to be uncertain or not assignable based on Klimisch Code criteria. Therefore, it is proposed that the PNEC<sub>saltwater\_st</sub> is based on the combined freshwater and saltwater dataset.

The lowest valid short-term toxicity value for freshwater invertebrates is a 48-hour EC50 of 2,000 µg a.i. l<sup>-1</sup> for the effects of technical grade dimethoate on the immobilisation of the water flea *Daphnia magna*. Lower short-term toxicity values have been reported in non-GLP studies, but these are considered to be unreliable due to the absence of measured concentration data and, in some instances, because of the collection of organisms from the field. Given the issues with the reliability of the data from the non-GLP studies, a larger precautionary assessment factor of 500 applied to the lowest valid toxicity value has been adopted resulting in a PNEC<sub>freshwater\_st</sub> of 4.0 µg l<sup>-1</sup>.

There is no current short-term EQS for saltwaters.

### PNECs for sediment

Since the log Kow of dimethoate is <3, the derivation of PNECs for the protection of benthic organisms is not required according to the TGD. Furthermore, although a sediment toxicity study is available, it is not considered appropriate for the derivation of a PNEC<sub>sediment</sub>.

### PNECs for secondary poisoning

Bioconcentration data (as BCF values) for dimethoate for the majority of aquatic organisms are low, with values for fish ranging from 1 to 6. Hence, the TGD BCF trigger of 100 is not exceeded and the derivation of a PNEC in whole fish for secondary poisoning of predators is not required.

### **Summary of proposed PNECs**

Receiving medium/exposure scenario	Proposed PNEC (µg l <sup>-1</sup> )	Existing EQS (µg l <sup>-1</sup> )
Freshwater/long-term	0.48	1.0
Freshwater/short-term	4.0	–
Saltwater/long-term	0.48	–
Saltwater/short-term	4.0	–
Sediment	Not required	–
Secondary poisoning	Not required	–

### **Analysis**

The data quality requirements are that, at one third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing gas chromatography/mass spectrometry (GC-MS) and capable of achieving detection limits as low as 50 ng l<sup>-1</sup> should offer adequate performance to analyse for dimethoate.

### **Implementation issues**

These PNECs are suitable for use as EQSs because analytical capability is adequate for compliance assessment purposes. Since additional testing to reduce uncertainty would likely result in standards that would not comply with the 'no deterioration' principle, adoption of the existing EQS as an interim value is not suitable. Instead, the proposed PNECs, similar in value to the existing EQS, are recommended.

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# 1 Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)<sup>1</sup> 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 dimethoate using the methodology described in Annex V of the Directive. There is an existing EQSs for dimethoate, but the method used to derive this is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for dimethoate, 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.<sup>2</sup> 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 of the WFD. 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 dimethoate.

## 1.1 Properties and fate in water

Dimethoate is an organophosphate insecticide used to kill mites and insects systemically and on contact. Dimethoate is not expected to persist in the water column due to biodegradation (based on a half-life of 8 days for degradation in river water). The substance is also not expected to persist in soil when released to the terrestrial compartment due to rapid degradation (77 per cent loss over 14 days). The low log K<sub>oc</sub> values of 1.26–1.56 indicate that dimethoate will not strongly sorb to soils, but would be subject to leaching. Given typical bioconcentration factor (BCF) values of <6, dimethoate is unlikely to accumulate in fish.

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<sup>1</sup> *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](http://www.eu.int/comm/environment/water/water-framework/index_en.html)

<sup>2</sup> Data quality assessment sheets are provided in Annex 1 of this report.

## 2 Results and observations

### 2.1 Identity of substance

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

**Table 2.1 Species covered by this report**

Name	CAS Number
dimethoate	60-51-5

### 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 (ECB 2003).

Section 2.6 summarises the effects data identified from the literature for dimethoate. 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	TGD deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater short-term	4.0 µg l <sup>-1</sup>	–	–
Freshwater long-term	0.48 µg l <sup>-1</sup>	Insufficient data	1.0 µg l <sup>-1</sup> (AA)
Saltwater short-term	4.0 µg l <sup>-1</sup>	–	–
Saltwater long-term	0.48 µg l <sup>-1</sup>	Insufficient data	*
Sediment	Not required	–	–
Secondary poisoning	Not required	–	–

AA = annual average

AF = assessment factor

SSD = species sensitivity distribution

\* No EQS proposed due to a lack of data, but the freshwater EQS should be adopted as interim guideline until further data become available,

### 2.3 Hazard classification

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

**Table 2.3 Hazard classification**

R-phrases and labelling	Reference
R20, 22, 51, 53 S 36, 60, 61	ECB 2006

## 2.4 Physical and chemical properties

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

**Table 2.4 Physical and chemical properties of dimethoate**

Property	Value	Reference
CAS number	60-51-5	Tomlin 2003
Substance name	O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate	Tomlin 2003
Molecular formula	C <sub>5</sub> H <sub>12</sub> NO <sub>3</sub> PS <sub>2</sub>	Tomlin 2003
Molecular structure		Chemfinder 2006
Molecular weight	229.30	Tomlin 2003
Colour/form	Pure dimethoate – colourless crystalline solid Technical dimethoate – off-white crystals to grey semi-crystalline material	Chemfinder 2006
Odour	Camphor (mercaptan)-like odour	HSDB 2006
Melting point (°C)	50.0–51.5 (99.5% purity)	EU Draft Assessment Report (EU DAR) 2005*
Boiling point (°C)	86°C at 0.0013 kPa	IPCS 2001
Vapour pressure	2.47 x 10 <sup>-4</sup> Pa at 25°C	EU DAR 2005
Density/specific gravity	1.31 (99.5% purity)	EU DAR 2005
Henry's Law constant	1.42 x 10 <sup>-6</sup> Pa m <sup>3</sup> mol <sup>-1</sup>	EU DAR 2005
Solubility	39.8 g l <sup>-1</sup> in water at 25°C (and pH 7) 1390 g l <sup>-1</sup> in acetone at 25°C 1500 g l <sup>-1</sup> in dichloromethane at 25°C 1590 g l <sup>-1</sup> in methanol at 25°C 313 g l <sup>-1</sup> in xylene at 25°C	EU DAR 2005

\* Prepared under Council Directive 91/414/EEC. Submitted by the rapporteur Member State (UK) for assessment on behalf of the European Commission to the Pesticide Risk Assessment Peer Review Unit (PRAPeR) of the European Food Safety Authority. Referred to subsequently in this report as EU DAR 2005.

The Food and Agricultural Organization (FAO) specification for plant protection products lists the main impurities of dimethoate as omethoate, isodimethoate and water, with dimethoate not being less than 950 g kg<sup>-1</sup> in the technical product in the 2005 specification for dimethoate (FAO 2005).

Dimethoate is less soluble in water than in organic solvents such as acetone, dichloromethane and methanol.

## 2.5 Environmental fate and partitioning

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

**Table 2.5 Environmental fate and partitioning of dimethoate**

Property	Value	Reference
Abiotic fate	The OH rate constant has been estimated at $7.9 \times 10^{-11} \text{ cm}^3 \text{ molecule sec}^{-1}$ at 25°C. The vapour phase half-life in the atmosphere is estimated at 2.83 days.	NIST 2005 Spectrum Laboratories 2006
Speciation	Dimethoate does not dissociate.	EU DAR 2005
Hydrolytic stability	Dimethoate is relatively stable in aqueous media at pH 5–7 with a half-life of 68–156 days (at 25°C), but hydrolyses in alkaline solutions with a half-life of 4.4 days at pH 9.	EU DAR 2005
Photostability	Dimethoate is photostable, with a half-life >175 days being reported at pH 5.	Tomlin 2003
Volatilisation	Volatilisation is not expected to be an important environmental fate process based on a Henry's Law constant of $1.42 \times 10^{-6} \text{ Pa m}^3 \text{ mol}^{-1}$ .	Tomlin 2003
Distribution in water/sediment systems (active substances)	Dimethoate is not expected to adsorb to sediments or suspended particles due to a low log K <sub>oc</sub> value (1.26–1.56).	Extoxnet 1996
Metabolites	When dimethoate hydrolyses in surface water with a pH >7.0, relatively high levels of both O-desmethyl dimethoate and O,O-dimethyl phosphorothioate would be expected to be formed.	EU DAR 2005
Degradation in soil	Dimethoate is of low persistence in soil with a half-life of 4–16 days. A loss of 77% of dimethoate has been reported in non-sterile clay loam soil after 2 weeks. Dimethoate weakly adsorbs to soil particles and therefore may be subject to leaching. It is degraded by hydrolysis, particularly in alkaline soils. Losses of 23–40% have been reported due to evaporation.	Extoxnet 1996
Biodegradation	Dimethoate was not readily biodegradable in a laboratory Organisation for Economic Co-operation and Development (OECD) 301 (D) test. In natural waters, a degradation half-life of 8 days has been reported.	EU DAR 2005 HSDB 2006

Property	Value	Reference
Octanol–water coefficient (Log Kow)	0.704	SRC 2006
Log Koc	1.26 in clay loam soil, 1.56 in clay soil	HSDB 2006
Bioaccumulation BCF	Bioconcentration factors ranging from 2.7–6.0 and 1.1–2.4 have been determined in <i>Cyprinus carpio</i> (carp) using exposure concentrations of 0.1 and 1.0 mg l <sup>-1</sup> dimethoate respectively. In another study, a BCF of 2 was reported. Dimethoate is therefore not expected to accumulate in fish.	HSDB 2006

In the aqueous environment, dimethoate may biodegrade in natural waters based on a half-life of 8 days for degradation in river water (HSDB 2006). It is not expected that dimethoate will adsorb to sediment given reported log Koc values of 1.26–1.56 – an assumption consistent with the results of a study by Heintze (2002) (see Section 2.6.3). In addition, dimethoate is unlikely to accumulate in fish given typical BCF values of <6.

Dimethoate is relatively hydrolytically stable in aqueous media at pH 5–7 with a half-life of 68–156 days (at 25°C), but hydrolyses in alkaline solutions with a half-life of 4.4 days at pH 9 (EU DAR 2005). Volatilisation is not expected to be an important environmental fate process based on a Henry's law constant of  $1.42 \times 10^{-6}$  Pa m<sup>3</sup> mol<sup>-1</sup> (Tomlin 2003). A half-life of 8 days in raw river water has been reported (Extoxnet 2006), which means that the substance is not expected to persist in the water column.

On the basis of log Koc values of 1.26–1.56, dimethoate would not adsorb to soils but would be subject to leaching. An important removal mechanism of dimethoate is by biodegradation, especially given that a 77 per cent loss was reported in clay loam soil after 2 weeks (Extoxnet 1996). However, the rate of biodegradation will depend on the soil type and the microorganisms present in the soil. Based on half-lives of approximately 4–20 days of dimethoate in soils and given that the substance readily hydrolyses in alkaline solutions, dimethoate may be susceptible to hydrolysis in moist, basic soils. As a result, dimethoate is not expected to persist in soil when released to the terrestrial compartment.

## 2.6 Effects data

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

Data collation followed a tiered approach.

First, critical freshwater and marine data were compiled from the existing EQS document. Further data published after derivation of the current UK EQS were then retrieved from:

- the US Environmental Protection Agency (US EPA) ECOTOX database;<sup>3</sup>
- the Draft Assessment Report prepared under Council Directive 91/414/EEC (EU DAR 2005);
- sources such as ScienceDirect<sup>4</sup> and the World Health Organization (WHO), e.g. Environmental Health Criteria (EHC) 90 (WHO 1989).

<sup>3</sup> <http://www.epa.gov/ecotox/>

<sup>4</sup> <http://www.sciencedirect.com/>

Dimethoate (an organophosphate insecticide which acts by combining with and inactivating the enzyme acetylcholinesterase) can be released to the environment in a number of chemical forms, for example, technical dimethoate or as part of a formulation. When evaluating the available data, it is therefore necessary to consider the form used to test toxicity to freshwater or saltwater organisms. It is also important to compare the toxicity of metabolites resulting from degradation in water such as:

- O-desmethyl dimethoate
- O,O-dimethyl phosphorothioate
- O,O-dimethyl phosphate

The descriptions of the large number of the toxicity studies cited in the existing EQS report (Murgatroyd and Patel 1994) do not indicate the form of dimethoate used in toxicity testing. This has limited the extent to which comparisons of toxicity can be made between forms of dimethoate.

## 2.6.1 Toxicity to freshwater organisms

Freshwater toxicity data on dimethoate are available for various taxonomic groups. Long-term toxicity data are available for eight taxonomic groups (algae, amphibians, crustaceans, fish, hydroids, insects, macrophytes and molluscs), with invertebrates and fish being more sensitive than algae. Short-term toxicity tests are available for eight taxonomic groups (algae, amphibians, annelids, crustaceans, fish, insects, molluscs and protozoa), with invertebrates and fish being more sensitive than other taxa.

Table 2.6 summarises the available data on the effects of different forms of dimethoate to freshwater organisms in long-term and short-term toxicity studies.

**Table 2.6 Summary of data availability**

Type of data	Form of substance	Taxonomic groups for which information is available
Long-term	Technical grade dimethoate	Algae, crustaceans, insects, fish and molluscs
	Roxion formulation*	Algae, crustaceans, fish
	Not stated	Algae, amphibians, crustaceans, fish, hydroids, insects and macrophytes
Short-term	Technical grade dimethoate	Algae, crustaceans, insects, fish, molluscs and protozoa
	Roxion formulation*	Algae, crustaceans and fish
	Rogor formulation**	Annelids, insects and fish
	Not stated	Algae, amphibians, crustaceans, insects and fish

\* Contains 38.9% weight/weight (w/w) dimethoate as the active ingredient (a.i.).

\*\* Typically contains 30% w/w dimethoate.

Overall, the available short-term and long-term toxicity test data indicate that aquatic invertebrates are more sensitive than algae and fish to both technical grade dimethoate and formulations containing dimethoate. The greater toxicity of technical grade dimethoate and the various formulations to aquatic invertebrates compared with other taxonomic groups (where data using comparable experimental designs are available) is consistent with the use of the substance as an insecticide.

Table 2.7 provides a comparison of the toxicity of technical grade dimethoate and the Roxion formulation (based on the concentration of active ingredient) to specific species – algal (*Selenastrum capricornutum*), invertebrate (*Daphnia magna*) and fish (*Oncorhynchus mykiss*) – measured in studies carried out using standardised procedures that were compliant with Good Laboratory Practice (GLP) (see Annex 1).

**Table 2.7 Relative toxicity of different formulations to three species**

Data type	Form of substance	Freshwater toxicity data*		
		Alga ( <i>S. capricornutum</i> )	Invertebrate ( <i>D. magna</i> )	Fish ( <i>O. mykiss</i> )
Long-term	Technical grade dimethoate	72-hour NOEC = 30,500 µg a.i. l <sup>-1</sup>	21-day NOEC = 40 µg a.i. l <sup>-1</sup>	21-day NOEC = 400 µg a.i. l <sup>-1</sup>
	Roxion formulation	72-hour NOEC = 56,600 µg a.i. l <sup>-1</sup>	21-day NOEC = 24 µg a.i. l <sup>-1</sup>	21-day NOEC = 290 µg a.i. l <sup>-1</sup>
Short-term	Technical grade dimethoate	72-hour EC50 = 90,400 µg a.i. l <sup>-1</sup>	48-hour EC50 = 2,000 µg a.i. l <sup>-1</sup>	96-hour LC50 = 30,200 µg a.i. l <sup>-1</sup>
	Roxion formulation	72-hour EC50 = 93,300 µg a.i. l <sup>-1</sup>	48-hour EC50 = 2,200 µg a.i. l <sup>-1</sup>	96-hour LC50 = 24,500 µg a.i. l <sup>-1</sup>

\* From Tables 2.8 and 2.9.

NOEC = no observed effect concentration

EC50 = concentration effective against 50% of the organisms tested

LC50 = concentration lethal to 50% of the organisms tested

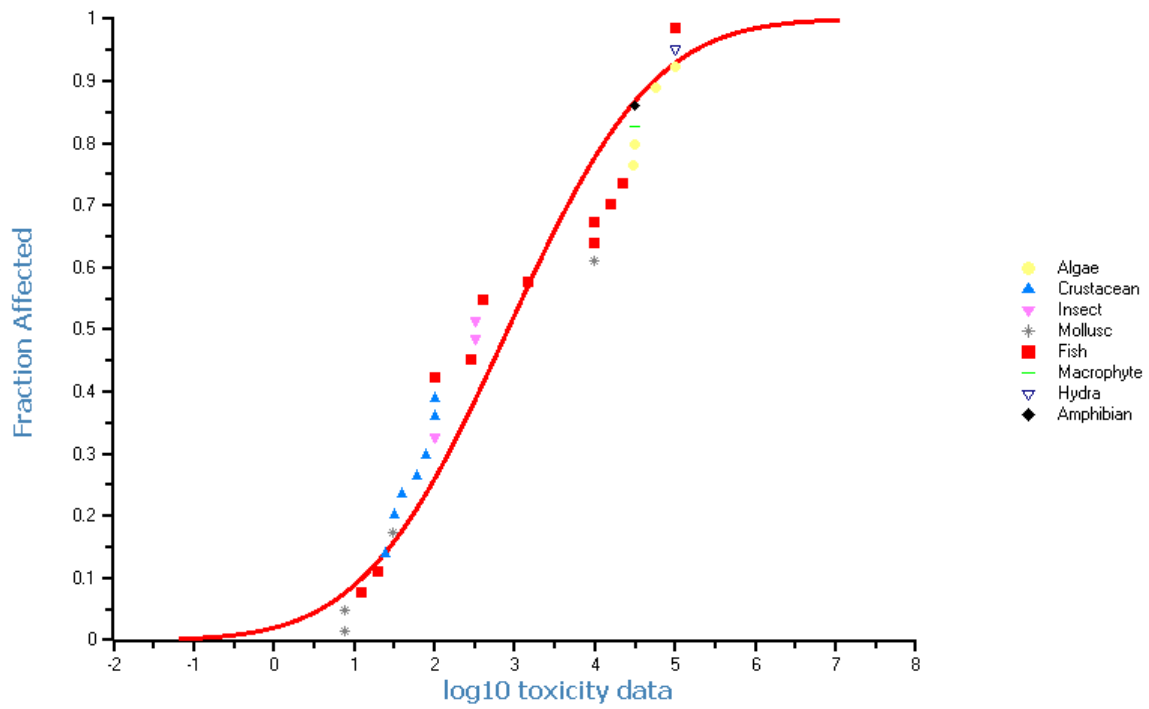
a.i. = active ingredient

These comparative data indicate that, based on the concentration of the active ingredient, the Roxion formulation is of similar short-term and long-term toxicity to the algal, invertebrate and fish species as technical grade dimethoate. However, the toxicity data set for different forms of dimethoate is variable. Therefore, a relatively large difference between the toxicity data for different forms of dimethoate would be needed before it can be concluded that one form of dimethoate was consistently more toxic than others. As there is not a clear difference between the toxicity of different forms of dimethoate, all the available data have been used to derive the PNECs and no distinction has been made between technical grade dimethoate and formulations containing dimethoate.

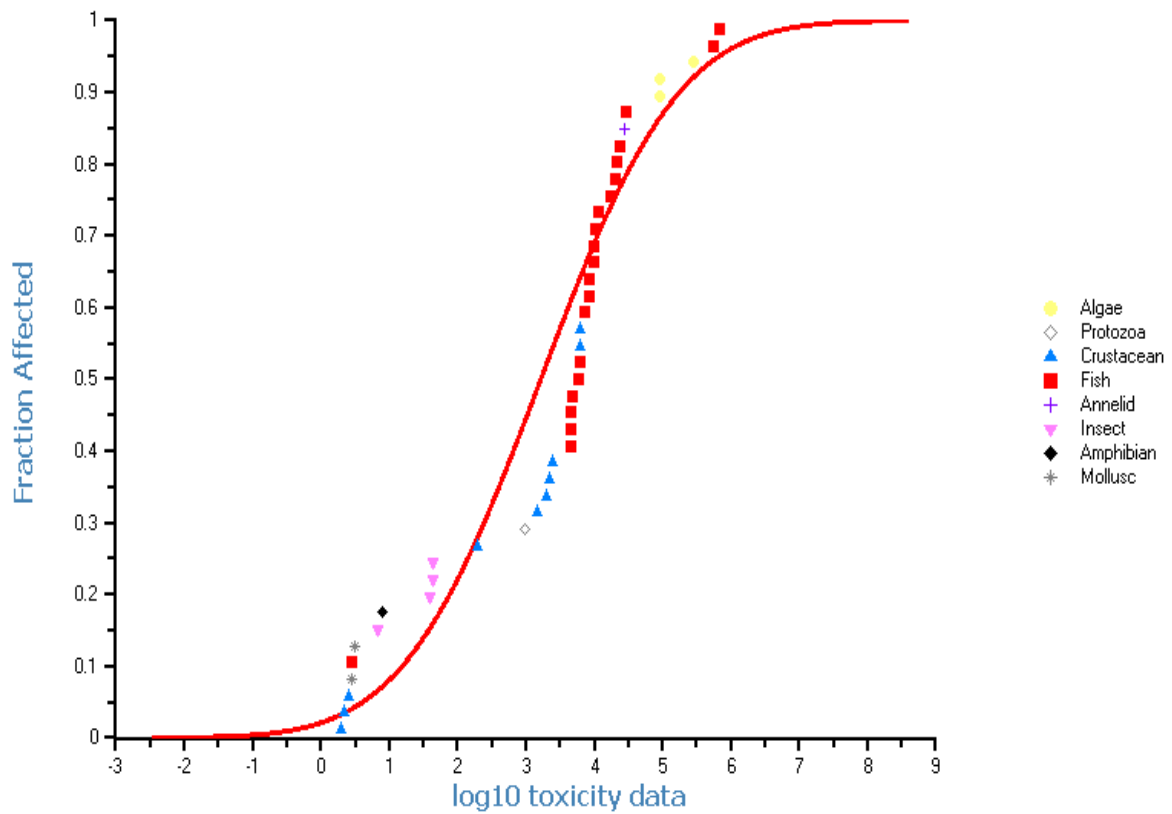
The available short-term data indicate that the breakdown metabolites (*O*-desmethyl dimethoate, *O,O*-dimethyl phosphorothioate and *O,O*-dimethyl phosphate) are of markedly lower toxicity than technical grade dimethoate. Short-term toxicity values for algae, crustaceans and fish in studies compliant with GLP were >70,000 µg a.i. l<sup>-1</sup> (the highest exposure concentration) in all cases (Hertl 2001a, Hertl 2001b, Hertl 2001c, Hertl 2002a, Hertl 2002b, Hertl 2002c, Hertl *et al.* 2002).

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for dimethoate 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 dimethoate PNECs. The lowest critical freshwater data for dimethoate are presented in Tables 2.8 (for long-term data) and 2.9 (for short-term data).

**Figure 2.1** Cumulative distribution function of freshwater long-term data ( $\mu\text{g a.i. l}^{-1}$ ) for dimethoate



**Figure 2.2** Cumulative distribution function of freshwater short-term data ( $\mu\text{g a.i. l}^{-1}$ ) for dimethoate



**Table 2.8 Most sensitive long-term aquatic toxicity data for freshwater organisms exposed to dimethoate**

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration	Conc. ( $\mu\text{g a.i. l}^{-1}$ )	Exposure <sup>1</sup>	Toxicant analysis <sup>2</sup>	Comments	Reliability (Klimisch Code*)	Reference
Dimethoate (technical grade)	<i>Selenastrum capricornutum</i>	Green algae	ALG	NOEC	Growth	72 hours	30,500	s	y		1	Caley <i>et al.</i> 1992c**
	<i>Daphnia magna</i>	Water flea	CRU	NOEC	Reproduction	21 days	40	ss	y		1	Wuthrich 1990b**
	<i>Helisoma trivolvis</i>	Snail	MOL	NOEC	Growth	63 days	7.5 (estimate)	ss	n	24°C	3	Aboul-Eta and Khalil 1987a
	<i>Helisoma trivolvis</i>	Snail	MOL	NOEC	Reproduction	21 days	7.5 (estimate)	ss	n	24°C	3	Aboul-Eta and Khalil 1987a
	<i>Helisoma trivolvis</i>	Snail	MOL	NOEC	Egg survival and hatching	14 days	7.5 (estimate)	s	n	24°C	3	Aboul-Eta and Khalil 1987a
	<i>Oncorhynchus mykiss</i>	Rainbow trout (juveniles)	FIS	NOEC	Growth	21 days	400	f	y	11–13.5°C	1	Wuthrich 1990a**
	<i>Oncorhynchus mykiss</i>	Rainbow trout (larvae)	FIS	NOEC	Growth	96 days	1,500	f	y	9.4–11.3°C	1	Strawn and Muckerman 1994**
Roxion formulation (400 a.i. g/l)	<i>Selenastrum capricornutum</i>	Green algae	ALG	NOEC	Growth	72 hours	56,600	s	y		1	Caley <i>et al.</i> 1992d**
	<i>Daphnia magna</i>	Water flea	CRU	NOEC	Growth	21 days	24	ss	y		1	Caley <i>et al.</i> 1992g**
	<i>Oncorhynchus mykiss</i>	Rainbow trout (juveniles)	FIS	NOEC	Physiology	21 days	290	f	y		1	Caley <i>et al.</i> 1992f**
Not stated	<i>Microcystis aeruginosa</i>	Blue green	ALG	NOEC	Growth rate	4 days	32,000	s	n	23°C	3	Sloof and Canton 1983
	<i>Lemna minor</i>	Duckweed	MAC	NOEC	Growth	7 days	32,000	s	n	23°C	3	Sloof and Canton 1983
	<i>Daphnia magna</i>	Water flea	CRU	LC0	Lethality	21 days	32	ss	n	19°C	3	Sloof and Canton 1983
	<i>Daphnia magna</i>	Water flea	CRU	NOEC	Reproduction	21 days	100	ss	n	19°C	3	Sloof and Canton 1983
	<i>Hydra oligactis</i>	Hydroid	HYD	NOEC	Growth	21 days	100,000	ss	n	18°C	3	Sloof and Canton 1983
	<i>Culex pipens</i>	Mosquito larvae	INS	NOEC	Development	25 days	320	ss	n	27°C	3	Sloof and Canton 1983
	<i>Danio rerio</i>	Zebrafish eggs	FIS	NOEC	Lethality	12 days	12.5	ss	n	ND	3	Grande <i>et al.</i> 1994
	<i>Salmo trutta</i>	Brown trout eggs	FIS	NOEC	Lethality	45 days	20	ss	n	9.5°C	3	Grande <i>et al.</i> 1994
	<i>Xenopus laevis</i>	Clawed toad tadpoles	AMP	NOEC	Development	100 days	32,000	ss	n	20°C	3	Sloof and Canton 1983

\* See Annex 1. \*\* Cited in EU DAR 2005; Estimate – the level of response at this concentration was not apparently different from that in the controls but was not confirmed.

<sup>1</sup> Exposure: s = static; ss = semi-static; f = flow-through. <sup>2</sup> Toxicant analysis: y = measured; n = nominal.

ALG = algae; AMP = amphibians; CRU = crustaceans; FIS = fish; HYD = hydra; INS = insects; MAC = macrophytes; MOL = molluscs

ND = no data

NOEC = no observed effect concentration

LC0 = concentration lethal to 0% of the organisms tested

**Table 2.9 Most sensitive short-term aquatic toxicity data for freshwater organisms exposed to dimethoate**

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. ( $\mu\text{g a.i. l}^{-1}$ )	Exposure <sup>1</sup>	Toxicant analysis <sup>2</sup>	Comments	Reliability (Klimisch Code*)	Reference
Dimethoate (technical grade)	<i>Selenastrum capricornutum</i>	Green algae	ALG	ECb50	Growth	72	90,400	s	y		1	Caley <i>et al.</i> 1992c**
	<i>Tetrahymena pyriformis</i>	Ciliate	PRO	LOEC	Growth	96	1,000	s	n	27±1°C	3	Kumar <i>et al.</i> 1989
	<i>Cyclops strensus</i>	Copepod	CRU	LC50	Lethality	96	2.0			27±2°C	3	Aboul-Eta and Khalil 1987b
	<i>Daphnia longispina</i>	Water flea	CRU	LC50	Lethality	96	2.6	s	n	27±2°C	3	Aboul-Eta and Khalil 1987b
	<i>Daphnia magna</i>	Water flea	CRU	EC50	Immobilisation	48	2,000	s	y		1	Hertl 2002d*
	<i>Gammarus pulex</i>	Amphipod	CRU	LC50	Lethality	96	2.2	s	n	27±2°C	3	Aboul-Eta and Khalil 1987b
	<i>Biomphalaria alexandrina</i>	Snail	MOL	LC50	Lethality	96	3.1	s	n	27±2°C	3	Aboul-Eta and Khalil 1987b
	<i>Bulinus truncatus</i>	Snail	MOL	LC50	Lethality	96	2.9	s	n	27±2°C	3	Aboul-Eta and Khalil 1987b
	Species of Mugilidae	Mullet	FIS	LC50	Lethality	96	2.3	s	n	27±2°C	3	Aboul-Eta and Khalil 1987b
	<i>Tilapia nilotica</i>	Nile tilapia	FIS	LC50	Lethality	96	5.2	s	n	27±2°C	3	Aboul-Eta and Khalil 1987b
	<i>Oncorhynchus mykiss</i>	Rainbow trout	FIS	LC50	Lethality	96	30,200	s	y		1	Bathe 1982
Roxion formulation (400 a.i. g/l)	<i>Selenastrum capricornutum</i>	Green algae	ALG	ECb50	Growth	72	93,300	s	y		1	Caley <i>et al.</i> 1992d**
	<i>Daphnia magna</i>	Water flea	CRU	EC50	Immobilisation	48	2,200	s	y		1	Caley <i>et al.</i> 1992e**
	<i>Lepomis macrochirus</i>	Bluegill sunfish	FIS	LC50	Lethality	96	17,600	ss	y		1	Caley <i>et al.</i> 1992a**
	<i>Oncorhynchus mykiss</i>	Rainbow trout	FIS	LC50	Lethality	96	24,500	ss	y		1	Caley <i>et al.</i> 1992b**
Rogor formulation	<i>Hirudo medicinalis</i>	Leech	ANN	LC50	Lethality	48	28,000	ND	ND	18–21°C	-	Koslovskaya <i>et al.</i> 1986
	<i>Baetis rhodani</i>	Mayfly larvae	INS	LC50	Lethality	96	7.0	s	n	15±1°C	3	Baekken and Aanes 1994
	<i>Saccobranchnus fossilis</i>	Singhi (5–10 g)	FIS	LC50	Lethality	96	4,570	s	n	18°C	-	Verma <i>et al.</i> 1982

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. ( $\mu\text{g a.i. l}^{-1}$ )	Exposure <sup>1</sup>	Toxicant analysis <sup>2</sup>	Comments	Reliability (Klimisch Code*)	Reference
Not stated	<i>Chlorella pyrenoidosa</i>	Green algae	ALG	EC50	Growth	48	290,000	s	n	23±2°C	-	Canton and Slooff 1979
	<i>Daphnia magna</i>	Water flea	CRU	EC50	Immobilisation	48	6400	s	y	19°C	-	Hermens <i>et al.</i> 1984
	<i>Pteronarcys californica</i>	Stonefly nymph	INS	LC50	Lethality	96	43	s	n	16°C	-	Cope 1965
	<i>Channa gachua</i>	Chingatta	FIS	LC50	Lethality	96	4480	s	n		-	Alabaster 1969
	<i>Rana hexadactyla</i>	Frog (Tadpoles)	AMP	LC50	Lethality	96	7.8	s	n	12–17°C	3	Khangarot <i>et al.</i> 1985

\* See Annex 1.

\*\* Cited in EU DAR 2005; Estimate – the level of response at this concentration was not apparently different from that in the controls but was not confirmed

<sup>1</sup> Exposure: s = static; ss = semi-static.

<sup>2</sup> Toxicant analysis: y = measured; n = nominal.

ALG = algae; AMP = amphibians; ANN = annelids; CRU = crustaceans; FIS = fish; INS = insects; MOL = molluscs; PRO = protozoans

ECb = effective concentration (biomass)

LOEC = lowest observed effect concentration

EC50 = concentration effective against 50% of the organisms tested

LC50 = concentration lethal to 50% of the organisms tested

ND = no data

## 2.6.2 Toxicity to saltwater organisms

Single species short-term toxicity data referring to marine organisms are available for five different taxonomic groups (i.e. algae, crustaceans, fish, macrophytes and molluscs), with crustaceans apparently being the most sensitive group. No long-term toxicity data were located for saltwater taxa.

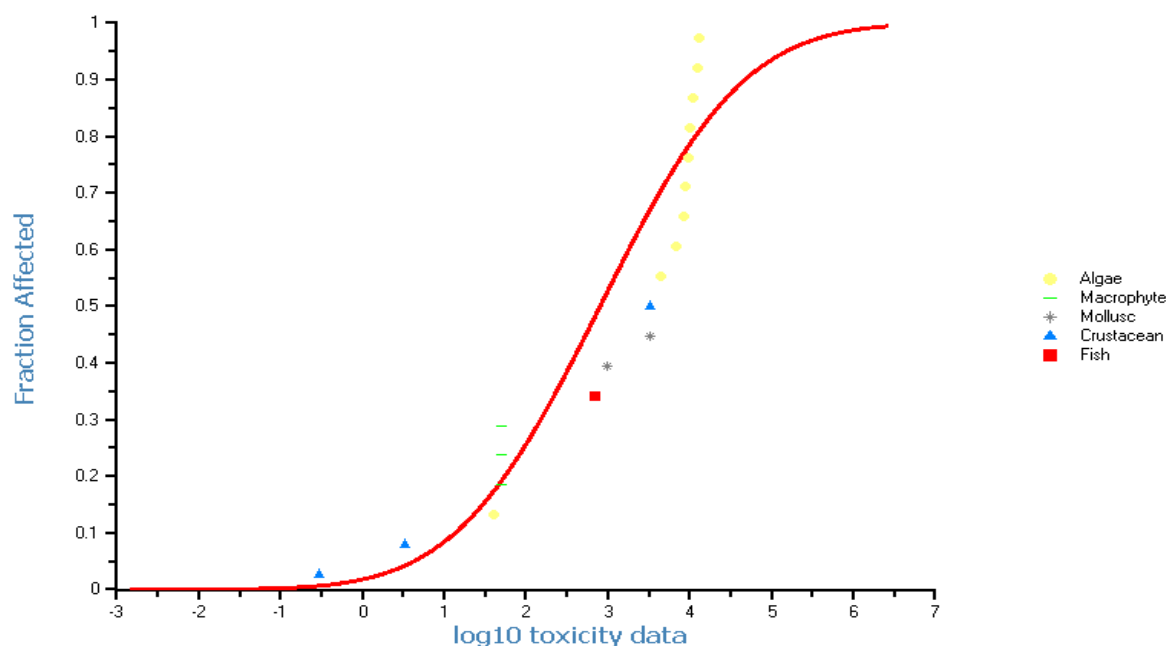
Several points can be made about the quality of the available short-term toxicity data:

- All the data were from studies where there was no analytical confirmation of the exposure concentrations. In addition, there was no indication for most studies of the form of dimethoate used in the toxicity test.
- Some of the studies showing low toxicity values were from investigations conducted using warm water species at temperatures of 28–30°C, which cannot be considered representative of potential impacts in the majority of European waters.

The lowest critical short-term toxicity data for marine species are summarised in Table 2.10. A diagrammatic representation of the available short-term saltwater data (cumulative distribution function) for dimethoate is presented in Figure 2.3. This diagram includes all data regardless of quality and provides an overview of the spread of the available data. The diagram is not a species sensitivity distribution and has not been used to set dimethoate PNECs.

No long-term data for the toxicity of dimethoate to saltwater organisms were located.

**Figure 2.3** Cumulative distribution function of saltwater short-term data ( $\mu\text{g a.i. l}^{-1}$ ) for dimethoate



**Table 2.10 Most sensitive short-term aquatic toxicity data for saltwater organisms exposed to dimethoate**

Form of substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration (hours)	Conc. ( $\mu\text{g a.i. l}^{-1}$ )	Exposure <sup>1</sup>	Toxicant analysis <sup>2</sup>	Comment	Reliability (Klimisch Code*)	Reference
Not stated	<i>Coscinodiscus concinnus</i>	Diatom	ALG	EC50	Growth rate	96	40	s	n	28°C; salinity 35‰	4	Ramachandran <i>et al.</i> 1980
	<i>Enteromorpha intestinalis</i>	Macrophyte	MAC	LOEC	Respiration	6	50	s	n	30°C; salinity 31‰	3	Ramachandran <i>et al.</i> 1984
	<i>Gracilaria verrucosa</i>	Macrophyte	MAC	LOEC	Respiration	6	50	s	n	30°C; salinity 31‰	3	Ramachandran <i>et al.</i> 1984
	<i>Grateloupia doryphora</i>	Macrophyte	MAC	LOEC	Respiration	6	50	s	n	30°C; salinity 31‰	3	Ramachandran <i>et al.</i> 1984
	<i>Crangon crangon</i>	Brown shrimp (adults)	CRU	LC50	Lethality	48	>0.3 (<)1.0	s	n	15°C	3	Portmann and Wilson 1971
Cygon formulation	<i>Crassostrea virginica</i>	Oyster (juveniles)	MOL	LOEC	Shell growth	96	1,000	f	n	20°C; salinity 31‰	3	Butler 1964
Rogor formulation	<i>Therapon jarbua</i>	6–9 months	FIS	LC50	Lethality	96	700	s	n	29°C; salinity 19‰	3	Lingaraja and Venugopalan 1978

\* See Annex 1.

<sup>1</sup> Exposure: s = static; f = flow-through.

<sup>2</sup> Toxicant analysis: n = nominal.

ALG = algae; AMP = amphibians; ANN = annelids; CRU = crustaceans; FIS = fish; INS = insects; MAC = macrophytes; MOL = molluscs; PLA = planarians; PRO = protozoans; ROT = rotifers

LOEC = lowest observed effect concentration

EC50 = concentration effective against 50% of the organisms tested

LC50 = concentration lethal to 50% of the organisms tested

### 2.6.3 Toxicity to sediment-dwelling organisms

Dimethoate's low log Kow of 0.704 (see Section 2.5) means that it is not expected to sorb to organic matter. This assumption is confirmed by the results of toxicity data from a study by Heintze (2002), which investigated the effects of technical grade dimethoate (purity 99.1 per cent) on larvae of the midge *Chironomus riparius* in an aerated static 28-day emergence test. The study was conducted according to draft OECD Guideline 219<sup>5</sup> and in compliance with GLP.

Artificial sediment consisting of 5 per cent sphagnum peat, 20 per cent kaolin clay and 75 per cent industrial sand was adjusted to pH 6.0 and used as a substrate. The water used throughout the study was a mixture of dechlorinated drinking water and deionised water. Test vessels containing a 2-cm layer of sediment and 15 cm of overlying water were prepared and allowed to acclimate for seven days before use. Twenty-five larvae of the first larval stage (2–3 days old) were added to each vessel 24 hours prior to treatment. Treatments consisted of a water control and nominal dimethoate concentrations of 50, 100, 200, 400, 800, and 1,600  $\mu\text{g l}^{-1}$ . Four replicates were used for each test substance concentration and six replicates for the water control. At the beginning of the test the aqueous solution of dimethoate was applied just below the water surface of the test vessels using a pipette and mixed gently without disturbing the sediment. The concentration of dimethoate in overlying water, sediment pore water and wet sediment was determined 1 hour, 7 days and 28 days after application.

Mean measured concentrations of dimethoate in the overlying water at day 0 were 90 and 1,500  $\mu\text{g l}^{-1}$  for the 100 and 1,600  $\mu\text{g l}^{-1}$  test vessels, respectively, and ranged from 87 to 94 per cent of nominal concentrations. After 7 days, only about half of the initial concentrations were found. After 28 days, the dimethoate levels in the water phase were found to be 23 and 300  $\mu\text{g l}^{-1}$  for the 100 and 1,600  $\mu\text{g l}^{-1}$  test vessels respectively, corresponding to 23 and 19 per cent of the initial concentrations of dimethoate in water. Mean measured concentrations of dimethoate in sediment at day 0 were below the limit of quantification (LOQ = 7  $\mu\text{g kg}^{-1}$ ) for the 100  $\mu\text{g l}^{-1}$  test vessels and 37  $\mu\text{g dimethoate kg}^{-1}$  for the highest water phase test concentration of 1,600  $\mu\text{g l}^{-1}$ . During the full 28-day period of the study, <6 per cent of the total amount of dimethoate in the test systems was transferred to the sediment.

The ecotoxicological endpoints from the study showed that the mean emergence rate was concentration dependent such that, at nominal water phase concentrations of  $\geq 400 \mu\text{g l}^{-1}$ , the emergence rate was <1.0 per cent, while at 200  $\mu\text{g l}^{-1}$  it was 29 per cent (compared with 96.7 per cent in the controls). At the lower exposure concentrations of 50 and 100  $\mu\text{g l}^{-1}$ , where the emergence rate was not considered to be affected by dimethoate exposure, there was also no delay in the rate of development of larvae. The 28-day emergence rate no observed effect concentration (NOEC) was calculated to be 100  $\mu\text{g l}^{-1}$  and the 28-day development rate NOEC was calculated to be 200  $\mu\text{g l}^{-1}$ . However, this study is not considered appropriate for the generation of a PNEC<sub>sediment</sub> due to the test method used, which involved application of the dimethoate to the water column rather than spiking into the sediment.

### 2.6.4 Endocrine-disrupting effects

Dimethoate has been shown to disrupt reproductive function in mammalian species. Although the pathogenesis of dimethoate-induced reproductive toxicity remains to be determined, a reduction in serum testosterone levels is thought to play an important

<sup>5</sup> See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1.00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1.00.html)

role in this process. Walsh *et al.* (2000), using the mouse MA-10 Leydig tumour cell line, found that dimethoate inhibited steroidogenesis in both a dose- and time-dependent manner without affecting total protein synthesis or protein kinase A activity. Although dimethoate exposure decreased the activity of the P450 side chain cleavage (P450 scc) enzyme, a reduction in the activity of this enzyme alone could not account for the level of Bu(2)cAMP-inhibited progesterone production. Instead the results suggested that dimethoate inhibited steroidogenesis primarily by blocking transcription of the steroidogenic acute regulatory (StAR) gene. This finding is considered to be significant since StAR protein mediates the rate-limiting and acutely regulated step in steroidogenesis, which is the transfer of cholesterol from the outer to the inner mitochondrial membrane.

A study by Aboul-Eta and Khalil (1987a) (see Table 2.8) on the chronic effects of technical grade dimethoate on the snail *Helisoma trivolvis* found that exposure to the insecticide not only caused a decrease in the number of eggs produced, but also changes in the shape of the eggs and the egg masses. It was found that, as early as the fourth or fifth day of the experiment, abnormal egg masses were evident in test vessels at all test concentrations (nominal values of 7.5, 30 and 120  $\mu\text{g l}^{-1}$ ) and many of these had eggs containing more than the single egg cell normally found. In other egg masses, only elements of the egg membrane were left and sometimes they were entirely absent. The egg cells were then surrounded only by the jelly mass and the outer egg-mass membrane. It was concluded that these results indicated a dimethoate-induced effect on the ability of parts of the oviductal tract to carry out their secretory function. In particular the pars contorta, which lays down these membranes, may be sensitive to insecticides such as dimethoate. There are issues with the reliability of this study as there was no analytical confirmation of the exposure concentrations and it needs to be recognised that these data are not necessarily evidence of endocrine disruption.

### **2.6.5 Mode of action of dimethoate**

Dimethoate is an organophosphate insecticide used to kill mites and insects systemically and on contact. Organophosphorus insecticides such as dimethoate act by combining with and inactivating the enzyme acetylcholinesterase (AChE). The inactivation of cholinesterase by such pesticides allows the accumulation of large amounts of the neurotransmitter acetylcholine and means neurons continue to fire. This causes overstimulation of the nervous systems of exposed organisms and ultimately results in death.

## **2.7 Mesocosm and field studies**

### **2.7.1 Freshwater mesocosm and field studies**

Baekken and Aanes (1994) reported on an investigation of the effects on spring and autumn benthic macroinvertebrate communities of exposure to a sub-lethal concentration of dimethoate (1  $\mu\text{g l}^{-1}$ ) in indoor experimental streams. For each of the spring and autumn tests, a total of 30 trays were filled with a defined combination of sand, gravel and pebbles and allowed to colonise by natural stream biota for five weeks. Ten randomly selected trays were placed in each of two indoor experimental streams. The remaining ten trays represented the invertebrate communities at the start of the experiment. Each stream was 5-m long and most of the water was recirculated, though there was continuous removal of 105  $\text{l h}^{-1}$ . One stream was continuously treated

with a concentration of  $1 \mu\text{g l}^{-1}$ , while the other was untreated. However, no analytical confirmation of the dimethoate exposure concentration was carried out during the study.

Some responses were common for the two tests:

- Drift rate (as well as the proportion of the populations found in drift) was higher in the dimethoate streams compared with the controls.
- Non-drifting movements away from the trays were higher in the dosed streams.
- Structural differences between the streams were small, but significant for some populations.

It was concluded for, both the spring and autumn communities, that exposure to a nominal dimethoate concentration of  $1 \mu\text{g l}^{-1}$  resulted in increased activity of the individuals and a reduction in the density of some populations. This results in an unbounded lowest observed effect concentration (LOEC) of  $1 \mu\text{g l}^{-1}$ , though the use of a single exposure concentration and the absence of analytical confirmation mean this value is of limited reliability.

Hessen *et al.* (1994) and Kallqvist *et al.* (1994) described the results of field studies on four pesticides (chlorosulfuron, dimethoate, glyphosphate and propiconazole) performed in 14 pelagic bags situated in the oligotrophic Lake Omdalsvann in south-east Norway. In both studies, each of the substances investigated was added to a separate bag at each of three concentrations ( $1$ ,  $10$  and  $100 \mu\text{g l}^{-1}$ ), with two bags acting as controls. At the start of the study, each bag received nitrate (as sodium nitrate) and phosphate (as potassium orthophosphate) equalling  $50$  and  $5 \mu\text{g l}^{-1}$  respectively.

In the Hessen *et al.* (1994) study, the bags were filled with surface water that was free of crustacean zooplankton before a vertical net tow ( $45 \mu\text{m}$  mesh,  $27\text{-cm}$  diameter) was added to each bag from a depth of  $6 \text{ m}$  (where the zooplankton were most dense) to ensure an equal inoculum of macrozooplankton in each bag. Subsequently, the nutrients and pesticides were added and the water was mixed thoroughly. Water samples for pesticide analysis were taken at the start and at the end of the experiment after  $15$  days. Qualitative phytoplankton and zooplankton samples were taken every three days. Due to the low concentrations of macrozooplankton, the effects on the crustacean zooplankton community could not be judged from the daily quantitative samples. Effects on this community were, therefore, based on the cumulative yield of quantitative samples and net hauls at the end of the study. The rotifers were persistently high in number in all bags throughout the study, allowing a day-to-day evaluation of responses. Photosynthetic activity was measured from  $^{14}\text{C}$  assimilation in  $25\text{-ml}$  bottles after  $2$ ,  $6$ ,  $9$  and  $13$  days.

Chemical analysis showed that the measured exposure concentrations in the bags were  $90\text{--}110$  per cent of nominal levels at the end of the study. The crustacean zooplankton species were the most susceptible organisms to dimethoate, with effects being evident on cladocerans at an exposure concentration of  $10 \mu\text{g l}^{-1}$ . In contrast, rotifers were not affected by exposure to any of the dimethoate concentrations. In general there was no correlation between total primary production, phytoplankton biomass and zooplankton. In the study the lowest observed effect concentration was determined to be  $10 \mu\text{g l}^{-1}$ , with a NOEC of  $1 \mu\text{g l}^{-1}$ .

In the Kallqvist *et al.* (1994) study, the bags were filled with surface water from  $0.5\text{--}1.0 \text{ m}$  depth, which was free of crustacean zooplankton, before a vertical net tow ( $45 \mu\text{m}$  mesh,  $27\text{-cm}$  diameter) was added to each bag from a depth of  $10 \text{ m}$  (taken when the zooplankton had migrated downwards). Subsequently, the nutrients and pesticides

were added and the water was mixed thoroughly. Water samples for pesticide analysis were taken at the start and at the end of the experiment after 16 days. Integrated samples were taken from 0–3.5 m depth and were analysed for water chemistry, chlorophyll a, phytoplankton species and numbers, and photosynthetic activity (measured from  $^{14}\text{C}$  assimilation in 25-ml bottles after 2, 6, 9 and 13 days).

Chemical analysis showed that the measured exposure concentrations in the bags were 90–110 per cent of nominal levels at the end of the study. The highest concentration of dimethoate of  $100\ \mu\text{g l}^{-1}$  affected biomass development (as measured by chlorophyll a). An initial slight depression of photosynthesis and a lower rate of nitrate assimilation were also evident. Effects on biomass development were also observed at  $10\ \mu\text{g l}^{-1}$ , where species diversity was reduced compared with the control enclosures. The authors concluded that the LOEC for effects on structural changes on phytoplankton communities was a dimethoate concentration of  $10\ \mu\text{g l}^{-1}$ , resulting in a NOEC of  $1\ \mu\text{g l}^{-1}$ .

### **2.7.2 Saltwater mesocosm and field studies**

No information on the effects of dimethoate on saltwater organisms from mesocosm and field studies was located.

# 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*

For the freshwater environment, data are available for the 'base set' of toxicity tests (i.e. tests with algae, crustaceans and fish) and, therefore, the EU Technical Guidance Document assessment factor (AF) method can be applied. Long-term (lt) toxicity data are available for eight taxonomic groups (algae, amphibians, crustaceans, fish, hydroids, insects, macrophytes and molluscs), with invertebrates and fish being more sensitive than algae.

In the open literature, there are four studies assessing the long-term toxicity of dimethoate which show NOEC values of  $<32 \mu\text{g a.i. l}^{-1}$  for crustaceans, fish and molluscs.

The lowest toxicity values for invertebrates were estimated from the study by Aboul-Eta and Khalil (1987a), which found that exposure of different age groups of the freshwater snail *Helisoma trivolvis* to technical grade dimethoate resulted in:

- an estimated 63-day NOEC of  $7.5 \mu\text{g a.i. l}^{-1}$  for effects on growth (9.7 per cent reduction from the controls was not apparently different but was not confirmed statistically);
- an estimated 21-day NOEC of  $7.5 \mu\text{g a.i. l}^{-1}$  for effects on reproduction (9.8 per cent reduction from the controls was not apparently different but was not confirmed statistically);
- an estimated 14-day NOEC of  $7.5 \mu\text{g a.i. l}^{-1}$  for effects on embryonic hatching and survival (0 per cent reduction from the controls)

The tests with growth and reproduction endpoints involved a semi-static design (with renewal of test solutions every 2–3 days), while the study on embryonic survival and hatching involved a static design. But in all the studies, there was limited replication and no analytical confirmation of the exposure concentrations. Furthermore, no statistical analysis of the data was presented to indicate the extent of the variability of the different datasets and it was not possible to derive statistically based NOEC values but only estimated ones.

A study of the effects of dimethoate on early-life stages of fish by Grande *et al.* (1994) reported a 12-day NOEC of  $12.5 \mu\text{g a.i. l}^{-1}$  for effects on the survival of the yolk-sac fry of zebrafish (*Danio rerio*) and a 45-day NOEC of  $20.0 \mu\text{g a.i. l}^{-1}$  for effects on the survival of the yolk-sac fry of brown trout (*Salmo trutta*). In both these studies, a semi-

static design was adopted with replacement of the test solutions every 24 hours. However, there was no analytical confirmation of the exposure concentrations.

Sloof and Canton (1983) assessed the long-term toxicity of dimethoate to a range of algal, macrophyte, invertebrate, fish and amphibian species. However, detailed information on the test methodologies and the results were not provided, and there was no analytical confirmation of the exposure concentrations in the various tests.

Overall, the limitations of the Sloof and Canton (1983), Aboul-Eta and Khalil (1987a) and Grande *et al.* (1994) studies mean they are considered to be insufficiently reliable for the derivation of the PNEC, but can be considered as supporting information.

The lowest valid long-term toxicity value for freshwater invertebrates is a 21-day NOEC of 24 µg a.i. l<sup>-1</sup> for effects of the Roxion formulation on the growth of the water flea *Daphnia magna* (Caley *et al.* 1992g). This NOEC value is consistent with a corresponding 21-day value of 40 µg a.i. l<sup>-1</sup> for the effects of technical grade dimethoate on the reproduction of *Daphnia* (Wuthrich 1990b). Both these studies were carried out to standardised procedures (OECD Guideline 202<sup>6</sup>) using a semi-static design and were completed to GLP requirements. In addition, there was analytical confirmation of the exposure concentrations, which indicated that these were typically within 20 per cent of the nominal values. The results of these studies are consistent with a 21-day LC0 and NOEC values of 32 and 100 µg a.i. l<sup>-1</sup> for effects of dimethoate on lethality and reproduction respectively of *Daphnia magna* (Sloof and Canton 1983). However, this semi-static study was not carried out to GLP and there was no analytical confirmation of the exposure concentrations.

Fish showed lower sensitivity to dimethoate than invertebrates, with the lowest relevant value being a 21-day NOEC of 290 µg a.i. l<sup>-1</sup> for effects of the Roxion formulation on the physiology of juvenile rainbow trout *Oncorhynchus mykiss* (Caley *et al.* 1992f). Wuthrich (1990a) reported a 21-day NOEC of 400 µg a.i. l<sup>-1</sup> for effects of technical grade dimethoate on the growth of juvenile rainbow trout *O.mykiss*. Both these studies were carried out to standardised procedures using a semi-static or flow-through design and were completed to GLP requirements. In addition, there was analytical confirmation of the exposure concentrations, which indicated that these were typically within 20 per cent of the nominal values.

The available long-term data indicated that algae were the least sensitive of the taxonomic groups tested, with NOECs for effects of dimethoate in the Roxion formulation and technical dimethoate on algal growth after 72 hours being 56,600 and 30,500 µg a.i. l<sup>-1</sup>, respectively (Caley *et al.* 1992c,d). Both these studies were carried out to standardised procedures (OECD Guideline 201<sup>6</sup>) and were completed to the requirements of GLP. In addition, there was analytical confirmation of the exposure concentrations which indicated that these were typically within 20 per cent of the nominal values.

Reliable long-term NOECs are available for algae, invertebrates and fish, and therefore an assessment factor of 10 could be applied to the lowest valid toxicity value based on the TGD. However, the application of this factor to the 21-day NOEC of 24 µg a.i. l<sup>-1</sup> for effects of the Roxion formulation on the growth of the water flea *Daphnia magna* would result in a higher PNEC<sub>freshwater\_lt</sub> higher than certain short-term toxicity data (which have been found to be in the range 2.0–7.8 µg l<sup>-1</sup>) – although it is accepted that the reliability of these short-term data [which are from studies that did not meet OECD principles of Good Laboratory Practice (GLP)] is questionable. This issue has been addressed by the application of larger precautionary assessment factor of 50, which provides further protection for freshwater organisms from the long-term effects of

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<sup>6</sup> See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1.00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1.00.html)

dimethoate. This results in the following value using the 21-day NOEC of 24 µg a.i. l<sup>-1</sup> for effects of the Roxion formulation on the growth of the water flea *Daphnia magna*:

$$\text{PNEC}_{\text{freshwater\_It}} = 24 \mu\text{g l}^{-1}/\text{AF (50)} = 0.48 \mu\text{g l}^{-1} \text{ dimethoate}$$

This PNEC would be consistent with data from the freshwater mesocosm and field studies (see Section 2.6.1), which indicate that effects on phytoplankton and zooplankton communities were evident at exposure concentrations of 10 µg l<sup>-1</sup> but not at 1 µg l<sup>-1</sup> (Hessen *et al.* 1994, Kallqvist *et al.* 1994).

If additional sensitive toxicity data become available, these could affect the resulting PNEC value.

### *PNEC accounting for transient concentration peaks*

Short-term (st) toxicity tests are available for eight taxonomic groups (algae, amphibians, annelids, crustaceans, fish, insects, molluscs and protozoa), with invertebrates and fish being more sensitive than the other taxa.

In the open literature there are a number of studies assessing the acute toxicity of dimethoate. These show L(E)C50 values of <10 µg a.i. l<sup>-1</sup> for amphibians, crustaceans, insects, fish and molluscs (e.g. Khangarot *et al.* 1985, Aboul-Eta and Khalil 1987b, Baekken and Aanes 1994).

The Aboul-Eta and Khalil (1987b) study reported 96-hour LC50 values of 2.0–5.2 µg a.i. l<sup>-1</sup> for static exposure tests of technical grade dimethoate on:

- a range of invertebrates:
  - the snails *Biomphalaria alexandrina* and *Bulinus truncatus*;
  - the copepods *Cyclops strensus* and *Daphnia longispina*;
  - the amphipod *Gammarus pulex*;
- fish – mullet of the Mugilidae species and the Nile tilapia *Tilapia nilotica*.

However, the collection of organisms from the field raises questions about their health prior to the start of the study. In addition, there was no analytical confirmation of exposure concentrations. As a result, it is not considered appropriate to derive the PNEC based on these data.

The other reported short-term studies showing L(E)C50 values of <10 µg a.i. l<sup>-1</sup> for dimethoate are a 96-hour LC50 of 7.0 µg a.i. l<sup>-1</sup> for effects on the survival of the larvae of the mayfly *Baetis rhodani* (Baekken and Aanes 1994) and a 96-hour LC50 of 7.8 µg a.i. l<sup>-1</sup> for effects on the survival of the frog tadpoles *Rana hexadactyla* (Khangarot *et al.* 1985). However, these studies were not carried out to standardised methodologies or GLP, and did not incorporate analytical confirmation of the exposure concentrations used. Furthermore, the Baekken and Aanes study reported higher 96-hour LC50 values of 23 and 81 µg a.i. l<sup>-1</sup> for effects of dimethoate on the larvae of *Hydropsyche siltalai* and *Heptagenia sulfurea* respectively. As a result, it is not considered appropriate to derive the PNEC based on these data.

The lowest valid short-term toxicity value for freshwater invertebrates is a 48-hour EC50 of 2,000 µg a.i. l<sup>-1</sup> for the effects of technical grade dimethoate on the immobilisation of the water flea *Daphnia magna* (Hertl 2002d). This EC50 value is consistent with a corresponding 48-hour value of 2,200 µg a.i. l<sup>-1</sup> for the effects of the Roxion formulation on the reproduction of *Daphnia* (Caley *et al.* 1992e). Both these studies were carried out to standardised procedures (OECD Guideline 202) and were

completed to GLP requirements. In addition, there was analytical confirmation of the exposure concentrations which indicated that these were typically within 20 per cent of the nominal values.

Hermens *et al.* (1984) conducted a study on the toxicity of dimethoate to *Daphnia magna* in which there was analytical confirmation of the exposure concentrations. This study resulted in a 48-hour EC50 value of 6,400 µg a.i. l<sup>-1</sup>, which is slightly higher than the values reported by Caley *et al.* (1992e) and Hertl (2002d).

Fish showed lower short-term toxicity to dimethoate than invertebrates. Caley *et al.* (1992a,b) reported 96-hour LC50 values of 17,600 and 24,500 µg a.i. l<sup>-1</sup> for effects of the Roxion formulation on the survival of bluegill sunfish *Lepomis macrochirus* and rainbow trout *Oncorhynchus mykiss* respectively. Bathe (1982a) reported a 96-hour LC50 of 30,200 µg l<sup>-1</sup> for effects of technical grade dimethoate on the survival of rainbow trout *O. mykiss*. Both these studies were carried out to standardised procedures (OECD Guideline 202) and were completed to GLP requirements. In addition, there was analytical confirmation of the exposure concentrations which indicated that these were typically within 20 per cent of the nominal values.

The available short-term data indicated that algae were the least sensitive of the taxonomic groups tested, with EC50 values for effects of technical grade dimethoate and dimethoate in the Roxion formulation on algal growth after 72 hours being 90,400 and 93,300 µg a.i. l<sup>-1</sup>, respectively (Caley *et al.* 1992c,d). Both these studies were carried out to standardised procedures (OECD Guideline 201) and were completed to GLP requirements. In addition, there was analytical confirmation of the exposure concentrations which indicated that these were typically within 20 per cent of the nominal values.

The available short-term toxicity data separate into two groups as follows:

- Non-GLP studies in which there was no analytical confirmation of the exposure concentrations that indicated short-term effects at concentrations of 2–10 µg a.i. l<sup>-1</sup> – a range overlapping the proposed PNEC<sub>freshwater\_lt</sub> values of 0.48–2.4 µg a.i. l<sup>-1</sup> (see above). Use of these data could potentially result in a PNEC<sub>freshwater\_st</sub> that was lower than the PNEC<sub>freshwater\_lt</sub>.
- GLP compliant studies in which there was analytical confirmation of exposure concentrations that indicated short-term effects at concentrations ≥2,000 µg a.i. l<sup>-1</sup>. These data indicate that there is an acute to chronic ratio of approximately 100 between the short-term and long-term data for sensitive species such as the crustacean *Daphnia magna*.

On this basis, the PNEC<sub>freshwater\_st</sub> should be derived using a 48-hour EC50 of 2,000 µg a.i. l<sup>-1</sup> for the effects of technical grade dimethoate on the immobilisation of the water flea *Daphnia magna*.

Based on the guidance given in the TGD on effects assessment for intermittent releases [Section 3.3.2 of Part II of the TGD (ECB 2003)], an assessment factor of 100 would normally be applied. But given the issues with the reliability of the data from the non-GLP studies, an alternative approach of applying a larger precautionary assessment factor of 500 to the lowest valid toxicity value has been adopted resulting in the following value:

$$\text{PNEC}_{\text{freshwater\_st}} = 2,000 \mu\text{g l}^{-1} / \text{AF (500)} = 4.0 \mu\text{g l}^{-1} \text{ dimethoate}$$

If additional sensitive toxicity data become available, these could affect the resulting PNEC value.

### 3.1.2 PNECs for saltwaters

The effects database for marine species is considerably smaller than that for freshwater organisms. Short-term toxicity data are available for five different taxonomic groups. However, no long-term data are available.

The short-term toxicity data of the marine taxa do not markedly differ from the range of values obtained for freshwater species (see Tables 2.9 and 2.10). However, the marine database is too small to draw firm conclusions on possible differences, particularly due to the absence of long-term effects data.

Based on the available data, it is proposed that:

- the TGD approach of using freshwater data within the marine effect assessment is used;
- suggested freshwater PNECs for setting of quality standards should be considered in deriving corresponding values for marine water bodies.

#### *PNEC accounting for the annual average concentration*

No long-term single species toxicity data for marine organisms are available. The absence of long-term data means that it is not possible to generate a  $PNEC_{\text{saltwater\_It}}$  based on the saltwater data alone and it is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation (see Section 3.1.1). This approach is consistent with that described in the TGD (ECB 2003).

Long-term NOECs are available for freshwater algae, invertebrates and fish but no toxicity data are available for exclusively marine taxa such as echinoderms. This would normally result in the application of an additional assessment factor of 10, resulting in a total assessment factor of 100. However, the available data indicate that the mode of toxic action of dimethoate should result in invertebrates such as crustaceans and insects being the most sensitive taxa to the substance.

The application of a factor of 10 to the 21-day NOEC of  $24 \mu\text{g a.i. l}^{-1}$  for effects of the Roxion formulation on the growth of the water flea *Daphnia magna* would result in a higher  $PNEC_{\text{saltwater\_It}}$  higher than certain short-term toxicity data (which have been found to be in the range  $2.0\text{--}7.8 \mu\text{g l}^{-1}$ ) the reliability of which is questionable. This issue has been addressed by the application of larger precautionary assessment factor of 50, which should provide further protection for freshwater organisms from the long-term effects of dimethoate. This results in the following value using the 21-day NOEC of  $24 \mu\text{g a.i. l}^{-1}$  for effects of the Roxion formulation on the growth of the water flea *Daphnia magna*:

$$PNEC_{\text{freshwater\_It}} = 24 \mu\text{g l}^{-1} / \text{AF (50)} = 0.48 \mu\text{g l}^{-1} \text{ dimethoate}$$

If additional sensitive toxicity data become available, these could affect the resulting PNEC value.

#### *PNEC accounting for transient concentration peaks*

Single species short-term toxicity data for marine organisms are available for five different taxonomic groups (algae, crustaceans, fish, macrophytes and molluscs), with crustaceans apparently being the most sensitive group. However, all these data were deemed to be uncertain or not assignable based on the Klimisch Code criteria.

Portmann and Wilson (1971) reported the lowest toxicity value for a saltwater species with an estimated 48-hour LC50 of 0.3–1.0 µg a.i. l<sup>-1</sup> for the effects of dimethoate on the survival of brown shrimp *Crangon crangon*. However, the lack of experimental data means these results cannot be fully assessed. Butler (1964) observed that a nominal dimethoate concentration of 1,000 µg a.i. l<sup>-1</sup> (tested using the Cygon formulation) caused 10 per cent inhibition of growth of juvenile oysters (*Crassostrea virginica*) after 96 hours exposure in a flow-through test.

For algae, Ramachandran *et al.* (1980) reported a 96-hour EC50 of 40 µg a.i. l<sup>-1</sup> for effects on the growth of the warm water diatom *Coscinodiscus concinnus*. Ramachandran *et al.* (1984) investigated the effects of exposure to 50 µg a.i. l<sup>-1</sup> dimethoate for six hours on four species of macroalgae and two species of plants. All the species were collected from warm (30°C) coastal waters of India. Small amounts of washed tissue (2–3 g) from each species were suspended 1-m below the surface of an estuary in a sealed vessel, and their photosynthetic and respiratory rates were measured by comparing oxygen balances in light and dark bottles. Respiration was not significantly affected in the macrophytes but was decreased by 24 per cent in the macroalga *Enteromorpha intestinalis* and, to a lesser degree, in other species. In contrast, macroalgal photosynthesis was only slightly reduced.

For the estuarine fish *Therapon jarbua*, Lingaraja and Venugopalan (1978) measured a 96-hour LC50 of 700 µg a.i. l<sup>-1</sup> in a test using the Rogor formulation.

However, all the available short-term data for saltwater species are from studies where there was no analytical confirmation of the exposure concentrations. As a result, there are issues with the reliability of these data in terms of deriving the PNEC<sub>saltwater\_st</sub>. Therefore, it is proposed that the PNEC<sub>saltwater\_st</sub> is based on the combined freshwater and saltwater dataset.

The TGD does not provide specific guidance for assessment of acute effects of intermittent releases to marine water bodies. Therefore, calculation of the PNEC is suggested accounting for effects following short-term exposure to dimethoate on the basis of the general guidance given in the TGD on the effects assessment for intermittent releases [Section 3.3.2 of Part II of the TGD (ECB 2003)]. This would normally result in application of an assessment factor of 100 being applied.

Since no data are available for exclusively marine species (e.g. echinoderms), an additional assessment factor of 10 would also normally be applied, resulting in a total assessment factor of 1,000. However, the mode of toxic action of dimethoate means that invertebrates such as insects would be expected to be the most sensitive taxa to the substance. Therefore, an assessment factor of 100 could be applied to the lowest valid toxicity value.

Given the issues with the reliability of the data from the non-GLP studies, an alternative approach of applying a larger precautionary assessment factor of 500 to the lowest valid toxicity value has been adopted resulting in the following value:

$$\text{PNEC}_{\text{saltwater\_st}} = 2000 \mu\text{g l}^{-1} / \text{AF (500)} = 4.0 \mu\text{g l}^{-1} \text{ dimethoate}$$

If additional sensitive toxicity data become available, these could affect the resulting PNEC value.

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

There are insufficient data to construct a species sensitivity distribution (SSD) based upon long-term exposure data.

### 3.3 Derivation of existing EQSs

The derivation of the proposed EQSs for dimethoate was described in a 1994 report to the Department of the Environment (Murgatroyd and Patel 1994).

In freshwaters, the available data were only considered sufficient to derive an annual average (AA) but no maximum allowable concentration (MAC). Applying safety factors of 100 and 10 to the most reliable acute and chronic data respectively, for aquatic crustaceans, suggested a 'safe' concentration in the range 10–60 µg l<sup>-1</sup>. However, the available data indicated that insects were one or two orders of magnitude more sensitive than crustaceans. Therefore, an EQS of around 1 µg dimethoate l<sup>-1</sup> was proposed for the protection of freshwater life, expressed as an annual average because of the large safety margin between this value and the more reliable acute toxicity data.

Given the limited database on the toxicity of dimethoate to saltwater organisms, it was proposed that the EQS set for the protection of freshwater life should also be adopted as an interim value for the protection of saltwater life.

### 3.4 Derivation of PNECs for sediment

Since the log K<sub>ow</sub> of dimethoate is <3 (see Section 2.5), the derivation of PNECs for the protection of benthic organisms is not required according to the TGD. Furthermore, although a sediment toxicity study is available (see Section 2.6.3), it is not considered appropriate for the derivation of a PNEC<sub>sediment</sub>.

### 3.5 Derivation of PNECs for secondary poisoning of predators

#### 3.5.1 Mammalian and avian toxicity data

A number of reviews have been published regarding dimethoate (WHO 1989, ACP 1990, ACP 1993, JMPR 1997, IUCLID 2000, EU DAR 2005). The most recent – the EU Draft Assessment Report and the IUCLID review – were assumed to contain the most sound and scientifically accurate mammalian data, particularly since many of the reported studies were carried out using OECD methods and to GLP. For this reason, these were the primary sources used though the other sources were consulted. Additional literature searches failed to locate any lower effect data since 2005. For avian data, the IUCLID (2000) datasheet was assumed to contain the most sound and scientifically accurate data.

Dimethoate is metabolised rapidly by mammals (Gallo and Lawryk 1991) with:

- rats excreting about 50–60 per cent of administered doses in urine, expired air and faeces within 24 hours;
- human volunteers excreting 76–100 per cent of the administered dimethoate within 24 hours

The rate of metabolism and elimination has been shown to vary between the mammalian species tested, with dimethoate appearing to be less toxic to those species with higher liver-to-body weight ratios and to those with the highest rate of dimethoate metabolism (Gallo and Lawryk 1991).

Dimethoate is moderately toxic to mammals by ingestion, inhalation and dermal adsorption. The reported acute oral LD50 values for the technical product range from 180 to 330 mg kg<sup>-1</sup> in the rat, while corresponding values in other species are 160 mg kg<sup>-1</sup> in mice, 350–400 mg kg<sup>-1</sup> in guinea pigs and 400–500 mg kg<sup>-1</sup> in rabbits (Gallo and Lawryk 1991).

The results of a number of short-term and long-term studies on the effects of dimethoate following oral exposure are summarized in Table 3.1.

**Table 3.1 Most sensitive mammalian and bird oral toxicity data relevant for the assessment of secondary poisoning**

Study and result	Details
<b>Sub-chronic toxicity to mammals</b>	
BASF 1983* Cited in IUCLID 2000 <b>Sub-chronic LOAEL = 0.5 mg/kg bw/day</b>	Wistar rats (eight per sex per group) received dimethoate orally via their diet for 28 days at doses of 0, 5, 25 or 75 mg/kg diet (equivalent to 0, 0.5, 2.5 or 7.5 mg/kg bw/day). This study was not conducted to GLP. The LOAEL was based on reduced brain and red blood cell cholinesterase in females. At higher doses, this effect was noted in both sexes, as well as reduced plasma cholinesterase and decreased body weight (bw).
DTF 1989* Cited in IUCLID 2000 <b>Sub-chronic LOAEL = 1.25 mg/kg bw/day</b> <b>Sub-chronic NOAEL = 0.25 mg/kg bw/day</b>	Beagle dogs (two per sex per group) received dimethoate orally via their diet for 28 days at doses of 0, 2, 10, 50, 250 or 1,250 mg/kg diet (equivalent to approximately 0, 0.05, 0.25, 1.25, 6.25 or 31.25 mg/kg bw/day). This study was conducted to GLP. The LOAEL and NOAEL were based on reduced brain and red blood cell cholinesterase.
Lamb 1994 Cited in EU DAR 2005 <b>Sub-chronic LOAEL = 3.33 mg/kg bw/day</b> <b>Sub-chronic NOAEL = 0.06 mg/kg bw/day</b>	Rats (strain and sex unspecified) received dimethoate in their diet for 13 weeks at doses that included 0, 1 and 50 parts per million (ppm) diet (equivalent to 0, 0.06 and 3.33 mg/kg bw/day) as part of a neurotoxicity study. This is considered to be a reliable study. The NOAEL and LOAEL were based on reduced red blood cell cholinesterase.
DTF 1959* Cited in IUCLID 2000 <b>Sub-chronic LOAEL = 0.25 mg/kg bw/day</b> <b>Sub-chronic NOAEL = 0.05 mg/kg bw/day</b>	Beagle dogs received dimethoate orally via their diet for 91 days at doses of 0, 2, 10 or 50 mg/kg diet (equivalent to approximately 0, 0.05, 0.25 or 1.25 mg/kg bw/day). The high dose was increased with time; 1500 mg/kg diet (37.5 mg/kg bw/day) for days 1-70, 2000 mg/kg diet (50 mg/kg bw/day) for days 71-77, 2500 mg/kg diet (62.5 mg/kg bw/day) for days 78-85 and 3000 mg/kg diet (75 mg/kg bw/day) for days 86-91. This study was not conducted to GLP. The LOAEL and NOAEL were based on reduced red blood cell cholinesterase.

Study and result	Details
<b>Chronic toxicity to mammals</b>	
DTF 1991* Cited in IUCLID 2000 <b>Chronic LOAEL = 3.75 mg/kg bw/day</b>	Beagle dogs (24 per sex per group) received dimethoate in their diet for 52 weeks at doses of 0, 5, 20 or 125 mg/kg diet (equivalent to approximately 0, 0.125, 0.5 or 3.125 mg/kg bw/day). This study was conducted to GLP. The LOAEL was based on decreased cholinesterase. No evidence of carcinogenicity was observed.
BASF 1986* Cited in IUCLID 2000 <b>Chronic LOAEL = 3.75 mg/kg bw/day</b>	B6C3F1 mice (50 per sex per group) received dimethoate in their diet for 78 weeks at doses of 0, 25, 100 or 200 mg/kg diet (equivalent to approximately 0, 3.75, 15 or 30 mg/kg bw/day). This study was not conducted to GLP. The LOAEL was based on a slightly increased body weight and decreased cholinesterase in both sexes. No evidence of carcinogenicity was observed.
Hellwig and Gembardt 1986 Cited in EU DAR 2005 and IUCLID 2000 <b>Chronic LOAEL = 0.2 mg/kg bw/day</b> <b>Chronic NOAEL = 0.04 mg/kg bw/day</b>	Wistar rats (65 per sex per group) received dimethoate in their diet for 24 months at doses of 0, 1, 5, 25 or 100 mg/kg diet (equivalent to 0, 0.04, 0.2, 1.2 or 5 mg/kg bw/day). This is considered to be a reliable, repeat dose study. The NOAEL and LOAEL were based on reduced brain and red blood cell cholinesterase. No evidence of carcinogenicity was observed.
<b>Effects on reproduction of mammals</b>	
Brooker <i>et al.</i> 1992 Cited in EU DAR 2005 and IUCLID 2000 <b>Reproductive NOAEL = 1.2 mg/kg bw/day</b> <b>Sub-chronic NOAEL = 0.08 mg/kg bw/day</b>	Cri:CD(SD)BR VAF/Plus rats received dimethoate in their diet for 70 days prior to mating and throughout two generations at doses of 0, 1, 15 or 65 mg/kg diet (equivalent to 0, 0.08, 1.2 or 5 mg/kg bw/day). This is considered to be a reliable, well-conducted and detailed study. The NOAEL, although stated to be difficult to determine (reasons not reported), was proposed for maternal and foetal effects, and was based on reduced fertility, litter size at birth, pup weight gain and pup mortality at the highest dose. A second NOAEL was also proposed for erythrocyte and brain cholinesterase inhibition in adults.
<b>Embryotoxicity and teratogenicity</b>	
Myers 2001 Cited in EU DAR 2005 <b>Developmental LOAEL = 0.5 mg/kg bw/day</b> <b>Developmental NOAEL = 0.1 mg/kg bw/day</b>	Rats (strain and sex unspecified) received dimethoate orally via gavage at unspecified doses as part of a developmental neurotoxicity study for an unstated duration. This is considered to be a reliable, repeat dose study. The NOAEL and LOAEL were based on mortality and poor condition in pups.

Study and result	Details
DTF 1984* Cited in IUCLID 2000 <b>Maternal NOAEL = 6 mg/kg bw/day</b> <b>Teratogenic NOAEL = 18 mg/kg bw/day</b>	Female CrL:COBS CD (SD)BR rats received dimethoate orally via gavage at doses of 0, 3, 6 or 18 mg/kg bw/day during days 5–15 of pregnancy. This study was conducted to GLP. The maternal NOAEL was based on hypersensitivity, body tremors, abnormal gait and reduced body weight. The teratogenic NOAEL was based on a lack of adverse foetal effects.
DTF 1984* Cited in IUCLID 2000 <b>Maternal NOAEL = 20 mg/kg bw/day</b> <b>Teratogenic NOAEL = 40 mg/kg bw/day</b>	Female New Zealand white rabbits received dimethoate orally via gavage at doses of 0, 10, 20 or 40 mg/kg bw/day during days 7–19 of pregnancy. This study was conducted to GLP. The maternal NOAEL was based on muscle tremors, unsteady gait and reduced body weight. The teratogenic NOAEL was based on a lack of adverse foetal effects.
<b>Neurotoxicity to mammals – no data located</b>	
<b>Endocrine disruption – see Section 2.5</b>	
<b>Sub-chronic and chronic toxicity to birds</b>	
DTF 1984* Cited in IUCLID 2000 <b>Sub-chronic NOEC = 30 mg/kg diet</b>	Bobwhite quails ( <i>Colinus virginianus</i> ) received dimethoate via their diet at doses of 0, 6, 18 or 30 mg kg <sup>-1</sup> diet for 28 days. Food consumption and body weight development were monitored and post-mortem examinations were performed. The NOEC was based on the lack of adverse effects in all treated birds.
DTF 1965* Cited in IUCLID 2000 <b>Sub-chronic NOEC = 260 mg/kg diet</b>	Hens (six per group) received dimethoate via their diet at doses of 0, 65, 130 or 260 mg/kg diet for four weeks. The NOEC was based on a lack of adverse effects on nerve fibres or their myelin sheaths in the brain, thoracic spinal cord and sciatic nerves in all treated birds.
<b>Effects on reproduction to birds</b>	
BASF 1986* Cited in IUCLID 2000 <b>Reproductive NOEC = 6 mg/kg diet</b>	Bobwhite quails ( <i>Colinus virginianus</i> ) received dimethoate via their diet at doses of 0, 6 or 30 mg/kg diet for 196 days (13 weeks during the pre-egg production period and 15 weeks of egg production). Weight, shell thickness, embryonic mortalities, hatchability and body weight gain of chicks were monitored until day-14 post-hatching. The NOEC was based on the reduced egg production and fertility rate that occurred at the higher dose.

Study and result	Details
BASF 1986* Cited in IUCLID 2000 <b>Reproductive NOEC = 30 mg/kg diet</b>	Mallard ducks ( <i>Anas platyrhynchos</i> ) received dimethoate via their diet at doses of 0, 6 or 30 mg/kg diet for 161 days (10 weeks during the pre-egg production period and 13 weeks of egg production). Adult food consumption, adult body weight, number of eggs laid and proportion damaged, egg weight, egg shell thickness, number of infertilities, embryonic mortalities, hatchability, number of 14-day-old surviving chicks, and chick body weight at hatching and 14 days after hatching were monitored until day-14 post-hatching. The NOEC was based on the lack of adverse effects in all treated birds.
No studies were available regarding the potential effects of dimethoate on avian development, nor on potential carcinogenicity.	

\* Unpublished study.

LOAEL = lowest observed adverse effect level

NOAEL = no observed adverse effect level

NOEC = no observed effect level

### 3.5.2 PNECs for secondary poisoning of predators

Bioconcentration data (as BCF values) for dimethoate for the majority of aquatic organisms are low, with values for fish ranging from 1 to 6 (see Section 2.5). Hence, the trigger of BCF values >100 is not met and the derivation of PNECs for secondary poisoning of predators is not required.

## 4 Analysis and monitoring

Analytical methods for dimethoate published before 1994 are discussed in Department of the Environment Report 3300/1 (Murgatroyd and Patel 1994).

The Draft Assessment Report (EU DAR 2005) states that water samples for analysis of dimethoate should be extracted by solid phase extraction (SPE) (using activated charcoal) and the compound should then be eluted from the charcoal with a mixture of dichloromethane and methanol. Dimethoate should be analysed using single ion mode gas chromatography–mass spectrometry (GC-MS). The reported LOQ was  $0.05 \mu\text{g l}^{-1}$ .

SPE techniques have also been proposed in several recent papers as possible methods of extracting a range of pesticides including dimethoate for analysis. Souza and Lancas (2003) proposed a solid phase micro-extraction technique coupled to high-resolution gas chromatography and mass spectrometry (SPME-HRGC-MS). The solid phase micro-extraction technique was validated using water samples spiked with several pesticides before it was applied to environmental samples. Tauler *et al.* (2001) also reported using a SPE technique followed by GC-MS in the analysis of organic pollutants in surface water samples. Over a period of six months, Tauler *et al.* (2001) sampled sites for 72 pollutants, including dimethoate. The SPE GC-MS method allowed analysis of samples below the detection limit in the Drinking Water Directive of  $0.1 \mu\text{g l}^{-1}$ .

Membrane extraction and gas chromatography has also been reported as a method of analysing dimethoate and other organophosphorus compounds in water samples (Xu *et al.* 2003). Xu *et al.* (2003) reported using a surface-modified acetic cellulose membrane to extract analytes, which are then back extracted into methanol. These methanol samples can then be analysed using gas chromatography with a pulsed flame photometric detector. Limits of detection for this technique were  $0.05 \mu\text{g l}^{-1}$ . Podhorniak *et al.* (2001) analysed organophosphorus pesticide residues in fruits and vegetables also using a gas chromatography and pulsed flame photometric technique. They extracted the pesticides using acetone extraction, SPE and sample clean-up using graphitised carbon black and propylsilane-bonded silica (PSA) cartridges, prior to analysis using a gas chromatography pulsed flame photometric detector.

Analysis of dimethoate in aquatic organisms has been performed using liquid chromatography (Hernández *et al.* 1998). Extraction of pesticide residues was performed using high-speed blending with acetonitrile/acetone (90:10 v/v). This was then concentrated and evaporated to dryness before being redissolved in *n*-hexane. One millilitre of this extract was then injected into a liquid chromatography system with a silica-gel column to allow lipid clean-up. The liquid chromatography elutant was then analysed using a gas chromatography system.

Gonçalves and Alpendurada (2005) examined the level of contamination of several pesticides, including dimethoate, in soil samples in a horticultural area using ultrasonic extraction and GC–MS. Residues were reported ranging from  $0.05$  to  $7.0 \mu\text{g kg}^{-1}$  with good precision and extraction efficiencies.

For water, proposed PNECs derived for dimethoate range from  $0.48$  to  $4.0 \mu\text{g l}^{-1}$ . The data quality requirements are that, at one-third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing GC-MS, which are capable of achieving detection limits as low as  $50 \text{ ng l}^{-1}$ , should offer adequate performance to analyse for dimethoate.

# 5 Conclusions

## 5.1 Availability of data

Long-term laboratory data are available for eight different freshwater taxonomic groups, i.e. algae, amphibians, crustaceans, fish, hydroids, insects, macrophytes and molluscs. Freshwater short-term toxicity data are available for eight taxonomic groups, i.e. algae, amphibians, annelids, crustaceans, fish, insects, molluscs and protozoa. Freshwater invertebrates and fish are more sensitive to than algae to both technical grade dimethoate and various dimethoate formulations. For marine organisms, single species short-term toxicity data are available for five different taxonomic groups (algae, crustaceans, fish, macrophytes and molluscs). However, no long-term toxicity data are available for saltwater taxa. Laboratory data are supplemented by freshwater mesocosm data, which confirm the greater sensitivity of crustaceans to dimethoate.

Dimethoate has been shown to disrupt reproductive function in mammalian species. Although the pathogenesis of dimethoate-induced reproductive toxicity remains to be determined, a reduction in serum testosterone levels is thought to play an important role in this process. Data for snails indicate there may be endocrine-mediated effects in egg production and development, but this hypothesis needs to be substantiated.

## 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

The lowest valid long-term toxicity value for freshwater invertebrates is a 21-day NOEC of 24  $\mu\text{g a.i. l}^{-1}$  for effects of the Roxion formulation on the growth of the water flea *Daphnia magna*. Reliable long-term NOECs are available for algae, invertebrates and fish; thus an assessment factor of 10 could be applied to the lowest valid toxicity value based on the EU Technical Guidance Document. However, the use of this factor would result in a  $\text{PNEC}_{\text{freshwater\_lt}}$  higher than certain short-term toxicity data (which have been found to be in the range 2.0–7.8  $\mu\text{g l}^{-1}$ ) – although it is accepted that the reliability of these short-term data [which are from studies that did not meet OECD principles of Good Laboratory Practice (GLP)], is questionable (see below). This issue has been addressed by the application of larger precautionary assessment factor of 50, which provides further protection for freshwater organisms from the long-term effects of dimethoate. This results in a  $\text{PNEC}_{\text{freshwater\_lt}}$  of 0.48  $\mu\text{g l}^{-1}$ .

This value is similar to the existing EQS of 1.0  $\mu\text{g l}^{-1}$ . This was derived by applying safety factors of 100 and 10 to the most reliable acute and chronic data respectively; for aquatic crustaceans a ‘safe’ concentration of in the range 10–60  $\mu\text{g l}^{-1}$  was suggested. However, the available data indicated that insects were one or two orders of magnitude more sensitive than crustaceans. Therefore, a lower EQS was proposed for the protection of freshwater life; this was expressed as an annual average because of the large safety margin between this value and the more reliable acute toxicity data.

### 5.2.2 Short-term PNEC for freshwaters

Reliable short-term (st) data are available for algal, invertebrate and fish species. The lowest valid short-term toxicity value for freshwater invertebrates is a 48-h EC50 of 2,000 µg a.i. l<sup>-1</sup> for the effects of technical grade dimethoate on the immobilisation of the water flea *Daphnia magna*. Lower short-term toxicity values have been reported in non-GLP studies, but these are considered to be unreliable due to the absence of measured concentration data and, in some instances, the collection of organisms from the field. Given the issues with the reliability of the data from the non-GLP studies, a larger precautionary assessment factor of 500 applied to the lowest valid toxicity value has been adopted, resulting in a PNEC<sub>freshwater\_st</sub> of 4.0 µg l<sup>-1</sup>.

There is no current short-term EQS for freshwaters.

### 5.2.3 Long-term PNEC for saltwaters

No long-term single species toxicity data for marine organisms are available. The absence of long-term data means that it is not possible to generate a PNEC<sub>saltwater\_lt</sub> based on the saltwater data alone, and it is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation. Reliable long-term NOECs are available for algae, invertebrates and fish; therefore an assessment factor of 10 could be applied to the lowest valid toxicity value based on the TGD. However, the application of this factor to the a 21-day NOEC of 24 µg a.i. l<sup>-1</sup> for effects of the Roxion formulation on the growth of the water flea *Daphnia magna*, would result in a PNEC<sub>freshwater\_lt</sub> higher than certain short-term toxicity data (which have been found to be in the range 2.0–7.8 µg l<sup>-1</sup>) – although it is accepted that the reliability of these short-term data, which are from studies that did not meet OECD principles of Good Laboratory Practice (GLP), is questionable. This issue has been addressed by the application of larger precautionary assessment factor of 50, which would provide further protection for saltwater organisms from the long-term effects of dimethoate. This results in a PNEC<sub>saltwater\_lt</sub> of 0.48 µg l<sup>-1</sup>.

There is no current long-term EQS for saltwaters.

### 5.2.4 Short-term PNEC for saltwaters

Single species short-term toxicity data for marine organisms are available for five different taxonomic groups, i.e. algae, crustaceans, fish, macrophytes and molluscs. However, all these data were deemed to be uncertain or not assignable based on Klimisch Code criteria. Therefore, it is proposed that the PNEC<sub>saltwater\_st</sub> is based on the combined freshwater and saltwater dataset.

The lowest valid short-term toxicity value for freshwater invertebrates is a 48-hour EC50 of 2,000 µg a.i. l<sup>-1</sup> for the effects of technical grade dimethoate on the immobilisation of the water flea *Daphnia magna*. Lower short-term toxicity values have been reported in non-GLP studies, but these are considered to be unreliable due to the absence of measured concentration data and, in some instances, the collection of organisms from the field. Given the issues with the reliability of the data from the non-GLP studies, a larger precautionary assessment factor of 500 applied to the lowest valid toxicity value has been adopted resulting in a PNEC<sub>freshwater\_st</sub> of 4.0 µg l<sup>-1</sup>.

There is no current short-term EQS for saltwaters.

### 5.2.5 PNEC for sediments

Since the log Kow of dimethoate is <3, the derivation of PNECs for the protection of benthic organisms is not required according to the TGD. Furthermore, although a sediment toxicity study is available, it is not considered appropriate for the derivation of a PNEC<sub>sediment</sub>.

### 5.2.6 PNEC for secondary poisoning

Bioconcentration data (as BCF values) for dimethoate for the majority of aquatic organisms are low, with values for fish ranging from 1 to 6. Hence, the TGD BCF trigger of 100 is not exceeded and the derivation of a PNEC in whole fish for secondary poisoning of predators is not required.

**Table 5.1 Summary of proposed PNECs**

Receiving medium/exposure scenario	Proposed PNEC ( $\mu\text{g l}^{-1}$ )	Existing EQS ( $\mu\text{g l}^{-1}$ )
Freshwater/long-term	0.48	1.0
Freshwater/short-term	4.0	–
Saltwater/long-term	0.48	–
Saltwater/short-term	4.0	–
Sediment	Not required	–
Secondary poisoning	Not required	–

## 5.3 Analysis

The data quality requirements are that, at one third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing GC-MS and capable of achieving detection limits as low as  $50 \text{ ng l}^{-1}$  should offer adequate performance to analyse for dimethoate.

## 5.4 Implementation issues

These PNECs are suitable for use as EQSs because analytical capability is adequate for compliance assessment purposes. Since additional testing to reduce uncertainty would likely result in standards that would not comply with the 'no deterioration' principle, adoption of the existing EQS as an interim value is not suitable. Instead, the proposed PNECs, similar in value to the existing EQS, are recommended.

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# List of abbreviations

AA	Annual average
AF	Assessment factor
a.i.	Active ingredient
BCF	Bioconcentration factor
Bw	body weight
CAS	Chemical Abstracts Service
DAR	Draft Assessment Report
ECb	Effective concentration (biomass)
EC50	concentration effective against 50% of the organisms tested
EHC	Environmental Health Criteria
EQS	Environmental Quality Standard
FAO	Food and Agricultural Organization
GC-MS	gas chromatography/mass spectrometry
GLP	Good Laboratory Practice (OECD)
LC50	concentration lethal to 50% of the organisms tested
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
LOQ	limit of quantitation
lt	long term
MAC	maximum allowable concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organization for Economic Co-operation and Development
PNEC	predicted no-effect concentration
ppm	parts per million
SSD	species sensitivity distribution
st	short term
TGD	Technical Guidance Document
UKTAG	UK Technical Advisory Group
WFD	Water Framework Directive
w/w	weight/weight

# ANNEX 1 Data quality assessment sheets

Identified and ordered by alphabetical order of references.

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/departement/0,2688,en\\_2649\\_34381\\_1\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/departement/0,2688,en_2649_34381_1_1_1_1_1,00.html)

<b>Reference</b>	Aboul-Eta and Khalil 1987a
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<b>Information on the test species</b>	
Test species used	Snail ( <i>Helisoma trivolvis</i> )
Source of the test organisms	Laboratory cultures
Holding conditions prior to test	Not stated
Life stage of the test species used	Growth test: spat (mean maximum diameter = 2.3 mm) Egg production test: adults (mean maximum diameter = 14.3±0.32 mm) Embryonic survival and hatching test: eggs

<b>Information on the test design</b>	
Methodology used	The methodology used is reasonably well described in the paper.
Form of the test substance	Technical grade dimethoate
Source of the test substance	Kafr El Zayat, Egypt
Type and source of the exposure medium	Not stated
Test concentrations used	Control, 7.5, 30, 120 and 480 µg l <sup>-1</sup>
Number of replicates per concentration	Growth test: 1 Egg production test: 1 Embryonic survival and hatching test: 1
Number of organisms per replicate	Growth test: 10 Egg production test: 4 Embryonic survival and hatching test: 40–60
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Growth test: semi-static, 63 days, feeding Egg production test: semi-static, 21 days, feeding Embryonic survival and hatching test: static, 14 days, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	The study is reasonably well described but there was limited replication and no analytical confirmation of the exposure concentrations.

<b>Reliability of study</b>	<b>Not reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>3</b>

<b>Reference</b>	Aboul-Eta and Khalil 1987b
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<b>Information on the test species</b>	
Test species used	Amphipod ( <i>Gammarus pulex</i> ) Copepods ( <i>Cyclops strennus</i> , <i>Daphnia longispina</i> , <i>Daphnia magna</i> ) Snails ( <i>Biomphalaria alexandrina</i> , <i>Bulinus truncate</i> ) Fish (mullet and Nile tilapia)
Source of the test organisms	Wadi El Rayan Lake or from El Wadi drain
Holding conditions prior to test	Not stated
Life stage of the test species used	<i>Biomphalaria alexandrina</i> : mean diameter = 11.6±0.33 mm <i>Bulinus truncate</i> : mean length = 10.8±0.40 mm <i>Cyclops strennus</i> : average length = 1.1 mm <i>Daphnia longispina</i> : average length = 1.3 mm <i>Gammarus pulex</i> : average length = 0.9 mm <i>Mullet</i> : average length = 5.2±1.2 mm <i>Nile tiapia</i> : average length = 15.3±2.3 mm

<b>Information on the test design</b>	
Methodology used	The methodology is reasonably well described in the paper.
Form of the test substance	Technical grade dimethoate
Source of the test substance	Kafr El Zayat, Egypt
Type and source of the exposure medium	Filtered lake water
Test concentrations used	10 (actual values not stated)
Number of replicates per concentration	2
Number of organisms per replicate	10–20 depending on species
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 96 hours, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	The study is reasonably well described but there was no analytical confirmation of the exposure concentrations.

<b>Reliability of study</b>	<b>Not reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>3</b>

<b>Reference</b>	Baekken and Aanes 1994
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<b>Information on the test species</b>	
Test species used	<i>Baetis rhodani</i> (Ephemeroptera) <i>Heptagenia sulfurea</i> (Ephemeroptera) <i>Hydropsyche siltalai</i> (Trichoptera) <i>Gammarus pulex</i> (Crustacea)
Source of the test organisms	Collected from the field (locations not given).
Holding conditions prior to test	Not stated
Life stage of the test species used	Larvae

<b>Information on the test design</b>	
Methodology used	The methodology is not described in detail in the paper.
Form of the test substance	Dimethoate insecticide Rogor L 20 containing 200 g l <sup>-1</sup> of active dimethoate
Source of the test substance	Not stated
Type and source of the exposure medium	Water typical of unpolluted lowland Norwegian water
Test concentrations used	0, 2.0, 20, 50, 200 and 2,000 µg l <sup>-1</sup>
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Flow-through, 96 hours, no feeding
Measurement of exposure concentrations	No
Measurement of water quality parameters	Yes (pH, conductivity and hardness)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	No
Overall comment on quality	The methodology is not described in detail in the paper and there was no analytical confirmation of the exposure concentrations.

<b>Reliability of study</b>	<b>Not reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>3</b>

<b>Reference</b>	Bathe 1982
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<b>Information on the test species</b>	
Test species used	Rainbow trout ( <i>Oncorhynchus mykiss</i> )
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Not stated in EU DAR 2005

<b>Information on the test design</b>	
Methodology used	BBA* Method No. 33
Form of the test substance	Technical dimethoate (purity not stated)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Not stated in EU DAR 2005
Test concentrations used	0 (control), 8,000, 15,000, 35,000, 50,000 and 100,000 µg l <sup>-1</sup>
Number of replicates per concentration	Not stated in EU DAR 2005
Number of organisms per replicate	Not stated in EU DAR 2005
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 96 hours, no feeding
Measurement of exposure concentrations	Yes (55–137% of nominal concentrations after 2 and 96 hours)
Measurement of water quality parameters	Not stated in EU DAR 2005
Test validity criteria satisfied	Not stated in EU DAR 2005
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	No
Overall comment on quality	Limited data on the test methodology is available but as a GLP compliant study it can be assumed that it was of good quality.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* Biologische Bundesanstalt (German Federal Biological Research Centre)

<b>Reference</b>	Butler 1964
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<b>Information on the test species</b>	
Test species used	Eastern Oyster ( <i>Crassostrea virginica</i> )
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Juveniles

<b>Information on the test design</b>	
Methodology used	There is only a limited description of the methodology.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Not stated
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Yes (salinity and temperature)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	The quality of the study is uncertain due to information presented in the paper.

<b>Reliability of study</b>	<b>Not reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>3</b>

<b>Reference</b>	Caley <i>et al.</i> 1992a
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<b>Information on the test species</b>	
Test species used	Rainbow trout ( <i>Oncorhynchus mykiss</i> )
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Not stated in EU DAR 2005

<b>Information on the test design</b>	
Methodology used	OECD Guideline 203*
Form of the test substance	Roxion (an EC formulation containing 38.9% w/w dimethoate)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Not stated in EU DAR 2005
Test concentrations used	0 (control), 16,500, 33,000, 65,400 and 129,000 $\mu\text{g l}^{-1}$
Number of replicates per concentration	Not stated in EU DAR 2005
Number of organisms per replicate	Not stated in EU DAR 2005
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Semi-static, 96 hours, no feeding
Measurement of exposure concentrations	Yes (122.7–132.6% of nominal concentrations after 0 and 48 hours)
Measurement of water quality parameters	Not stated in EU DAR 2005
Test validity criteria satisfied	Not stated in EU DAR 2005
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	Yes
Overall comment on quality	Limited data on the test methodology is available but as a GLP compliant study it can be assumed that it was of good quality.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1,00.html)

<b>Reference</b>	Caley <i>et al.</i> 1992b
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<b>Information on the test species</b>	
Test species used	Bluegill sunfish ( <i>Lepomis macrochirus</i> )
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Not stated in EU DAR 2005

<b>Information on the test design</b>	
Methodology used	OECD Guideline 203*
Form of the test substance	Roxion (an EC formulation containing 38.9% w/w dimethoate)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Not stated in EU DAR 2005
Test concentrations used	0 (control), 14,000, 28,700, 57,200 and 109,000 $\mu\text{g l}^{-1}$
Number of replicates per concentration	Not stated in EU DAR 2005
Number of organisms per replicate	Not stated in EU DAR 2005
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Semi-static, 96 hours, no feeding
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Not stated in EU DAR 2005
Test validity criteria satisfied	Not stated in EU DAR 2005
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	Yes
Overall comment on quality	Limited data on the test methodology is available but as a GLP compliant study it can be assumed that it was of good quality.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1,00.html)

<b>Reference</b>	Caley <i>et al.</i> 1992c
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<b>Information on the test species</b>	
Test species used	<i>Selenastrum capricornutum</i>
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Growth phase

<b>Information on the test design</b>	
Methodology used	OECD Guideline 201*
Form of the test substance	Roxion (an EC formulation containing 38.9% w/w dimethoate)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Not stated in EU DAR 2005
Test concentrations used	Not stated in EU DAR 2005
Number of replicates per concentration	Not stated in EU DAR 2005
Number of organisms per replicate	Not stated in EU DAR 2005
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 72 hours, no feeding
Measurement of exposure concentrations	Yes (40.3–100.8% of nominal concentrations after 0 and 72 hours)
Measurement of water quality parameters	Not stated in EU DAR 2005
Test validity criteria satisfied	Not stated in EU DAR 2005
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	Yes
Overall comment on quality	Limited data on the test methodology is available but as a GLP compliant study it can be assumed that it was of good quality.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1,00.html)

<b>Reference</b>	Caley <i>et al.</i> 1992d
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<b>Information on the test species</b>	
Test species used	<i>Selenastrum capricornutum</i>
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Growth phase

<b>Information on the test design</b>	
Methodology used	OECD Guideline 201*
Form of the test substance	Technical dimethoate (96.7% purity)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Nutrient media
Test concentrations used	Not stated in EU DAR 2005
Number of replicates per concentration	Not stated in EU DAR 2005
Number of organisms per replicate	Not stated in EU DAR 2005
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 72 hours, no feeding
Measurement of exposure concentrations	Yes (22.2–112.1% of nominal concentration after 0 and 72 hours)
Measurement of water quality parameters	Not stated in EU DAR 2005
Test validity criteria satisfied	Not stated in EU DAR 2005
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	Yes
Overall comment on quality	Limited data on the test methodology is available but as a GLP compliant study it can be assumed that it was of good quality.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1,00.html)

<b>Reference</b>	Caley <i>et al.</i> 1992e
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<b>Information on the test species</b>	
Test species used	Water flea ( <i>Daphnia magna</i> )
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Not stated in EU DAR 2005

<b>Information on the test design</b>	
Methodology used	OECD Guideline 202*
Form of the test substance	Roxion (an EC formulation containing 38.9% w/w dimethoate)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Not stated in EU DAR 2005
Test concentrations used	0 (control), 3,470, 11,100, 36,500 and 111,000 $\mu\text{g l}^{-1}$
Number of replicates per concentration	Not stated in EU DAR 2005
Number of organisms per replicate	Not stated in EU DAR 2005
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 48 hours, no feeding
Measurement of exposure concentrations	Yes (77–112% of nominal concentrations after 0 and 48 hours)
Measurement of water quality parameters	Not stated in EU DAR 2005
Test validity criteria satisfied	Not stated in EU DAR 2005
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	Yes
Overall comment on quality	Limited data on the test methodology is available but as a GLP compliant study it can be assumed that it was of good quality.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1,00.html)

<b>Reference</b>	Caley <i>et al.</i> 1992f
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<b>Information on the test species</b>	
Test species used	Rainbow trout ( <i>Oncorhynchus mykiss</i> )
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Juveniles at start of the test, length 4–6 cm

<b>Information on the test design</b>	
Methodology used	OECD Guideline 204*
Form of the test substance	Roxion (an EC formulation containing 38.9% w/w dimethoate)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Not stated in EU DAR 2005
Test concentrations used	0 (control), 200, 600, 2,000, 20,000 and 60,000 $\mu\text{g l}^{-1}$
Number of replicates per concentration	1
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Semi-static system (with renewal of test solutions every 72 hours), 21 days, feeding
Measurement of exposure concentrations	Yes (old and new concentrations measured at changeovers)
Measurement of water quality parameters	Not stated in EU DAR 2005
Test validity criteria satisfied	Not stated in EU DAR 2005
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	Yes
Overall comment on quality	The study is of good quality having been conducted to a standardised methodology under GLP.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1,00.html)

<b>Reference</b>	Caley <i>et al.</i> 1992g
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<b>Information on the test species</b>	
Test species used	Water fleas ( <i>Daphnia magna</i> )
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Juveniles

<b>Information on the test design</b>	
Methodology used	OECD Guideline 202*
Form of the test substance	Roxion (an EC formulation containing 38.9% w/w dimethoate)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Not stated in EU DAR 2005
Test concentrations used	0 (control), 50, 160, 500, 1,600 and 5,000 µg l <sup>-1</sup>
Number of replicates per concentration	4
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Semi-static system (with renewal of exposure concentrations every 48 hours), 21 days, feeding with a mixture of baker's yeast and algae ( <i>Scenedesmus subspicatus</i> )
Measurement of exposure concentrations	Yes (old and new concentrations measured at changeovers)
Measurement of water quality parameters	Yes (pH, temperature, dissolved oxygen)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Yes
Overall comment on quality	The study is of good quality having been conducted to a standardised methodology under GLP.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1,00.html)

<b>Reference</b>	Grande <i>et al.</i> 1994
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<b>Information on the test species</b>	
Test species used	Brown trout ( <i>Salmo trutta</i> ) Zebrafish ( <i>Danio rerio</i> )
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Brown trout: eyed egg stage at start of the test Zebrafish: newly fertilised eggs

<b>Information on the test design</b>	
Methodology used	The methodology is reasonably well described in the paper.
Form of the test substance	Not stated
Source of the test substance	Delivered from the Norwegian Plant Protection Institute as solutions.
Type and source of the exposure medium	Water from a nearby lake
Test concentrations used	Not stated
Number of replicates per concentration	Brown trout: 1 Zebrafish: Not stated but followed method of Dave <i>et al.</i> (1987)
Number of organisms per replicate	Brown trout: 50 Zebrafish: Not stated but followed method of Dave <i>et al.</i> (1987)
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Brown trout: semi-static, 12 days, no feeding Zebrafish: semi-static, 12 days, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	The study is reasonably well described but there was no analytical confirmation of the exposure concentrations.

<b>Reliability of study</b>	<b>Not reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>3</b>

<b>Reference</b>	Heintze 2002
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<b>Information on the test species</b>	
Test species used	Midge larvae ( <i>Chironomus riparius</i> )
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	First larval stage (2–3 days old)

<b>Information on the test design</b>	
Methodology used	OECD Guideline 219 (draft)*
Form of the test substance	Technical dimethoate (99.1% purity)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	A mixture of dechlorinated drinking water and deionised water Artificial sediment consisting of 5% sphagnum moss, 20% kaolin clay and 75% industrial sand adjusted to pH 6.0.
Test concentrations used	0 (control), 50, 100, 200, 400, 800 and 1,600 µg l <sup>-1</sup>
Number of replicates per concentration	6 for the controls and four for each treatment concentration
Number of organisms per replicate	25
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 29 day, feeding
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Not stated in EU DAR 2005
Test validity criteria satisfied	Not stated in EU DAR 2005
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	Yes
Overall comment on quality	The study is of good quality having been conducted to a standardised methodology under GLP.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1,00.html)

<b>Reference</b>	Hertl 2002d
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<b>Information on the test species</b>	
Test species used	Water flea ( <i>Daphnia magna</i> )
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Not stated in EU DAR 2005

<b>Information on the test design</b>	
Methodology used	OECD Guideline 202*
Form of the test substance	Technical dimethoate (99.1% purity)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Not stated in EU DAR 2005
Test concentrations used	0 (control), 500, 1,000, 2,000, 4,000, 8,000 and 16,000 µg l <sup>-1</sup>
Number of replicates per concentration	Not stated in EU DAR 2005
Number of organisms per replicate	Not stated in EU DAR 2005
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 48 hours, no feeding
Measurement of exposure concentrations	Yes (86–96% of nominal concentrations after 0 and 48 hours)
Measurement of water quality parameters	Not stated in EU DAR 2005
Test validity criteria satisfied	Not stated in EU DAR 2005
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	Yes
Overall comment on quality	Limited data on the test methodology is available but as a GLP compliant study it can be assumed that it was of good quality.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1.00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1.00.html)

<b>Reference</b>	Khangorot <i>et al.</i> 1985
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<b>Information on the test species</b>	
Test species used	Frog ( <i>Rana hexadactyla</i> )
Source of the test organisms	Collected from natural breeding grounds.
Holding conditions prior to test	Not stated
Life stage of the test species used	Tadpoles (mean length = 20 mm and mean wet weight = 500 mg)

<b>Information on the test design</b>	
Methodology used	There is only a limited description of the methodology.
Form of the test substance	Rogor 30 EC
Source of the test substance	Rallis India Ltd, India
Type and source of the exposure medium	Natural water
Test concentrations used	Control, acetone control and 7–10 concentrations based on results of preliminary assays
Number of replicates per concentration	3
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Semi-static, 96 hours, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Yes (dissolved oxygen, hardness, pH and temperature)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	The quality of the study is uncertain from the information presented in the paper.

<b>Reliability of study</b>	<b>Not reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>3</b>

<b>Reference</b>	Kumar <i>et al.</i> 1989
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<b>Information on the test species</b>	
Test species used	<i>Tetrahymena pyriformis</i> (syngen-1)
Source of the test organisms	Department of Biochemistry, University of Hull, UK
Holding conditions prior to test	Subcultured axenically in sterilised medium consisting of 1% proteose peptone supplemented with 0.5% NaCl and 0.3% yeast extract
Life stage of the test species used	-

<b>Information on the test design</b>	
Methodology used	There is only a limited description of the methodology.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Sterilised nutrient media (consisting of 1% proteose peptone supplemented with 0.5% NaCl and 0.3% yeast extract)
Test concentrations used	Control (0.1% acetone), 1, 10 50 and 100 mg l <sup>-1</sup>
Number of replicates per concentration	3
Number of organisms per replicate	Initial cell density = 2 x 10 <sup>4</sup> cells ml <sup>-1</sup>
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	The study is not well described and the exposure concentrations used were not measured

<b>Reliability of study</b>	<b>Not reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>3</b>

<b>Reference</b>	Lingaraja and Venugopalan 1978
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<b>Information on the test species</b>	
Test species used	Fish ( <i>Therapon jarba</i> )
Source of the test organisms	Collected from the Vellar estuary, Porto Novo, India.
Holding conditions prior to test	Acclimatised in the laboratory for 7 days and fed for 5 days with juvenile prawns from the estuary
Life stage of the test species used	6–9 months old (length = 6.9–9.2 cm, weight = 6.5–13.1 g)

<b>Information on the test design</b>	
Methodology used	The method is reasonably well described.
Form of the test substance	Dimethoate
Source of the test substance	Rallis India Ltd, India
Type and source of the exposure medium	Estuarine water
Test concentrations used	Control, ethyl alcohol control and five test concentrations
Number of replicates per concentration	1
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 96 hours, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Yes (dissolved oxygen, pH, salinity and temperature)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	The study is of limited quality due to the absence of analytical confirmation of exposure concentrations.

<b>Reliability of study</b>	<b>Not reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>3</b>

<b>Reference</b>	Portmann and Wilson 1971
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<b>Information on the test species</b>	
Test species used	Brown shrimp ( <i>Crangon crangon</i> )
Source of the test organisms	Collected from nearby estuary 2–3 days before the test.
Holding conditions prior to test	Not stated
Life stage of the test species used	Adults

<b>Information on the test design</b>	
Methodology used	The methodology is not described in the paper but in Portmann (1968).
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 48 hours, no feeding
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	The quality of the study is uncertain due to information presented in the paper, but there was no analytical confirmation of the exposure concentration.

<b>Reliability of study</b>	<b>Not reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>3</b>

<b>Reference</b>	Ramachandran <i>et al.</i> 1980
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<b>Information on the test species</b>	
Test species used	Marine diatom <i>Coscinodiscus concinnus</i>
Source of the test organisms	Paper not available
Holding conditions prior to test	Paper not available
Life stage of the test species used	Paper not available

<b>Information on the test design</b>	
Methodology used	Paper not available
Form of the test substance	Paper not available
Source of the test substance	Paper not available
Type and source of the exposure medium	Paper not available
Test concentrations used	Paper not available
Number of replicates per concentration	Paper not available
Number of organisms per replicate	Paper not available
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Paper not available
Measurement of exposure concentrations	Paper not available
Measurement of water quality parameters	Paper not available
Test validity criteria satisfied	Paper not available
Water quality criteria satisfied	Paper not available
Study conducted to GLP	Paper not available
Overall comment on quality	Not possible to assess as paper not available

<b>Reliability of study</b>	<b>Not assessed</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>4</b>

<b>Reference</b>	Ramachandran <i>et al.</i> 1984
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<b>Information on the test species</b>	
Test species used	Six macrophytes including <i>Chaetomorpha linum</i> , <i>Enteromorpha intestinalis</i> , <i>Gracilaria verrucosa</i> , <i>Grateloupia doryphora</i> , <i>Halophila ovalis</i> and <i>Halodule uninervis</i>
Source of the test organisms	Collected from littoral region of the marine zone of the Vellar estuary, India.
Holding conditions prior to test	Not relevant
Life stage of the test species used	Not relevant

<b>Information on the test design</b>	
Methodology used	The methodology is reasonably well described.
Form of the test substance	Dimethoate (96% purity)
Source of the test substance	Rallis India Ltd, India
Type and source of the exposure medium	Filtered seawater
Test concentrations used	Control, acetone control, 50 µg l <sup>-1</sup>
Number of replicates per concentration	3
Number of organisms per replicate	2–3 g of a species per 200 ml bottle
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 6 hours, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	The quality of the study is uncertain due to information presented in the paper, but there was no analytical confirmation of the exposure concentration.

<b>Reliability of study</b>	<b>Not reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>3</b>

<b>Reference</b>	Sloof and Canton 1983
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<b>Information on the test species</b>	
Test species used	Bacteria ( <i>Pseudomonas fluorescens</i> ), cyanobacteria ( <i>Microcystis aeruginosa</i> ), algae ( <i>Scenedesmus pannonicus</i> ), plants ( <i>Lemna minor</i> ), crustaceans ( <i>Daphnia magna</i> ), insects ( <i>Culex pipiens</i> ), hydrozoans ( <i>Hydra oligactis</i> ), molluscs ( <i>Lymnaea stagnalis</i> ), viviparous and oviparous fish ( <i>Poecilia reticulata</i> and <i>Oryzias latipes</i> ) and amphibians ( <i>Xenopus laevis</i> )
Source of the test organisms	Laboratory cultures
Holding conditions prior to test	Not stated
Life stage of the test species used	<i>Pseudomonas fluorescens</i> : Log phase <i>Microcystis aeruginosa</i> : Log phase <i>Scenedesmus pannonicus</i> : Log phase <i>Lemna minor</i> : - <i>Daphnia magna</i> : <24 hours old <i>Culex pipiens</i> : 1st instar <i>Hydra oligactis</i> : budless <i>Lymnaea stagnalis</i> : 5 months <i>Lymnaea stagnalis</i> : eggs <i>Poecilia reticulata</i> : 3–4 weeks old <i>Oryzias latipes</i> : eggs <i>Xenopus laevis</i> : <2 days old

<b>Information on the test design</b>	
Methodology used	There is only a limited description of the methodology.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Artificial media
Test concentrations used	Not stated
Number of replicates per concentration	<i>Pseudomonas fluorescens</i> : 3 <i>Microcystis aeruginosa</i> : 3 <i>Scenedesmus pannonicus</i> : 3 <i>Lemna minor</i> : 2 <i>Daphnia magna</i> : 2 <i>Culex pipiens</i> : 2 <i>Hydra oligactis</i> : 5 <i>Lymnaea stagnalis (growth)</i> : 1 <i>Lymnaea stagnalis (hatching)</i> : 1 <i>Poecilia reticulata</i> : 1 <i>Oryzias latipes</i> : 1 <i>Xenopus laevis</i> : 1

Number of organisms per replicate	<i>Pseudomonas fluorescens</i> : ~10 <sup>10</sup> <i>Microcystis aeruginosa</i> : 1.5 x 10 <sup>6</sup> <i>Scenedesmus pannonicus</i> : 1.5 x 10 <sup>6</sup> <i>Lemna minor</i> : 2 fronds <i>Daphnia magna</i> : 25 <i>Culex pipiens</i> : 30 <i>Hydra oligactis</i> : 2 <i>Lymnaea stagnalis (growth)</i> : 20 <i>Lymnaea stagnalis (hatching)</i> : 5 <i>Poecilia reticulata</i> : 25 <i>Oryzias latipes</i> : 35 <i>Xenopus laevis</i> : 75
Nature of test system (Static, Semi-static or, Flow-through, duration, feeding)	<i>Pseudomonas fluorescens</i> : static, 8 hours, no feeding <i>Microcystis aeruginosa</i> : static, 96 hours, no feeding <i>Scenedesmus pannonicus</i> : static, 96 hours, no feeding <i>Lemna minor</i> : static, 7 days, no feeding <i>Daphnia magna</i> : semi-static, 21 days feeding <i>Culex pipiens</i> : semi-static, 25 days, feeding <i>Hydra oligactis</i> : semi-static, 21 days, feeding <i>Lymnaea stagnalis (growth)</i> : semi-static, 40 days, feeding <i>Lymnaea stagnalis (hatching)</i> : static, 7 days, no feeding <i>Poecilia reticulata</i> : semi-static, 28 days, feeding <i>Oryzias latipes</i> : semi-static, 40 days, feeding <i>Xenopus laevis</i> : semi-static, 100 days, feeding
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	
<b>Reliability of study</b>	<b>Not reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>3</b>

<b>Reference</b>	Strawn and Muckerman 1994
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<b>Information on the test species</b>	
Test species used	Rainbow trout ( <i>Oncorhynchus mykiss</i> )
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Early life stages, eggs from female rainbow trout milt from adult brood stock were used to produce newly fertilised eggs for the study

<b>Information on the test design</b>	
Methodology used	EPA Guideline E 72-4
Form of the test substance	Technical dimethoate (99.1% purity)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Not stated in EU DAR 2005
Test concentrations used	0 (control), 0 (solvent control) 380, 750, 1,500, 3,000 and 6,000 µg l <sup>-1</sup>
Number of replicates per concentration	4 chambers per concentration
Number of organisms per replicate	120 (30 eggs per chamber), on day 40 (4 days post-hatch) sac-fry were thinned to 15 animals per replicate
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Flow-through, 96 days duration (60 days post-hatch), live brine shrimp nauplii ( <i>Artemia</i> sp.) was introduced on day 50 and a commercially prepared salmon starter was added to the diet on day 60.
Measurement of exposure concentrations	Yes (each concentration was measured 19 times during the test period)
Measurement of water quality parameters	Yes (temperature and dissolved oxygen)
Test validity criteria satisfied	Not stated in EU DAR 2005
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	Yes
Overall comment on quality	The study is of good quality having been conducted to a standardised methodology under GLP.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

<b>Reference</b>	Wuthrich 1990a
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<b>Information on the test species</b>	
Test species used	Rainbow trout ( <i>Oncorhynchus mykiss</i> )
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Juveniles at start of the test: mean weight = 2.35 g, mean length = 62 mm,

<b>Information on the test design</b>	
Methodology used	OECD Guideline 204*
Form of the test substance	Technical dimethoate (99.0% purity)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Not stated in EU DAR 2005
Test concentrations used	0 (control), 80, 400, 2,000, 10,000 and 50,000 $\mu\text{g l}^{-1}$
Number of replicates per concentration	1
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Flow-through system, 21 days, Feeding
Measurement of exposure concentrations	Yes (lowest, intermediate and highest concentrations were measured after 1, 8 and 21 days)
Measurement of water quality parameters	Not stated in EU DAR 2005
Test validity criteria satisfied	Not stated in EU DAR 2005
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	Yes
Overall comment on quality	The study is of good quality having been conducted to a standardised methodology under GLP.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1,00.html)

<b>Reference</b>	Wuthrich 1990b
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<b>Information on the test species</b>	
Test species used	Water fleas ( <i>Daphnia magna</i> )
Source of the test organisms	Not stated in EU DAR 2005
Holding conditions prior to test	Not stated in EU DAR 2005
Life stage of the test species used	Juveniles

<b>Information on the test design</b>	
Methodology used	OECD Guideline 202*
Form of the test substance	Technical dimethoate (99.0% purity)
Source of the test substance	Not stated in EU DAR 2005
Type and source of the exposure medium	Not stated in EU DAR 2005
Test concentrations used	0 (control), 40, 100, 250, 600 and 1,500 µg l <sup>-1</sup>
Number of replicates per concentration	8
Number of organisms per replicate	5
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Semi-static system (with renewal of exposure concentrations on days 2,5, 7, 9, 12, 16 and 19), 21 days, feeding with a mixture of baker's yeast and algae ( <i>Scenedesmus subspicatus</i> )
Measurement of exposure concentrations	Yes (lowest, intermediate and highest concentrations were measured after 1, 8 and 21 days)
Measurement of water quality parameters	Not stated in EU DAR 2005
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Not stated in EU DAR 2005
Study conducted to GLP	Yes
Overall comment on quality	The study is of good quality having been conducted to a standardised methodology under GLP.

<b>Reliability of study</b>	<b>Reliable</b>
<b>Relevance of study</b>	<b>Relevant</b>
<b>Klimisch Code</b>	<b>1</b>

\* See [http://www.oecd.org/document/62/0,2340,en\\_2649\\_34377\\_2348862\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/62/0,2340,en_2649_34377_2348862_1_1_1_1,00.html)



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