Proposed EQS for Water Framework Directive Annex VIII substances: methiocarb *(For consultation)*

by
Water Framework Directive - United Kingdom Technical Advisory Group (WFD-UKTAG)

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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 the Environment Agency, 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 methiocarb using the methodology described in Annex V of the Directive. There are existing EQSs for methiocarb, 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 methiocarb. 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

Methiocarb is a carbamate compound with insecticidal, acaricidal and molluscicidal activity. Methiocarb is moderately soluble and is expected to undergo some adsorption to sediment and suspended solids but it is not expected to accumulate in sediments. Volatilisation is not expected to occur, based on a Henry's Law constant of 1.2×10^{-4} $Pa.m^3.mole^{-1}$ at 20°C. The hydrolysis of methiocarb is pH dependent with first-order DT50s of 321, 24 and 0.21 days having been calculated at pH 5, 7 and 9, respectively. In aerobic natural water, 80% breakdown of methiocarb has been reported after 3 days. Given reported bioconcentration factor (BCF) values of 11-90, methiocarb is unlikely to accumulate in fish.

Availability of data

Long-term freshwater toxicity data are available for three taxonomic groups, i.e. algae, crustaceans and fish, with crustaceans being more sensitive than algae and fish. Short-term toxicity tests are available for six taxonomic groups, i.e. algae, amphibians, crustaceans, fish, insects and molluscs, with insects and crustaceans being more sensitive than other taxa. For marine organisms, single species short-term toxicity data are available for three different taxonomic groups; crustaceans, fish and molluscs. However, no long-term toxicity data are available for saltwater taxa.

No information on the effects of methiocarb on freshwater or saltwater organisms from mesocosm or field studies was located.

Derivation of PNECs

Long-term PNEC for freshwaters

The lowest valid long-term toxicity value for freshwater invertebrates is a 21-day No Observed Effect Concentration (NOEC) of 0.1 µg active ingredient (a.i.) l^{-1} for effects on the reproduction of the waterflea *Daphnia magna*. Reliable long-term NOECs are available for algae, invertebrates and fish. Therefore, based on the EU Technical Guidance Document (TGD) methodology, an assessment factor of 10 could be applied to the lowest valid toxicity value. This results in a $PNEC_{freshwater\_lt}$ of 0.01 µg l^{-1}.

The current long-term EQS for freshwater is 0.01 µg l^{-1} and was derived by applying an assessment factor of 150 to the then most reliable short-term data (24-hour LC50 of 1.6 µg l^{-1}) obtained for the midge (*Chironomus tentans*). This endpoint is no longer considered reliable because there was high control mortality in the test, and several test details are unreported.

Short-term PNEC for freshwaters

Reliable short-term data are available for algal, invertebrate and fish species. The lowest valid acute toxicity value for freshwater invertebrates is a 48-hour EC50 of 7.7 µg a.i. l^{-1} for the effects of technical grade methiocarb on the immobilisation of the waterflea *Daphnia magna.* Lower short-term values have been reported in non-GLP studies, but these are considered to be unreliable due to the use of field collected organisms. Based on the EU Technical Guidance Document (TGD) methodology and a large body of acute data for methiocarb, an assessment factor of 10 could be applied to the lowest valid toxicity value. This results in a $PNEC_{freshwater\_lt}$ of 0.77 µg l^{-1}.

The current short-term EQS for freshwaters is 0.16 µg l^{-1} and was derived by applying an assessment factor of 10 to the then most reliable short-term data (24-hour LC50 of 1.6 µg l^{-1}) obtained for the midge (*Chironomus tentans*). This study is not considered to be of sufficient reliability and has therefore not been used to derive the PNEC.

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 $PNEC_{saltwater\_lt}$ based on the saltwater data alone, and it is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation. Reliable chronic NOECs are available for algae, invertebrates and fish. The lowest long-term toxicity value in the combined dataset is a 21-day NOEC of 0.1 µg active ingredient (a.i.) l^{-1} for effects on the reproduction of the waterflea *Daphnia magna*. This is consistent with the greater sensitivity expected in insects and crustaceans given the mode of action of methiocarb. However, since no long-term data is available for marine species such as echinoderms and molluscs it is proposed that an assessment factor of 100 is applied. This results in a $PNEC_{saltwater\_lt}$ of 0.001 µg l^{-1}.

This current long-term EQS for saltwaters is 0.01 µg l^{-1} and was derived by applying an assessment factor of 150 to the then most reliable short-term data (24-hour LC50 of 1.6 µg l^{-1}) obtained for the midge (*Chironomus tentans*). This study is not considered to be of sufficient reliability and has therefore not been used to derive the PNEC.

Short-term PNEC for saltwaters

Single species short-term toxicity data for marine organisms are available for three different taxonomic groups, i.e. crustaceans, fish and molluscs. Therefore, it is proposed that the $PNEC_{saltwater\_st}$ 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 7.7 µg a.i. l^{-1} for the effects of technical grade methiocarb on the

immobilisation of the waterflea *Daphnia magna.* This is consistent with the greater sensitivity expected in insects and crustaceans given the mode of action of methiocarb. However, since no short-term data is available for marine species such as echinoderms it is proposed that an assessment factor of 50 is applied. This results in a $PNEC_{saltwater\_st}$ of 0.15 µg a.i. l^{-1}.

The current short-term EQS for saltwaters is 0.16 µg l^{-1} and was derived by applying an assessment factor of 10 to the then most reliable short-term data (24-hour LC50 of 1.6 µg l^{-1}) obtained for the midge (*Chironomus tentans*).

PNECs for sediment

Since the log Kow of methiocarb is 3.08, the derivation of PNECs for the protection of benthic organisms is required according to the TGD. However, although a sediment toxicity study is available, it is not considered appropriate for the derivation of a $PNEC_{sediment}$.

PNECs for secondary poisoning

Bioconcentration data (as BCF values) for methiocarb are low, with values for fish ranging from 11 to 90. 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^{-1})**	**Existing EQS (µg l^{-1})**
Freshwater/long-term	0.01	0.01
Freshwater/short-term	0.77	0.16
Saltwater/long-term	0.001	0.01
Saltwater/short-term	0.15	0.16
Sediment	Insufficient data	-
Secondary poisoning	Not required	–

Analysis

For water, proposed PNECs derived for methiocarb range from 0.001 to 0.77 µg 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 gas chromatography/mass spectrometry (GC-MS) and capable of achieving detection limits as low as 0.02 µg l^{-1} do not offer adequate performance to analyse for methiocarb against all proposed PNECs**.**

Implementation issues

Based on consideration of the information collated within the report and the proposed PNECs the following comments are made re: implementation:-

- Current analytical methods are not sensitive enough to assess compliance with the lowest proposed PNECs. This will require further investigation.

Contents

1 Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)[1] is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. UKTAG has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes Predicted No-Effect Concentrations (PNECs) for methiocarb using the methodology described in Annex V of the Directive. There is an existing EQSs for methiocarb, 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 methiocarb. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data.[2] Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V 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.

1.1 Properties and fate in water

Methiocarb is a carbamate compound with insecticidal, acaricidal and molluscicidal activity. Methiocarb is moderately soluble and is expected to undergo some adsorption to sediment and suspended solids. Volatilisation is not expected to occur, based on a Henry's Law constant of 1.2 $x10^{-4}$ $Pa.m^3.mole^{-1}$ at 20°C (EU DAR, 2005). The hydrolysis of methiocarb is pH dependent with first-order DT50s of 321, 24 and 0.21 days having been calculated at pH 5, 7 and 9, respectively (EFSA, 2006). In aerobic natural water, 80% breakdown of methiocarb has been reported after 3 days. Given reported bioconcentration factor (BCF) values of 11-90, methiocarb is unlikely to accumulate in fish (PSD, 1998).

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

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

Table 2.1 Species covered by this report

Name	CAS Number
Methiocarb	2032-65-7

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 methiocarb. 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	0.77 µg l^{-1}	Insufficient data	0.16 µg l^{-1} (MAC)
Freshwater long-term	0.01 µg l^{-1}	Insufficient data	0.01 µg l^{-1} (AA)
Saltwater short-term	0.15 µg l^{-1}	Insufficient data	0.16 µg l^{-1} (MAC)
Saltwater long-term	0.001 µg l^{-1}	Insufficient data	0.01 µg l^{-1} (AA)
Sediment	Insufficient data	–	–
Secondary poisoning	Not required	–	–

AA = Annual Average
AF = Assessment Factor
SSD = Species Sensitivity Distribution
TGD = Technical Guidance Document

2.3 Hazard classification

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

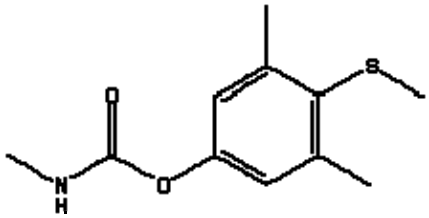
Table 2.3 Hazard classification

R-phrases and labelling	Reference
R25-50/53 S 1/2, 22, 37, 45, 60, 61	ECB – ESIS (Accessed December 2008)

2.4 Physical and chemical properties

Table 2.4 summarises the physical and chemical properties of methiocarb.

Table 2.4 Physical and chemical properties of methiocarb

Property	Value	Reference
CAS number	2032-65-7	Chemfinder 2007
Substance name	Mercaptodimethur	Chemfinder 2007
Molecular formula	$C_{11}H_{15}NO_2S_2$	Chemfinder 2007
Molecular structure		Chemfinder 2007
Molecular weight	225.3	Chemfinder 2007
Colour/form	Colourless, white to beige crystalline powder	EU DAR, 2005
Odour	Odourless or phenol-like	EU DAR, 2005
Melting point (°C)	119	EU DAR, 2005
Boiling point (°C)	311 (estimate)	EU DAR, 2005
Vapour pressure	1.5 $x10^{-5}$ Pa at 20ºC (extrapolated) 3.6 $x10^{-5}$ Pa at 25ºC (extrapolated)	EU DAR, 2005
Density/specific gravity	1.25 (99.5% purity)	EU DAR, 2005
Henry's Law constant	1.2 $x10^{-4}$ $Pa.m^3.mol^{-1}$ at 20ºC	EU DAR, 2005
Solubility	27 mg l^{-1} in water at 20ºC 0.57 g l^{-1} in n-heptane at 20ºC >250 g l^{-1} in dichloromethane at 20ºC	EU DAR, 2005

2.5 Environmental fate and partitioning

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

Table 2.5 Environmental fate and partitioning of methiocarb

Property	Value	Reference
Abiotic fate	The OH rate constant has been estimated at 1.35×10^{-11} cm^3.molecule.sec^{-1} at 25°C.	SRC 2007
Speciation	pKa -0.82 at 25°C.	SRC 2007
Hydrolytic stability	The hydrolysis of methiocarb is pH dependent. First-order DT50s of 321, 24 and 0.21 days are reported at pH 5, 7 and 9, respectively.	EFSA 2006
Photostability	A photochemical half-life of 128 days has been calculated at pH5. Methiocarb will be photochemically degraded in the atmosphere with a half-life of 13.8 hours.	PSD 1998 EFSA 2006
Volatilisation	Volatilisation is not expected to be an important environmental fate process, based on a Henry's Law constant of 1.2×10^{-4} Pa.m³.$mole^{-1}$ at 20°C.	Tomlin 2006
Distribution in water/sediment systems (active substances)	Methiocarb is expected to undergo some adsorption to sediment and suspended solids, based on a log Kow of 3.08.	Tomlin 2006
Metabolites	Methiocarb sulphoxide, methiocarb sulphoxide phenol, methiocarb sulphone phenol, methiocarb methoxy sulphone and methiocarb phenol have all been identified as metabolites of methiocarb.	EFSA 2006
Degradation in soil	The rate of degradation of methiocarb in aerobic soil is dependent on soil pH, organic matter and the methiocarb concentration. The degradation rate increases with increasing pH, but becomes slower with increasing organic matter content and increasing methiocarb concentration. In sunny or warm conditions methiocarb is likely to be rapidly degraded in soils (with half lives in the range of 4 to 64 days) while in cold climates or cold conditions methiocarb may not degrade for more than a year. Degradation in anaerobic conditions is reported to be slower than in aerobic soil. Overall for soils the available data indicate that the main abiotic degradation processes are hydrolysis and oxidation. Degradation is more rapid in alkaline soils and at lower application rates	EFSA 2006 Murgatroyd *et al*. 1997 EFSA 2006 Murgatroyd *et al*. 1997

Property	Value	Reference
Biodegradation	-	-
Octanol–water coefficient (Log Kow)	3.08 3.11 at pH 4 and 20°C 3.18 at pH 7 and 20°C	Tomlin 2006 EFSA 2006 EFSA 2006
Log Koc	Kocs of 1000, 632, 600 and 408 (log Koc values of 3.00, 2.80, 2.78 and 2.61) have been reported in sand, sand-loam, silt-loam and clay-loam soils, respectively.	PSD 1998
Bioaccumulation BCF	Bioconcentration factors of 11 and 60-90 have been determined in *Lepomis macrochirus* (bluegill sunfish) at methiocarb concentrations of 0.03 and 0.1 mg l^{-1} respectively. Methiocarb is therefore not expected to accumulate in fish.	Lamb 1974, cited in EU DAR, 2005 Dorgerloh *et al.* 2002, cited in EU DAR, 2005

Methiocarb is moderately soluble (27 mg l^{-1} at 20°C) (Tomlin, 2006). In the aqueous environment, methiocarb is expected to undergo some adsorption to sediment and suspended solids, based on a log Kow of 3.08 (Tomlin, 2006). However, accumulation in sediments is not expected to occur. Volatilisation is not expected to occur, based on a Henry's Law constant of 1.2 x10^{-4} Pa.m³.$mole^{-1}$ at 20°C (Tomlin, 2006).

The hydrolysis of methiocarb has been shown to be pH dependent in a degradation study conducted in dark conditions at 25°C where phenyl-1-^{14}C-labelled methiocarb was incubated in buffered aqueous solutions at pH 5, 7 and 9. At pH 5, 93% of the applied radioactivity was still present as methiocarb after 51 days. After 30 days at pH 7, approximately 50% of the applied radioactivity was present as methiocarb whilst 46% was identified as the metabolite methiocarb phenol. At pH 9, approximately 18% of the applied radioactivity was present as methiocarb after 3 days whilst 82% of the applied radioactivity was identified as methiocarb phenol. Methiocarb sulphoxide phenol was also detected as a metabolite (maximum 1.5% of applied radioactivity at day 1). First-order DT50s of 321, 24 and 0.21 days were calculated at pH 5, 7 and 9, respectively (EFSA, 2006). These data indicate that hydrolysis is not a major loss route for methiocarb under normal pH conditions.

In an aqueous photolysis study, solutions of radiolabelled methiocarb in sterile acetate buffer at pH 5 were placed in quartz tubes immersed in a deionised water bath and exposed to direct sunlight in Lexington, Kentucky, USA during January and February 1988. The mean temperature of the solutions was 24.9°C. After 30 days, 83.8% of the applied radioactivity was present as methiocarb in irradiated samples, compared to 94.7% in dark controls. The photolysis half-life in the irradiated solution was calculated to be 88 days. However, as some hydrolysis had occurred, a corrected photochemical half-life of 128 days was calculated (PSD, 1998). These data indicate that photolysis is not a major loss route for methiocarb.

The fate of methiocarb has been studied in aerobic and anaerobic natural water. Pond and sediment water were collected from a natural pond in Stanley, Kansas, USA. The sediment was characterised as silty-clay-loam. The pH of the water was reported to be 8. No further material characterisation was performed. In the aerobic study, 100 ml of water was inoculated with 2 mg radiolabelled methiocarb l^{-1}. In the anaerobic study, pond sediment and pond water containing glucose (2 mg ml^{-1}) and calcium nitrate (8.4 mg ml^{-1}) were stored in glass jars and left for 33 days, after which the water was discarded and replaced with fresh pond water. Radiolabelled methiocarb was applied at a concentration of 2 mg l^{-1}. Under aerobic conditions,

mainly organosoluble materials were detected throughout the study. After 3 days, 80% of the radioactivity in the sample was present as the breakdown product methiocarb phenol, which was subsequently converted to methiocarb sulphoxide (63% of applied radioactivity after 14 days). In anaerobic conditions, with sediment present, much more of the radioactivity was associated with the sediment. The conversion of methiocarb to methiocarb phenol occurred in both the water and sediment phase. As the concentration of methiocarb phenol in the sediment decreased, the concentration of an unidentified residue bound to the humic and fulvic acid fractions increased (PSD, 1998).

Volatilisation of methiocarb from moist soil surfaces is not expected to occur, based on a Henry's Law constant of 1.2 $x10^{-4}$ $Pa.m^3.mole^{-1}$ at 20°C (Tomlin 2006). Koc values of 1000, 632, 600 and 408 have been reported in sand, sand-loam, silt-loam and clay-loam soils, respectively (mean Koc 660), indicating methiocarb is moderately mobile in soil (PSD, 1998).

Degradation of radiolabelled methiocarb in aerobic soils has been investigated in laboratory studies conducted in four German soils (EFSA, 2006). These soils covered a pH range of 6.0-8.4, clay content of 5.0-13.8% and organic matter content of 1.2-4.5%. The main degradation products were methiocarb sulphoxide, methiocarb sulphoxide phenol, methiocarb sulphone phenol and methiocarb methoxy sulphone. The rate of degradation of methiocarb in aerobic soil was dependent on soil pH, organic matter and the methiocarb concentration. The degradation rate increases with increasing pH, but becomes slower with increasing organic matter content and increasing methiocarb concentration. Other studies indicate that in sunny or warm conditions methiocarb is likely to be rapidly degraded in soils (with half lives of 4 to 69 days) while in cold climates or cold conditions methiocarb may persist for more than a year (Murgatroyd *et al*. 1997).

In a soil degradation study conducted in anaerobic conditions at 24°C in sandy-loam soil (pH 6.7, 10% clay, 1% organic matter), mineralisation was reported to be slower than in aerobic soil with similar degradation products being produced as in aerobic soils (EFSA, 2006).

In studies of the photolysis of radiolabelled methiocarb conducted in soil, methiocarb sulphoxide was identified as the major degradation product and represented 57.2 and 42.8% of the applied radioactivity after 1 and 15 days, respectively (EFSA, 2006). The results of the studies suggested that photodegradation in soil will contribute to the degradation of methiocarb to a certain extent under environmental conditions.

Overall for soils, the available data indicate that the main abiotic degradation processes are hydrolysis and oxidation. Degradation is more rapid in alkaline soils and at lower application rates (Murgatroyd *et al*. 1997).

Methiocarb will be photochemically degraded in the atmosphere with a half-life of 13.8 hours (EFSA, 2006).

Bioconcentration factor (BCF) values of 11-90 have been reported in studies using bluegill sunfish (*Lepomis macrochirus*). Lamb (1974, cited in EU DAR, 1995) measured the accumulation and persistence of residues of ^{14}C-methiocarb in Bluegill (*Lepomis macrochirus*) in a flow-through test over 34 days. Eighty fish (weight: 0.1-0.5 g) were continuously exposed to a concentration of 0.01 mg ^{14}C-methiocarb l^{-1} or held in a control (test volume 50 litres) after 2 days of acclimatisation. On day 34, the fish were exposed to uncontaminated water. The fish were fed a maintenance diet during the exposure and withdrawal period. The study was conducted to US EPA Guideline 72-32 and to GLP. In the study ^{14}C residues accumulated to a maximum level of 60 to 90 fold in bluegill fish exposed to 0.01 mg ^{14}C-methiocarb l^{-1}. Therefore, the BCF for bluegill sunfish was determined to be 60 - 90.

In a later study in bluegill sunfish Dorgerloh *et al.* (2002, cited in EU DAR, 1995) assessed the bioconcentration, and depuration of residues of [phenyl-1-^{14}C]-methiocarb-phenol, (radiochemical purity: 98.5%, chemical purity: 97.6%). Sixty four young bluegill sunfish (*Lepomis macrochirus*, with a mean body weight of 4.3 g and a mean body length of 6.6 cm) were exposed at each treatment level and one control. A dosing system was used to maintain a mean water concentration (nominal) of 30 µg [^{14}C]-methiocarb-phenol l^{-1} for a 28-day exposure period. After exposure the test fish were placed in clean water for 14 days in order to determine the depuration of [^{14}C]-methiocarb-phenol. The study was performed in accordance with OECD 305 and to GLP. In the study ^{14}C residues accumulated to a maximum level of 14.5 fold in bluegill fish exposed to 30 µg [^{14}C]-methiocarb-phenol l^{-1} for a 28-day exposure period. Normalised to 6% lipid content the BCF for bluegill sunfish was determined to be 10.9. On this basis methiocarb is unlikely to accumulate in fish.

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;[3]
- the Draft Assessment Report prepared under Council Directive 91/414/EEC (EU DAR, 2005) and the EFSA Scientific Report Conclusion on the Peer Review of Methiocarb (EFSA, 2006),
- sources such as ScienceDirect®[4].

All relevant data has been quality assessed as part of the preparation of this report (see relevant Klimisch codes in data tables), even if it has already undergone prior quality assessment by other organisations.

Methiocarb is a carbamate compound with insecticidal, acaricidal and molluscicidal activity. It also has a repellent effect against birds. Methiocarb acts by combining with and inactivating the enzyme acetylcholinesterase (AChE), causing an accumulation of the neurotransmitter acetylcholine, producing an overstimulation of the exposed organism's nervous systems and ultimately results in death. As a result certain invertebrate taxa (crustaceans, insects and molluscs) would be expected to be sensitive to exposure to methiocarb.

2.6.1 Toxicity to freshwater organisms

Freshwater toxicity data on methiocarb are available for various taxonomic groups. Long-term toxicity data are available for three taxonomic groups (algae, crustaceans and fish), with crustaceans being more sensitive than algae and fish. Short-term toxicity tests are available for six taxonomic groups (algae, amphibians, crustaceans, fish, insects and molluscs), with insects and crustaceans being more sensitive than other taxa. Overall, the available short-term and long-term toxicity test data indicate that insects and crustaceans are more sensitive than algae and fish to technical grade methiocarb. The indicated greater toxicity of technical grade methiocarb to

[3] http://www.epa.gov/ecotox/
[4] http://www.sciencedirect.com/

arthropods compared with other taxonomic groups (where data using comparable experimental designs are available) is consistent with the use of the substance as an insecticide.

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for methiocarb 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 methiocarb PNECs. The lowest critical freshwater data for methiocarb are presented in Tables 2.6 (for long-term data) and 2.7 (for short-term data).

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

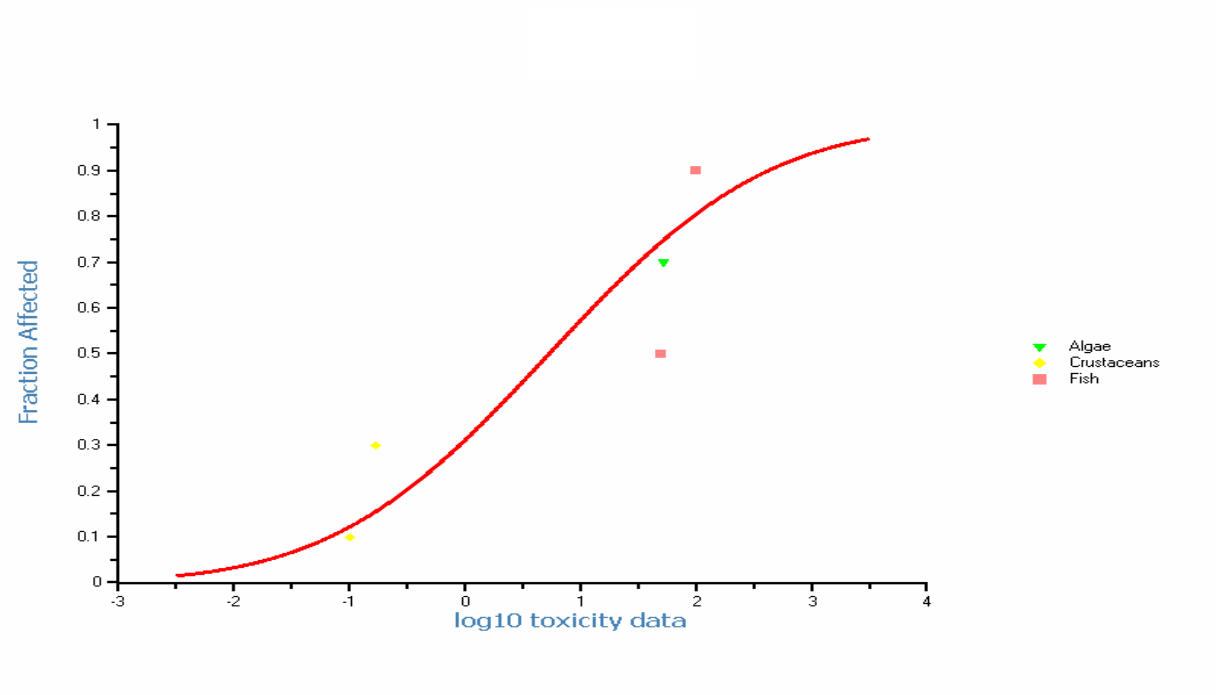


Figure 2.2 Cumulative distribution function of freshwater short-term data (µg a.i. l^{-1}) for methiocarb

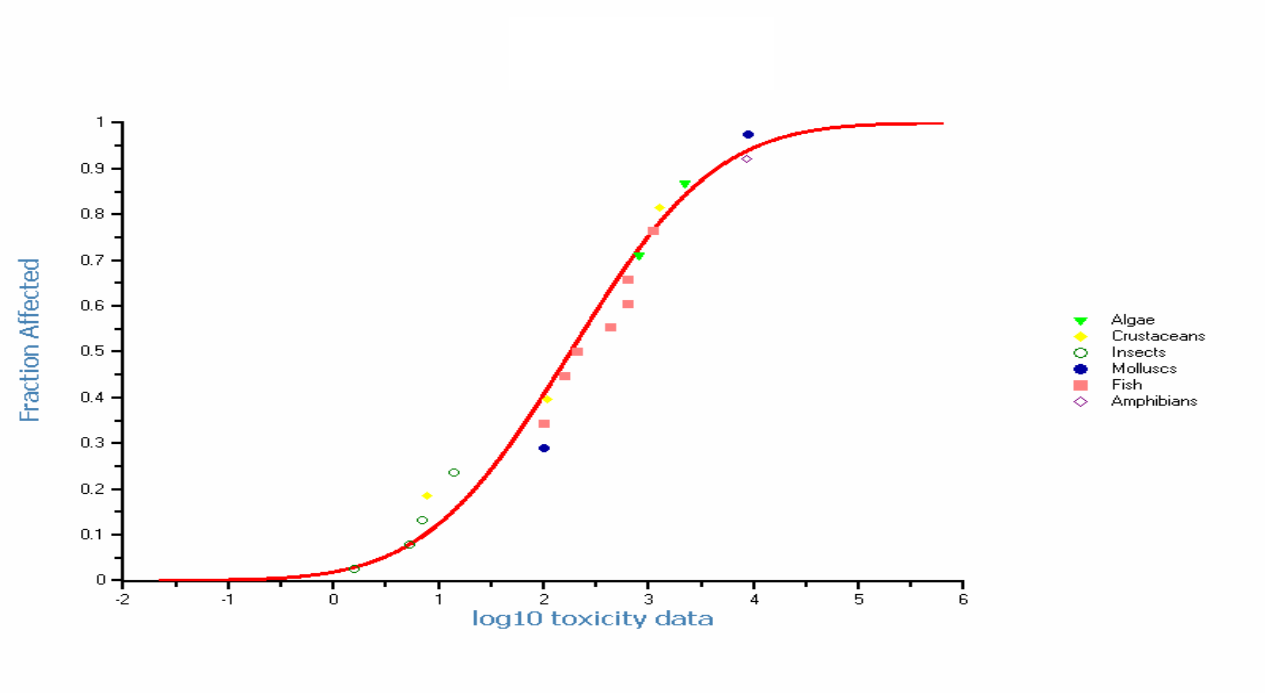


Table 2.6 Most sensitive long-term aquatic toxicity data for freshwater organisms exposed to methiocarb

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (days)	Conc. (µg a.i. l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Algae												
Technical grade (99.3-99.4% purity)	*Scenedesmus subspicatus*	Green algae	ALG	NOEC	Growth inhibition (Biomass)	3	52	s	y	Conducted to OECD 203 and EEC C3	1	Peither (2000a), cited in EU DAR (2005)
Invertebrates												
Technical grade (99.7% purity)	*Daphnia magna* (<24 h old)	Water flea	CRU	NOEC	Reproduction	21	0.1	f	y	Temperature = 20±2ºC, 16/8 hour light/dark cycle, fed, pH = 8.2-8.4, DO = 8.0-8.7 mg/l. Conducted according to OECD Guideline 210, US EPA 72-4 and GLP	1	Forbis (1988), cited in EU DAR (2005)
Technical grade (99.7% purity)	*Daphnia magna* (<24 h old)	Water flea	CRU	NOEC	Adult growth	21	0.17	f	y	Temperature = 20±2ºC, 16/8 hour light/dark cycle, fed, pH = 8.2-8.4, DO 8.0-8.7 mg/l . Conducted according to OECD Guideline 210, US EPA 72-4 and GLP	1	Forbis (1988), cited in EU DAR (2005)
Fish												
Technical grade (97% purity)	*Oncorhynchus mykiss* (fry)	Rainbow trout	FIS	NOEC	Growth and biomass reduction (body weight) and fry survival	56	100	f	y	Temperature = 7-12ºC, 16/8 hour light/dark cycle, fed, pH = 6.2-8.0. Conducted according to OECD Guideline 210, US EPA 72-4 and GLP	1	Carlisle (1985), cited in EU DAR (2005)
Technical grade (97% purity)	*Oncorhynchus mykiss* (fry)	Rainbow trout	FIS	NOEC	Intoxication	56	50	f	y	Temperature = 7-12ºC, 16/8 hour light/dark cycle, fed, pH = 6.2-8.0. Conducted according to OECD Guideline 210, US EPA 72-4 and GLP	1	Carlisle (1985), cited in EU DAR (2005)

* See Annex 1.
[1] Exposure: s = static; ss = semi-static; f = flow-through. [2] Toxicant analysis: y = measured; n = nominal.
ALG = algae; CRU = crustaceans; FIS = fish
ND = no data
NOEC = No Observed Effect Concentration

Table 2.7 Most sensitive short-term aquatic toxicity data for freshwater organisms exposed to methiocarb

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. (µg a.i. l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Algae												
Technical grade (99.3-99.4% purity)	*Scenedesmus subspicatus*	Green algae	ALG	EC50	Growth inhibition (growth rate)	72	2200 (1500-3700)[a]	s	y	Conducted to OECD 203 and EEC C3	1	Peither (2000a), cited in EU DAR (2005)
Technical grade (99.3-99.4% purity)	*Scenedesmus subspicatus*	Green algae	ALG	EC50	Growth inhibition (biomass)	72	820 (380-1700)[a]	s	y	Conducted to OECD 203 and EEC C3	1	Peither (2000a), cited in EU DAR (2005)
Invertebrates												
Technical grade (99.3-99.4% purity)	*Daphnia magna* (<24 h old)	Water flea	CRU	EC50	Immobilisation	48	7.7 (6.7-8.8)[a]	ss	y	Conducted to OECD 203 and EEC C2	1	Peither (2000b), cited in EU DAR (2005)
Methiocarb (90% purity as Mesurol)	*Chironomus tentans* (3/4 instar larvae)	Midge	INS	LC50	Lethality	24	1.6	s	n	Temperature = 22 °C	3	Karnak and Collins (1974)
Technical grade (purity not stated)	*Hydropysche sp.*(larvae)	Caddisfly	INS	LC50	Lethality	96	14	s	n	Conducted according to ASTM methodology with unspecified modifications, Temperature = 16 ºC, pH = 7.1, Hardness = 44 mg $CaCO_3$ l^{-1}	3	Marking and Chandler (1981)
Technical grade (purity not stated)	*Isonychia sp.* (nymphs)	Mayfly	INS	LC50	Lethality	96	7.0 (5-11)[a]	s	n	Conducted according to ASTM methodology, with unspecified modifications, Temperature = 12 ºC, Hardness = 44 mg $CaCO_3$ l^{-1},	3	Marking and Chandler (1981)
Technical grade (99% purity as Bayer 37344)	*Pteronarcys californica* (nymphs, 30-35mm)	Stonefly	INS	LC50	Lethality	96	5.4 (4.6 – 6.4)[a]	s	n	Temperature = 15 ºC, pH = 7.1, Hardness = 44 mg $CaCO_3$ l^{-1}	3	Sanders and Cope (1968)

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. (µg a.i. l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Technical grade (purity not stated)	*Oxytrema cateria* (adults)	River horn snail	MOL	LC50	Lethality	96	100	s	n	Temperature = 16 ºC, pH = 7.1, Hardness = 44 mg $CaCO_3$ l^{-1}, Conducted according to ASTM methodology with unspecified modifications	3	Marking and Chandler (1981),
Fish												
Technical grade (purity not stated)	*Lepomis macrochirus*	Bluegill sunfish	FIS	LC50	Lethality	96	100	s	ND	Weight 0.97 g. Temperature 24°C	3	Cope (1965)
Technical grade (99% purity)	*Lepomis macrochirus*	Bluegill sunfish	FIS	LC50	Lethality	96	210 (121 – 364) [a]	s	ND	Conducted according to ASTM methodology. Weight = 1.0 g. pH = 7.2-7.5, Hardness 44 mg $CaCO_3$ l^{-1}, temperature = 24 ºC.	C	Office of Pesticide Programs (2007)
Methiocarb (purity not stated)	*Lepomis macrochirus*	Bluegill sunfish	FIS	LC50	Lethality	96	160	ND	ND	-	4	Fingas *et al* (1991)
Technical grade (99.3-99.4% purity)	*Lepomis macrochirus*	Bluegill sunfish	FIS	LC/EC50	NR	96	650 (440-960) [a]	f	n	Conducted to OECD 203 and EEC C1	1	Peither (2000c), cited in EU DAR (2005)
Technical grade (99.3-99.4% purity	*Oncorhynchus mykiss*	Rainbow trout	FIS	LC50	Lethality	96	1100 (730 – 1600) [a]	ss	y	Conducted to OECD 203 and EEC C1	1	Peither (2000d), cited in EU DAR (2005)
Technical grade (purity not stated)	*Oncorhynchus mykiss*	Rainbow trout	FIS	LC50	Lethality	96	640	s	ND	Weight 1.32 g. Temperature 12.8 ºC	3	Cope (1965)
Technical grade (98% purity)	*Oncorhynchus mykiss*	Rainbow trout	FIS	LC50	Lethality	96	436 (356-536) [a]	s	ND	Conducted according to EPA guidelines . Weight = 0.8-1.8 g, length = 40-50 mm, pH = 7.15-7.23, water hardness = 40-41 mg.l^{-1}, dissolved oxygen = 9.68-9.71 mg.l^{-1}.	C	Office of Pesticide Programs (2007)

Form of substance	Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. (µg a.i. l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Amphibians												
Technical grade (purity not stated)	*Rana sphenocephala (larvae)*	Leopard frog	AMP	LC50	Lethality	96	8700 (7800 – 9600) [a]	s	n	Conducted according to ASTM methodology with unspecified modifications Hardness = 24 mg $CaCO_3$ l^{-1}, temperature = 16 ºC.	3	Marking and Chandler (1981)

* See Annex 1;
C = Core data, which can be considered to be reliable (i.e. equivalent to Klimisch code 1 or 2 described in Annex 1)
[1] Exposure: s = static; ss = semi-static.
[2] Toxicant analysis: y = measured; n = nominal.
[a] Confidence Limits
ALG = algae; AMP = amphibians; CRU = crustaceans; FIS = fish; INS = insects; MOL = molluscs
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 three different taxonomic groups (crustaceans, fish and molluscs), with crustaceans apparently being the most sensitive group. No long-term toxicity data were located for saltwater taxa.

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 methiocarb used in the toxicity test.

The lowest critical short-term toxicity data for marine species are summarised in Table 2.8. A diagrammatic representation of the available short-term saltwater data (cumulative distribution function) for methiocarb 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 methiocarb PNECs.

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

Figure 2.3 Cumulative distribution function of saltwater short-term data (µg a.i. l^{-1}) for methiocarb

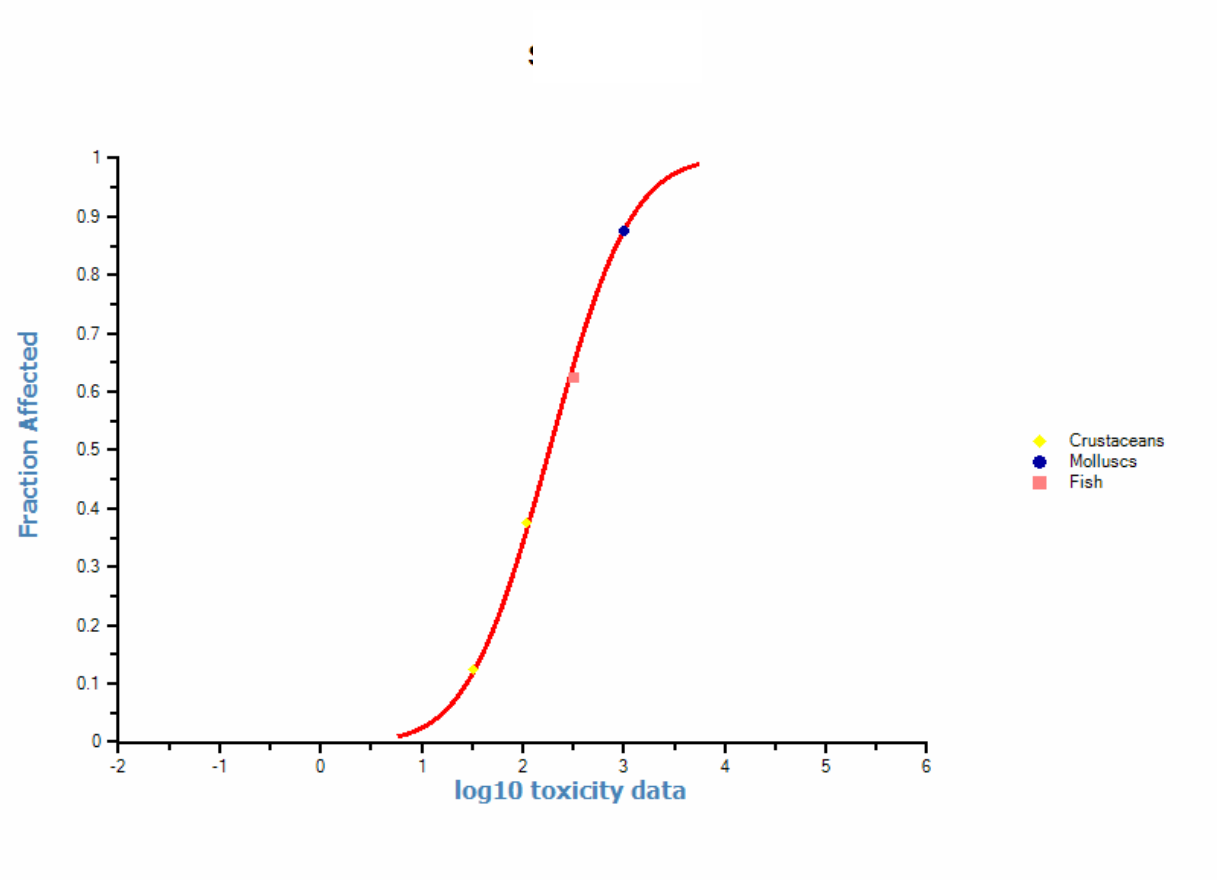


Table 2.8 Most sensitive short-term aquatic toxicity data for saltwater organisms exposed to methiocarb

Form of substance	Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration (hours)	Conc. (μg a.i. l^{-1})	Exposure [1]	Toxicant analysis [2]	Comment	Reliability (Klimisch Code*)	Reference
Invertebrates												
Technical grade (purity not stated	*Palaemonetes kadiakensis*	Glass shrimp	CRU	LC50	Lethality	96	110 (100 – 130) [a]	s	ND	Temperature = 16°C,	3	Marking and Chandler (1981)
Technical grade (99% purity)	*Penaeus duorarum* (Adults)	Northern pink shrimp	CRU	EC50	Equilibrium	48	55	s	n	Temperature = 27°C, salinity = 27 g l^{-1}	C	Butler (1964) , cited in Office of Pesticide Programs (2007)
Technical grade (99% purity)	*Crassostrea gigas* (Juveniles)	Eastern oyster	MOL	EC50	Not stated	96	>1000	f	ND		C	Office of Pesticide Programs (2007)
Not stated	*Paracentrotus lividus* (fertilised eggs)	Sea urchin	ECH	NR	Abnormal development	48	100-200	ND	ND	-	4	Tchounwou *et al.* (1991)
Fish												
Technical grade (99% purity)	*Fundulus similis* (Juveniles)	Longnose killifish	FIS	LC50	Lethality	96	320	f	n	Temperature 16°C	C	Butler (1963), cited in Office of Pesticide Programs (2007)

* See Annex 1: C = Core data, which can be considered to be reliable (i.e. equivalent to Klimisch code 1 or 2 described in Annex 1)
[1] Exposure: s = static; f = flow-through.
[2] Toxicant analysis: n = nominal; ND = No data
[a] Confidence Limits
CRU = crustaceans; ECH = echinoderm; FIS = fish; MOL = molluscs
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

The log Kow of methiocarb is 3.08 (see Section 2.5), therefore it is expected to undergo some adsorption to sediment and suspended solids. The toxicity of methiocarb to sediment dwelling organisms must therefore be considered. However, only one study is available which is not sufficient for the derivation of a PNEC (see Section 3.4)

Pery *et al*. (2004) investigated the toxicity of methiocarb to the sediment dwelling organism *Chironomus riparius* (chironomid). Test beakers were filled with artificial sediment (silicate; 0.1 l) and 0.3 l water taken from an uncontaminated spring. The water had a pH of 7.7, a specific conductivity of 40 µS cm^{-1} and contained only trace concentrations of chemicals, with the exception of aluminium and atrazine, which were detected at concentrations of 21 and 0.03 µg l^{-1}, respectively. Three days before the study, beakers were placed in a water bath at 21°C with a 16:8 hour light:dark photoperiod and the test water was gently aerated. Specific conductivity, temperature, pH, dissolved oxygen, nitrates and ammonia concentrations were measured daily. Test organisms were maintained on Tetramin® fish food and received 0.6 mg $larvae^{-1}$ day^{-1}, which corresponded to *ad libitum* conditions. Methiocarb (purity not reported) was introduced into the beakers by adding 0.1 l water containing nominal concentrations of the pesticide of 0, 10, 20, 30, 60, 80 or 100 µg l^{-1}. This water was introduced slowly to avoid disturbance of the sediment. Sediment methiocarb concentrations were not determined. At day 0, ten organisms were introduced into each glass beaker, twenty organisms were also randomly selected and measured at the beginning of the test. Growth tests were performed with 2^{nd}, 3^{rd} and 4^{th} instar larvae. They were two, four and six days old, respectively at day 0. The test was conducted for three days. Organisms were killed for measurement using a solution of 20% formaldehyde and 80% water. They were kept in this solution for less than 10 seconds to avoid distortion of their shape. Survival was also noted during this test. No information was given on the sediment concentration.

During the experiment, temperature was maintained at 21°C ± 0.5°C, pH was maintained at 8.1-8.4, specific conductivity was 300-400 µS cm^{-1}, dissolved oxygen was always above 80% and nitrate and ammonia levels were always below 2 mg l^{-1}.

Survival of 2^{nd} instar chironomids was lower than that of the 3^{rd} and 4^{th} instar. NOECs for survival of 30, 60 and 60 µg l^{-1} and LOECs of 60, 80 and 80 µg l^{-1} were reported in the 2^{nd}, 3^{rd} and 4^{th} instar, respectively. In terms of growth the second and third instar larvae showed marked reductions in length after 3 days methiocarb exposure at nominal concentrations of 60 and 80 µg l^{-1}, respectively. At lower exposure concentrations (20 and 30 µg l^{-1} for second instars and 30 µg l^{-1} for third and fourth instars).

Pery *et al*. (2004) also studied the effects of methiocarb on chironomid survival with or without tube building. Fourth instar chironomid larvae (6 days since hatching) were separated into two groups per methiocarb concentration, the first group were put into their beaker one day before the test to allow them to build tubes under clean water conditions. The second group (7 days since hatching) were introduced directly to beakers containing pesticide without the opportunity to build tubes. The test was conducted for two days with nominal methiocarb concentrations of 0, 67.5, 125, 250 500 and 1000 µg l^{-1}.

No significant differences were observed between the two groups and all of the dead organisms were found at the surface of the sediment. All control organisms survived. In the groups where the organisms had been introduced directly into the pesticide, no tubes were found. However, in the groups where the organisms had been placed in

clean water for one day before exposure to methiocarb, tubes were found. The authors concluded that these data indicate that organisms had left their tubes to die suggesting that tube formation in insect larvae is not protective against mortality following methiocarb exposure.

Although this study used relevant sediment dwelling organisms it is not considered to be suitable on its own to derive a sediment PNEC as sediment methiocarb concentrations were not determined.

2.6.4 Endocrine-disrupting effects

There are no data available on the endocrine disrupting effects of methiocarb to aquatic organisms.

2.6.5 Mode of action of methiocarb

Methiocarb is a carbamate compound with insecticidal, acaricidal and molluscicidal activity. It also has a repellent effect against depredating birds.

Carbamate compounds, such as methiocarb, 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 exposed organism's nervous systems and ultimately results in death. Invertebrates are particularly susceptible to this mode of action compared with most vertebrates.

2.7 Mesocosm and field studies

2.7.1 Freshwater mesocosm and field studies

No information on the effects of methiocarb on freshwater organisms from mesocosm or field studies was located.

2.7.2 Saltwater mesocosm and field studies

No information on the effects of methiocarb on saltwater organisms from mesocosm or 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 toxicity data are available for three taxonomic groups (algae, crustaceans and fish), with crustaceans being more sensitive than algae and fish.

A valid study of the effects of methiocarb to algae by Peither (2000a) (which is cited in the EU DAR, 2005) reported a 72 hour NOEC of 52 µg l^{-1} for growth inhibition (using the biomass endpoint) in green algae (*Scenedemus subspicatus*). No value was reported in the study for growth inhibition using the growth rate endpoint and no reason is given for the omission. The study was conducted according to OECD Guideline 203 and EEC Guideline C1 and was carried out to GLP. This study adopted a static design, with analytical confirmation of exposure concentrations.

The lowest reliable long-term toxicity value for crustaceans was in a study by Forbis (1988) (which is cited in the EU DAR, 2005) that reported a 21 day NOEC of 0.1 µg l^{-1} based on effects on reproductive in the waterflea (*Daphnia magna*). This study was conducted according to OECD Guideline 210 and US EPA Guideline 72-4 and was carried out to GLP. A flow-through exposure system was used and there was analytical confirmation of the exposure concentrations.

The lowest reported long-term toxicity data for fish was obtained in the rainbow trout (*Oncorhynchus mykiss*) carried out by Carlisle (1985) (and cited in EU DAR, 2005). The study reported 56 day NOECs of 50 µg l^{-1} for intoxication and 100 µg l^{-1} for growth, biomass reduction and fry survival, respectively. The study was conducted according to OECD Guideline 210 and US EPA Guideline 72-4 and was carried out to GLP. It used a flow-through system and included analytical confirmation of exposure concentrations.

Reliable long-term NOECs are available for algae, crustaceans and fish, and therefore an assessment factor of 10 could be applied to the lowest valid toxicity value based on the TGD approach. Using the 21-day NOEC of 0.1 µg a.i. l^{-1} for effects of the methiocarb on the reproduction of the waterflea (*Daphnia magna*) results in:

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

PNEC accounting for transient concentration peaks

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

A valid study of the effects of methiocarb to algae by Peither (2000a) (which is cited in the EU DAR, 2005) reported a 72 hour EC50 of 820 µg l^{-1} (95% confidence intervals of 380-1700 µg l^{-1}) based on growth inhibition (using biomass as an endpoint) and a 72 hour EC50 of 2200 µg l^{-1} (95% confidence intervals of 1500-3700 µg l^{-1}) based on growth inhibition (using growth rate as an endpoint). The study was conducted according to OECD Guideline 203 and EEC Guideline C1 and was carried out to GLP. This study adopted a static design, with analytical confirmation of exposure concentrations.

The lowest toxicity value for invertebrates was a 24-hour LC50 of 1.6 µg l^{-1} (0.9 µg l^{-1} uncorrected for control mortality) reported by Karnak and Collins (1974) for the midge (*Chironomus tentans*). This study was conducted in static conditions and did not involve analytical analysis of exposure concentrations. There are several limitations within this study, notably the high control mortality of 20%, that make the reliability of this result questionable.

In a study by Sanders and Cope (1968) (cited in OPP, 2007) a 96-hour LC50 of 5.4 µg l^{-1} (95% confidence intervals of 4.6-6.4 µg l^{-1}) was reported for mortality effects in nymphs of the stonefly (*Pteronarcys californica*). The study was carried out according to ASTM methodology using a static exposure regime. There was no analytical confirmation of the exposure concentrations. This study is not considered suitable for derivation of the PNEC since the study used field collected organisms, the history of which is unknown. However, it does provide supporting information which can be used alongside the definitive data.

In a study by Marking and Chandler (1981) (cited in OPP, 2007) a 96-hour LC50 of 7.0 µg l^{-1} (95% confidence intervals of 5-11 µg l^{-1}) was reported for mortality effects in nymphs of the mayfly (*Isonychia sp*). The study was carried out according to ASTM methodology using a static exposure regime. There was no analytical confirmation of the exposure concentrations. This study is not considered suitable for derivation of the PNEC since the study used field collected organisms, the history of which is unknown. However, it does provide supporting information which can be used alongside the definitive data.

Peither (2000b) (cited in EU DAR, 2005) reported a 48-hour LC50 of 7.7 µg l^{-1} (95% confidence intervals of 6.7-8.8 µg l^{-1}) for effects on immobilisation of the waterflea (*Daphnia magna*). This valid study was conducted according to OECD Guideline 203 and EEC C2 and carried out to GLP. It was carried out under semi-static conditions with analytical measurements of the exposure concentrations.

Several short-term studies were located for freshwater molluscs. The lowest reported short-term toxicity study was conducted in the River horn snail (*Oxytrema cateria*) by Marking and Chandler (1981). A 96 hour LC50 of 100 µg l^{-1} was determined in this study. Two other short-term studies reported higher LC50s of 3400 and 8800 µg l^{-1}. Although these results are considered unreliable, they suggest that freshwater molluscs are no more sensitive to methiocarb than other aquatic organisms.

Several short-term toxicity studies have been reported in fish. The lowest reported short-term LC50 in fish was reported by Cope (1965) and involved a 96 hour LC50 of 100 µg l^{-1} using the bluegill sunfish (*Lepomis macrochirus*). Limited details of this study are reported by Cope (1965), therefore it is considered to be unreliable. The lowest

valid short-term LC50 in fish was a 96-hour LC50 value of 210 µg l^{-1} for *L. macrochirus* that was reported in the US OPP database (OPP, 2007).

Overall the lowest reliable toxicity value is a 48 hour LC50 of 7.7 µg l^{-1} for effects on immobilisation of the waterflea (*Daphnia magna*) measured in a semi-static exposure regime (Peither, 2000b). Based on the guidance given in the TGD on effects assessment for intermittent releases, and the large body of acute toxicity data for methiocarb it is proposed to apply an assessment factor of 10 resulting in:

$PNEC_{freshwater\_st}$ = 7.7 µg l^{-1}/AF (10) = 0.77 µg l^{-1}

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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 three different taxonomic groups (crustaceans, fish and molluscs). However, no long-term data are available.

The short-term toxicity data of the marine taxa do not indicate markedly lower values from the range of values obtained for freshwater species (see Tables 2.6 and 2.7). 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 $PNEC_{saltwater\_lt}$ 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 marine taxa such as echinoderms. The lowest long-term toxicity value in the combined dataset is a 21-day NOEC of 0.1 µg active ingredient (a.i.) l^{-1} for effects on the reproduction of the waterflea (*Daphnia magna*). This is consistent with the greater sensitivity expected in insects and crustaceans given the mode of action of methiocarb. However, since no long-term data is available for marine species such as echinoderms and molluscs it is proposed that an assessment factor of 100 is applied. This results in

$PNEC_{saltwater\_lt}$ = 0.1 µg l^{-1}/AF (100) = 0.001 µg l^{-1}

PNEC accounting for transient concentration peaks

Single species short-term toxicity data for marine organisms are available for three different taxonomic groups (crustaceans, fish and molluscs), with crustaceans apparently being the most sensitive group.

Butler (1964) reported the lowest short-term toxicity value for a saltwater species to be a 48-hour EC50 (equilibrium) of 55 µg l^{-1} in the northern pink shrimp (*Penaeus duorarum*) under static conditions. This study also reported a 96-hour LC50 of 320 µg l^{-1} in the longnose killifish (*Fundulus similis*).

A reference was found to data for echinoderms (hhtp;//www. pesticideinfo.org/List_AquireAll.jsp?Rec_Id=PC35108&Taxa_Group=Echinoderms) but this only relates to a single record for sea urchins with a 'toxic dose' in the range of 10-200 µg l^{-1} for abnormal development. However, the endpoint is not properly defined and there is no other information on the study (i.e. species used, duration of the test) so it has not been included in the data tables.

All the available short-term data for saltwater species described 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 $PNEC_{saltwater\_st}$. Therefore, it is proposed that the $PNEC_{saltwater\_st}$ is based on the combined freshwater and saltwater dataset.

The TGD does not provide specific guidance for the assessment of short-term effects of intermittent releases to marine water bodies. Therefore, it is suggested that the calculation of the PNEC takes into account effects following short-term exposure to methiocarb 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)].

The lowest valid short-term toxicity value for the combined fresh and saltwater dataset is for freshwater invertebrates is a 48-hour EC50 of 7.7 µg a.i. l^{-1} for the effects of technical grade methiocarb on the immobilisation of the waterflea (*Daphnia magna*). This is consistent with the greater sensitivity expected in insects and crustaceans given the mode of action of methiocarb. However, although data is available for molluscs as no short-term data are available for marine species such as echinoderms it is proposed that an assessment factor of 50 is applied. This results in:

$PNEC_{saltwater\_st}$ = 7.7 µg l^{-1}/AF (50) = 0.15 µg l^{-1}

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 methiocarb was described in a 1997 report to the Department of the Environment (Murgatroyd *et al*., 1997).

In freshwaters, the available data were considered sufficient to derive an annual average (AA) and a maximum allowable concentration (MAC). An Annual Average EQS of 0.01 µg total methiocarb l^{-1} was proposed by applying a safety factor of 150 to

the most reliable acute data (24-hour LC50 of 1.6 µg l^{-1}) obtained for the midge (*Chironomus tentans*). Since there were no chronic toxicity data for the midge, the safety factor was derived using the acute/chronic ratio calculated from the data available for the waterflea (*Daphnia magna*).

In order to protect against short-term episodic exposure a maximum allowable concentration of 0.16 µg total methiocarb l^{-1} was proposed which was derived by applying a safety factor of 10 to the lowest acute value (24-hour LC50 of 1.6 µg l^{-1}) obtained for the midge.

The current long-term and short-term EQSs for saltwaters are the same as those for freshwaters.

3.4 Derivation of PNECs for sediment

Since the log Kow of methiocarb is 3.08, the derivation of PNECs for the protection of benthic organisms is required according to the TGD. However, only one sediment toxicity study is available and this is not considered sufficient data for the derivation of a $PNEC_{sediment}$.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

Several reviews have been published regarding methiocarb (JMPR, 1981; JMPR, 1983; JMPR, 1998, EC PPP, 2006). As the most recent, the EC PPP has been assumed to contain the most sound and scientifically accurate mammalian data. For this reason, this was the primary source used. However, the JMPR reviews were also consulted. Additional literature searches were performed from 2006 to the present day to locate any lower effect data since 2006, however, none were located.

For avian data, due to the lack of relevant data in the EC PPP reviews, the JMPR reviews were consulted. However, as for to the mammalian data, a comprehensive literature search was also performed from 1998 to the present day to locate any lower effect data since 1998.

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

Type of study, reference & result	Details
Sub-chronic toxicity to mammals	
Hixson (1981) Cited in JMPR (1998) **Sub-chronic NOAEL = 0.5 mg/kg bw/day**	Female Sprague-Dawley rats (15/group) received methiocarb (purity 97%) orally via gavage at doses of 0, 0.5 or 2 mg/kg bw/day five days a week for 4 weeks. The NOAEL was based on erythrocyte acetylcholinesterase inhibition that occurred at the higher dose.
	Beagle dogs (numbers per sex and group unspecified) received methiocarb orally (specific route unspecified) for

Anon Cited in EC PPP (2006) **Sub-chronic NOAEL = 1.33 mg/kg bw/day**	90 days at doses that included 0 and 1.33 mg/kg bw/day (exact doses unspecified). The NOAEL was based on clinical signs, reduced bodyweight gain, erythrocyte cholinesterase inhibition and retinal cholinesterase inhibition that occurred at higher doses. It is unclear in the review as to the identity of the original paper.
Chronic toxicity to mammals	
Anon Cited in EC PPP (2006) **Chronic NOAEL = 2.2 mg/kg bw/day**	Beagle dogs (numbers per sex and group unspecified) received methiocarb orally via their diet for 2 years at doses that included 0, 2.2 and 8.6 mg/kg bw/day (exact doses unspecified). The NOAEL was based on impaired food consumption, vomiting, clinical signs and cholinesterase inhibition that occurred at 8.6 mg/kg bw/day. It is unclear in the review as to the identity of the original paper.
Krötlinger *et al.* (1981) and Krötlinger (1990) Cited in JMPR (1998) **Chronic NOAEL = 3.3 mg/kg bw/day**	Male and female Wistar TNO W.74 rats (number per group unspecified) received methiocarb orally via their diet for 2 years at doses of 0, 3.3, 9.3 or 29 mg/kg bw/day for males, and 0, 5, 14 and 42 mg/kg bw/day for females. The NOAEL was based on haematological changes (increased leukocyte count, increased reticulocyte count, decreased mean corpuscular haemoglobin concentration, decreased red blood cell count, and decreased haemoglobin and haematocrit values) that occurred at higher doses.
Hoffman and Schilde (1980) Cited in JMPR (1998) and JMPR (1981) **Chronic NOAEL = 1.5 mg/kg bw/day**	Male and female Beagle dogs (4/sex/group) received methiocarb (purity 98.4%) orally via their diet at doses of 0, 0.12, 1.5 or 6 mg/kg bw/day for 104 weeks. The NOAEL was based on clinical signs (mild weakness of the hind limbs, trembling, reduced alertness and some vomiting), which occurred at the higher doses.
Effects on reproduction of mammals	
Anon Cited in EC PPP (2006) Parental, reproductive and offspring **NOAEL = 4.3 mg/kg bw/day**	In a multigeneration study, rats (strain and numbers per sex and group unspecified) were administered methiocarb orally (route and duration unspecified) at doses that included 0, 4.3 and >12 mg/kg bw/day (exact doses unspecified). The NOAEL was based on reduced body weights, reduced number of pups per litter and impaired lactation that occurred at >12 mg/kg bw/day and in the presence of maternal toxicity. It is unclear in the review as to the identity of the original paper.
Anon Cited in EC PPP (2006) **Parental NOAEL = 0.5 mg/kg bw/day** **Developmental NOAEL = 10 mg/kg bw/day**	In a multigeneration study, rabbits (strain and number per sex and group unspecified) were administered methiocarb orally (route and duration unspecified) at doses that included 0, 0.5 and 10 mg/kg bw/day (exact doses unspecified). It is not clear upon what the parental NOAEL was based. No developmental toxicity was noted; the developmental NOAEL was the highest dose tested. It is unclear in the review as to the identity of the original paper.

No evidence of carcinogenicity has been reported in mice and rats (EC PPP, 2006) and methiocarb is not genotoxic (JMPR, 1998).	
Acute toxicity to birds	
ECOTOX (2007) **5 day LC50 = 929, 1071, 2082, 4113 mg/kg diet** **8 day LC50 = 929, 1071, 2082, 4113, 7469 mg/kg diet** **14 day LD50 = 7.1, 9.4, 12.8 mg/kg bw/day**	Mallard ducks (*Anas platyrhynchos*) (sex and number unspecified) were administered methiocarb either in their diet (for the 5 and 8 day LC50s) or orally via an unspecified route (for the 14 day LD50).
ECOTOX (2007) **5 day LC50 = 1120 mg/kg diet** **8 day LC50 = 3849 mg/kg diet** **14 day LD50 = 4.67 mg/kg bw/day**	Red-winged blackbirds (*Agelaius phoeniceus*) (sex and number unspecified) were administered methiocarb either in their diet (for the 5 and 8 day LC50s) or orally via an unspecified route (for the 14 day LD50).
ECOTOX (2007) **30 day LC50 = 630 mg/kg diet**	Mourning doves (*Zenaida macroura*) (sex and number unspecified) were administered methiocarb in their diet.
ECOTOX (2007) **14 day LD50 = 1.33 mg/kg bw/day**	Budgerigars (*Melopsittacus undulatus*) (sex and number unspecified) were administered methiocarb orally via an unspecified route.
Strankowski and Minor (1976) Cited in JMPR (1998) and JMPR (1981) **28 day NOEL = 2.5 mg/kg bw/day (methiocarb and methiocarb sulphoxide)**	Chickens (*Gallus gallus* Babcock 300; sex and number per group unspecified) were administered methiocarb and methiocarb sulphoxide (in a mixture of a 9:1 ratio) orally via their diet for 28 days at doses of 0, 2.5, 7.5, 15 and 45 mg/kg bw/day. The NOEL was based on decreased food consumption and decreased plasma cholinesterase activity. No effects on egg production were observed.
No evidence of delayed neurotoxicity has been reported in hens (EC PPP, 2006; JMPR, 1998). No studies were available regarding the potential effects of methiocarb on avian reproduction, development or potential carcinogenicity.	

3.5.2 PNECs for secondary poisoning of predators

Bioconcentration data (as BCF values) for methiocarb are low, with reported values for bluegill sunfish ranging from 11-90 (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

Two methods for the analysis of methiocarb in river and drinking waters have been produced as “Blue Books” by the Standing Committee of Analysts (SCA) (HMSO, 1987). In the first method a one litre sample is acidified to pH 3 with dilute sulphuric acid and extracted sequentially with 50 ml and 25 ml portions of dichloromethane (DCM). The combined solvent extract is evaporated to dryness and reconstituted in 500 µl of the initial high performance liquid chromatography (HPLC) mobile phase before being analysed by normal phase HPLC. The limit of detection is 0.13 µg l^{-1} and the range of application up to 100 µg l^{-1}. In the second method the sample is extracted and concentrated as in method A, but the extract is analysed by reverse phase HPLC The limit of detection is 0.04 µg l^{-1} and the range of application is typically 0-20 µg l^{-1}.

Yang (2004) reported on a LC/MS/MS method for the analysis of N-Methyl carbamates in water. The method did not require any sample enrichment and resulted in a reported Limit of Quantitation (LOQ) of 0.02 µg l^{-1}.

EFSA (2006) reported that methiocarb and its sulfoxide metabolite could be determined in water by HPLC/MS/MS, with an LOQ of 0.1 µg l^{-1} (for each analyte).

For water, proposed PNECs derived for methiocarb range from 0.001 to 0.77 µg 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 0.02 µg l^{-1}, do not offer adequate performance to analyse for methiocarb against all proposed PNECs.

5 Conclusions

5.1 Availability of data

Long-term laboratory data are available for three freshwater taxonomic groups, i.e. algae, crustaceans and fish. Freshwater short-term toxicity data are available for six taxonomic groups, i.e. algae, amphibians, crustaceans, fish, insects and molluscs. Freshwater insects and crustaceans are more sensitive to methiocarb than other taxa. For marine organisms, short-term toxicity data are available for three taxonomic groups, i.e. crustaceans, fish and molluscs. No long-term toxicity data are available for saltwater taxa.

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 No Observed Effect Concentration (NOEC) of 0.1 µg active ingredient (a.i.) l^{-1} for effects on the reproduction of the waterflea (*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. This results in a $PNEC_{freshwater\_lt}$ of 0.01 µg l^{-1}.

The current long-term EQS for freshwater is 0.01 µg l^{-1} and was derived by applying assessment factor of 150 to the then most reliable acute data (24-hour LC50 of 1.6 µg l^{-1}) obtained for the midge (*Chironomus tentans*). This endpoint is no longer considered reliable because there was high control mortality in the test, and several test details are unreported.

5.2.2 Short-term PNEC for freshwaters

Reliable short-term 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 7.7 µg a.i. l^{-1} for the effects of technical grade methiocarb on the immobilisation of the waterflea (*Daphnia magna*). Lower short-term toxicity values have been reported in non-GLP studies, but these are considered to be unreliable due to the use of field collected organisms. Based on the EU Technical Guidance Document (TGD) methodology and a large body of acute data for methiocarb, an assessment factor of 10 could be applied to the lowest valid toxicity value. This results in a $PNEC_{freshwater\_lt}$ of 0.77 µg l^{-1}.

The current short-term EQS for freshwater is 0.16 µg l^{-1} and was derived by applying assessment factor of 10 to the then most reliable short-term data (24-hour LC50 of 1.6 µg l^{-1}) obtained for the midge (*Chironomus tentans*).

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 $PNEC_{saltwater\_lt}$ based on the saltwater data alone, and it is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation. Reliable chronic NOECs are available for algae, invertebrates and fish. The lowest long-term toxicity value in the combined dataset is a 21-day NOEC of 0.1 µg active ingredient (a.i.) l^{-1} for effects on the reproduction of the waterflea *Daphnia magna*. This is consistent with the greater sensitivity expected in insects and crustaceans given the mode of action of methiocarb. However, since no long-term data is available for marine species such as echinoderms and molluscs it is proposed that an assessment factor of 100 is applied. This results in a $PNEC_{saltwater\_lt}$ of 0.001 µg l^{-1}.

The current long-term EQS for saltwater is 0.01 µg l^{-1} and was derived by applying assessment factor of 150 to the then most reliable short-term data (24-hour LC50 of 1.6 µg l^{-1}) obtained for the midge (*Chironomus tentans*).

5.2.4 Short-term PNEC for saltwaters

Single species short-term toxicity data for marine organisms are available for three different taxonomic groups, i.e. crustaceans, fish and molluscs. Therefore, it is proposed that the $PNEC_{saltwater\_st}$ 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 7.7 µg a.i. l^{-1} for the effects of technical grade methiocarb on the immobilisation of the waterflea *Daphnia magna.* This is consistent with the greater sensitivity expected in insects and crustaceans given the mode of action of methiocarb. However, since no short-term data is available for marine species such as echinoderms it is proposed that an assessment factor of 50 is applied. This results in a $PNEC_{saltwater\_st}$ of 0.15 µg a.i. l^{-1}.

The current short-term EQS for saltwaters is 0.16 µg l^{-1} and was derived by applying assessment factor of 10 to the then most reliable short-term data (24-hour LC50 of 1.6 µg l^{-1}) obtained for the midge (*Chironomus tentans*).

5.2.5 PNEC for sediments

Since the log Kow of methiocarb is 3.08, the derivation of PNECs for the protection of benthic organisms is required according to the TGD. However, although a sediment toxicity study is available, it is not considered appropriate for the derivation of a $PNEC_{sediment}$.

5.2.6 PNEC for secondary poisoning

Bioconcentration data (as BCF values) for methiocarb are low, with values for bluegill sunfish ranging from 11-90 (see Section 2.5). Hence, the TGD trigger of BCF values >100 is not met and the derivation of PNECs for secondary poisoning of predators is not required.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1})	Existing EQS (µg l^{-1})
Freshwater/long-term	0.01	0.01
Freshwater/short-term	0.77	0.16
Saltwater/long-term	0.001	0.01
Saltwater/short-term	0.15	0.016
Sediment	Insufficient data	–
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 0.02 µg l^{-1} do not offer adequate performance to analyse for methiocarb against all proposed PNECs.

5.4 Implementation issues

Based on consideration of the information collated within the report and the proposed PNECs the following comments are made re: implementation:-

- Current analytical methods are not sensitive enough to assess compliance with the lowest proposed PNECs. This will require further investigation.

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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). All relevant data has been quality assessed as part of the preparation of the report, even if it has already undergone prior quality assessment by other organisations.

Table A1 Klimisch Criteria*

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

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

Reference	Butler (1963) cited in OPP (2007)

Information on the test species	
Test species used	*Fundulus similis*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Juvenile

Information on the test design	
Methodology used	Not stated
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	At least five concentrations used
Number of replicates per concentration	Not stated
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	No (Nominal concentrations used)
Measurement of water quality parameters	Yes (Temperature = 16 °C)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Poorly documented study protocols

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**C (in OPP database)**

Reference	Butler (1964) cited in OPP (2007)

Information on the test species	
Test species used	*Penaeus duorarum*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Adults

Information on the test design	
Methodology used	Not stated
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	0.001-10.0 $mg.l^{-1}$ (2-Propanone solvent used)
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
Measurement of exposure concentrations	No (Nominal concentrations used)
Measurement of water quality parameters	Yes (Temperature = 16 °C, salinity = 27‰)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Poorly documented study protocols

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**C (in OPP database)**

Reference	Carlisle (1985)

Information on the test species	
Test species used	*Oncorhynchus mykiss*
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	Newly hatched larvae

Information on the test design	
Methodology used	OECD guideline 201 and US EPA 72-4
Form of the test substance	97% pure
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	Control, 25, 50, 100, 200 and 400 $\mu g.l^{-1}$ (as nominals)
Number of replicates per concentration	Five replicate test chambers
Number of organisms per replicate	100 eggs per chamber
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Flow-through, 56 days, fed starter feed (10 g per replicate) three times per day.
Measurement of exposure concentrations	Yes (Water analysed weekly except for weeks 1 and 4, mean measured concentrations ranged from 88 – 111 % of nominals)
Measurement of water quality parameters	Yes (pH = 6.2 - 8.0, temperature = 7 - 12°C, dissolved oxygen = 6.5 - 11.9 $mg.l^{-1}$
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.

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

Reference	Cope (1965)

Information on the test species	
Test species used	*Lepomis macrochirus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Weight = 0.97 g

Information on the test design	
Methodology used	Not stated
Form of the test substance	Technical grade
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
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Yes (Temperature = 24 °C)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Study is poorly reported, lacking details on methodology and exposure conditions.

Reliability of study	**Not reliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Cope (1965)

Information on the test species	
Test species used	*Oncorhynchus mykiss*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Weight = 1.32 g

Information on the test design	
Methodology used	Not stated
Form of the test substance	Technical grade
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
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Yes (Temperature = 12.8 °C)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Study is poorly reported, lacking details on methodology and exposure conditions.

Reliability of study	**Not reliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Fingas *et al*. (1991)

Information on the test species	
Test species used	*Lepomis macrochirus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
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	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Report contains a table of results for many chemicals, but no study details. Therefore it is not possible to assess the quality of this study

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

Reference	Forbis (1988)

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated in EU DAR, 2005
Holding conditions prior to test	Temperature = 20±2ºC, 16:8 hour light:dark cycle, pH = 8.2-8.4, DO = 8.0-8.7 $mg.l^{-1}$ (92-100% saturation)
Life stage of the test species used	First instar, <24 hours old

Information on the test design	
Methodology used	OECD Guideline 210 and US EPA 72-4
Form of the test substance	99.7% pure
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	Nominal concentrations of 0.079, 0.13, 0.28, 0.47 and 1.1 $\mu g.l^{-1}$, a control and a solvent control. Measured concentrations were 0.1, 0.17, 0.32, 0.5 and 1.3 $\mu g.l^{-1}$.
Number of replicates per concentration	Four
Number of organisms per replicate	Ten
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Flow-through, 21 days, fed
Measurement of exposure concentrations	Yes (Measured concentrations were 106-131% of nominal)
Measurement of water quality parameters	Yes (pH and dissolved oxygen measured on days 0, 4, 7, 14 and 21).
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.

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

Reference	Karnak and Collins (1974)

Information on the test species	
Test species used	*Chironomus tentans*
Source of the test organisms	Adults collected from Jackson Pike sewage treatment plant, Columbus, Ohio, USA
Holding conditions prior to test	Fed Hartz Mountain Dog Kisses(R) and reared according to Biever (1965), using glass wool as a substrate
Life stage of the test species used	3/4 instar larvae

Information on the test design	
Methodology used	Not stated
Form of the test substance	90% purity
Source of the test substance	Not stated
Type and source of the exposure medium	Study was performed at 22°C in glass containers containing mineral wool and conducted with conditioned tap water (aged 24 hours with vigorous aeration). Solutions were gently aerated with air stones throughout the assay.
Test concentrations used	Not stated
Number of replicates per concentration	Three
Number of organisms per replicate	Ten
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 96 hours, fed 150 mg powdered Hartz Mountain Dog Kisses(R) to avoid unusually high control mortality.
Measurement of exposure concentrations	No (Nominal concentrations used)
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	Study lacks many details and suffered from high control mortality.

Reliability of study	**Not reliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Marking and Chandler (1981)

Information on the test species	
Test species used	*Hydropysche sp.*
Source of the test organisms	Obtained from small streams and rivers near Warm Springs, Georgia, USA
Holding conditions prior to test	Not stated
Life stage of the test species used	Larvae

Information on the test design	
Methodology used	ASTM Committee E-35 on Pesticides (1980) methodology with minor, unspecified modifications.
Form of the test substance	Technical methiocarb
Source of the test substance	Not stated
Type and source of the exposure medium	Test material dissolved in water or acetone and volumetrically added to test vessels to the correct concentration. Water in the test vessels was reconstituted spring water and had a hardness of 24 mg $CaCO_3$ l^{-1}. Temperature was maintained at 16 ºC by immersion of test vessels in a water bath.
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
Measurement of exposure concentrations	Nominal
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	Study was conducted to an established methodology, however, unspecified changes were made. Limited details of test conditions are reported and no details of doses and replicates are provided.

Reliability of study	**Not reliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Marking and Chandler (1981)

Information on the test species	
Test species used	*Isonychia sp.*
Source of the test organisms	Obtained from small streams and rivers near Warm Springs, Georgia, USA
Holding conditions prior to test	Not stated
Life stage of the test species used	Nymph

Information on the test design	
Methodology used	ASTM Committee E-35 on Pesticides (1980) methodology with minor, unspecified modifications.
Form of the test substance	Technical methiocarb
Source of the test substance	Not stated
Type and source of the exposure medium	Test material dissolved in water or acetone and volumetrically added to test vessels to the correct concentration. Water in the test vessels was reconstituted spring water and had a hardness of 24 mg $CaCO_3$ l^{-1}. Temperature was maintained at 12 ºC by immersion of test vessels in a water bath.
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
Measurement of exposure concentrations	Nominal
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	Study was conducted to an established methodology, however, unspecified changes were made. Limited details of test conditions are reported and no details of doses and replicates are provided.

Reliability of study	**Not reliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Marking and Chandler (1981)

Information on the test species	
Test species used	*Oxytrema cateria*
Source of the test organisms	Obtained from small streams and rivers near Warm Springs, Georgia, USA
Holding conditions prior to test	Not stated
Life stage of the test species used	Adult

Information on the test design	
Methodology used	ASTM Committee E-35 on Pesticides (1980) methodology with minor, unspecified modifications.
Form of the test substance	Technical methiocarb
Source of the test substance	Not stated
Type and source of the exposure medium	Test material dissolved in water or acetone and volumetrically added to test vessels to the correct concentration. Water in the test vessels was reconstituted spring water and had a hardness of 24 mg $CaCO_3$ l^{-1}. Temperature was maintained at 16ºC by immersion of test vessels in a water bath.
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
Measurement of exposure concentrations	Nominal
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	Study was conducted to an established methodology, however, unspecified changes were made. Limited details of test conditions are reported and no details of doses and replicates are provided.

Reliability of study	**Not reliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Marking and Chandler (1981)

Information on the test species	
Test species used	*Rana sphenocephala*
Source of the test organisms	Egg clusters were collected from ponds at the Warm Springs Hatchery, Georgia, USA, within 16 hours of deposition. Egg masses were teased into approximately equal clusters.
Holding conditions prior to test	Egg clusters were placed into individual test vessels at 16°C.
Life stage of the test species used	Larvae

Information on the test design	
Methodology used	ASTM Committee E-35 on Pesticides (2000) methodology with minor, unspecified modifications.
Form of the test substance	Technical methiocarb
Source of the test substance	Not stated
Type and source of the exposure medium	Test material dissolved in water or acetone and volumetrically added to test vessels to the correct concentration. Water in the test vessels was reconstituted spring water and had a hardness of 24 mg $CaCO_3$ l^{-1}. Temperature was maintained at 16 or 22°C by immersion of test vessels in a water bath.
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
Measurement of exposure concentrations	Nominal
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	Study was conducted to an established methodology, however, unspecified changes were made. Limited details of test conditions are reported and no details of doses and replicates are provided.

Reliability of study	**Not reliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Office of Pesticide Programs (2007)

Information on the test species	
Test species used	*Lepomis macrochirus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Weight 1 g

Information on the test design	
Methodology used	Conducted according to the methodology of the American Society for Testing and Materials (1980) and the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975).
Form of the test substance	90% pure
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Control, 0.01, <0.1, 0.1, <1.0, 1.0, <10, 10, <100, 100, <1000, 1000, <10000, 10000, <100000, ≥100000 $\mu g.l^{-1}$
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, 96 hours, fed
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Yes (Hardness = 44 mg $CaCO_3$ l^{-1}, temperature = 24 ºC, pH = 7.1)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Yes
Overall comment on quality	Conducted according to standard methodologies, however, several details have not been reported.

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**C (in OPP database)**

Reference	Office of Pesticide Programs (2007)

Information on the test species	
Test species used	*Oncorhynchus mykiss*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Weight = 0.8-1.8 g, length = 40-50 mm

Information on the test design	
Methodology used	EPA guidelines
Form of the test substance	98% pure
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
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Yes (pH = 7.15-7.23, water hardness = 40-41 $mg.l^{-1}$, alkalinity = 31-33 mg CaCO3 l^{-1}, dissolved oxygen = 9.68-9.71 $mg.l^{-1}$.
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Conducted to EPA guidelines, but many study details are missing

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**C (in OPP database)**

Reference	Peither (2000a)

Information on the test species	
Test species used	*Scenedesmus subspicatus*
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	Cells in the growth phase

Information on the test design	
Methodology used	OECD 203 and EEC C3
Form of the test substance	99.3-99.4% pure
Source of the test substance	Not stated in EU DAR, 2005
Type and source of the exposure medium	Nutrient media
Test concentrations used	Solvent control, 0.052, 0.18, 0.6, 1.5 and 4.6 mg a.i. l^{-1} (as measured concentrations)
Number of replicates per concentration	6 in solvent control and 3 in each treatment
Number of organisms per replicate	1 x 10^4 cells ml^{-1} at test initiation
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static, 72 hours, not fed
Measurement of exposure concentrations	Yes (Mean measured concentrations were 72 – 90% of nominals at the start of the test and 13 – 33% of nominal et end of the test)
Measurement of water quality parameters	pH = 8.1 – 8.7, temperature = 23 - 24 ^oC
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.

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

Reference	Peither (2000b)

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	In-house cultures
Holding conditions prior to test	Not stated in EU DAR, 2005
Life stage of the test species used	<24 hour old neonates

Information on the test design	
Methodology used	OECD 203 and EEC C2
Form of the test substance	99.3-99.4% pure
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	Control, solvent control (DMF), 2.2, 4.6, 10, 22 and 46 mg l^{-1} (as nominals)
Number of replicates per concentration	2
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Semi-static (renewal after 24 hours), 48 hours, not fed
Measurement of exposure concentrations	Yes (Mean measured concentrations were 76 – 104% in the freshly prepared solutions and 59 – 72% of nominal in the aged solutions)
Measurement of water quality parameters	Yes (pH = 7.8 – 8.2, temperature = 20 -21 ^{o}C, dissolved oxygen = 8.1 to 8.9 mg l^{-1})
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.

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

Reference	Peither (2000c)

Information on the test species	
Test species used	*Lepomis macrochirus*
Source of the test organisms	Not stated in EU DAR, 2005
Holding conditions prior to test	Fish were acclimated for more than one week prior to the start of the test
Life stage of the test species used	Organisms of mean body length of 3.4 cm and mean body weight of 0.49 g at the start of the test

Information on the test design	
Methodology used	OECD 203 and EEC C1
Form of the test substance	99.3-99.4% pure
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	Control, 0.08, 0.16, 0.36, 0.8, 1.6, 3.6 and 8.0 mg a.i. l^{-1} (as nominals)
Number of replicates per concentration	1
Number of organisms per replicate	7
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Flow-through (renewal every 48 hours), 96 hours, not fed
Measurement of exposure concentrations	Yes (Mean measured concentrations were 88 – 98% of nominals in the fresh solutions and 87 – 99% of nominal in the aged solutions)
Measurement of water quality parameters	Yes (pH = 7.7 – 7.8, temperature = 23 ^{o}C, dissolved oxygen = 7.5 t o 8.5 mg l^{-1})
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.

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

Reference	Peither (2000d)

Information on the test species	
Test species used	*Oncorhynchus mykiss*
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	Fish were acclimated for two weeks prior to the start of the test
	Organisms of mean body length of 5.0 cm and mean body weight of 1.5 g at the start of the test

Information on the test design	
Methodology used	OECD 203 and EEC C1
Form of the test substance	99.3-99.4% pure
Source of the test substance	Not stated in EU DAR, 2005
Type and source of the exposure medium	Control, 0.16, 0.35, 0.8, 1.6, 3.6 and 8.0 mg a.i. l^{-1} (as nominals)
Test concentrations used	1
Number of replicates per concentration	7
Number of organisms per replicate	Flow-through (renewal every 48 hours), 96 hours, not fed
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Yes (Mean measured concentrations were 82 – 91% of nominals in the fresh solutions and 80 – 89% of nominal in the aged solutions)
Measurement of exposure concentrations	Yes (pH = 7.4 – 7.9, temperature = 14 - 15 ^{o}C, dissolved oxygen = 8.0 t o 9.6 mg l^{-1})
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.

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

Reference	Sanders and Cope (1968)

Information on the test species	
Test species used	*Pteronarcys californica*
Source of the test organisms	Mountain streams near the Fish-Pesticide Research Laboratory, Denver, Colorado, USA
Holding conditions prior to test	Acclimated in aerated aquaria at 15.5°C for 48 hours prior to test. Compressed air was introduced through air stones to oxygenate the water and several pieces of driftwood were added to aquaria to provide food and a place of attachment.
Life stage of the test species used	Nymphs, 30-35 mm

Information on the test design	
Methodology used	Not stated
Form of the test substance	Technical grade
Source of the test substance	Not stated
Type and source of the exposure medium	Aquaria containing 4 litres reconstituted water (1 l deionised water containing 30 mg $CaCO_3$, 30 mg $MgSO_4$, 48 mg $NaHCO_3$ and 2 mg KCl), pH = 7.1, dissolved oxygen = 7 mg l^{-1} at beginning of test, 5 mg l^{-1} after 24 hours and 3 mg l^{-1} after 96 hours. Aquaria were not aerated during study.
Test concentrations used	Four or five unstated concentrations plus an untreated control
Number of replicates per concentration	Not stated
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No (Nominal concentrations used)
Measurement of water quality parameters	Yes (Dissolved oxygen)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Dissolved oxygen was considered to be adequate throughout the exposure period.
Study conducted to GLP	Not stated
Overall comment on quality	Although several details are lacking, the study is generally well documented.

Reliability of study	**Not Reliable**
Relevance of study	**Relevent**
Klimisch Code	**3**