Proposed EQS for Water Framework Directive Annex VIII substances: 2,4-dichlorophenol *(For consultation)*

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

Publisher: **Water Framework Directive - United Kingdom Technical Advisory Group (WFD-UKTAG)**
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www.wfduk.org

May 2012

This report is the result of research commissioned and funded by the Environment Agency.

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Research performed:
2009

Dissemination Status:
Publicly available

Keywords:
2,4-dichlorophenol, Water Framework Directive, specific pollutants, predicted no-effect concentrations, freshwater, saltwater

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Environment Agency Science Project Number:
SC080021/5a(xi)

Use of this report

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

This technical document has been developed through a project managed by the Environment Agency and has involved members and partners of UKTAG. It provides background information to support the ongoing development of the standards and classification methods.

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

Note:

This report is an update of report Number SCHO0407BLVR-E-E 'Proposed EQSs for Water Directive Annex VIII substances: 2,4-dichlorophenol' produced in 2007 as part of a programme of work commissioned by the UK Technical Advisory Group (UKTAG) to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). The original report proposed PNECs derived according to the Annex V methodology but because of a lack of certain data, large assessment factors were used in their derivation. This led to the UKTAG concluding that the values were unsuitable for use as EQSs since they were subject to excessive uncertainty, but that this uncertainty may be reduced by appropriate additional ecotoxicity testing (SP Report 2007). Consequently an ecotoxicity study on the alga *Pseudokirchneriella subcapitata* (Environment Agency 2008) was commissioned with the aim of reducing the data gap, assessment factors and ultimately the uncertainty in the PNEC values. This report incorporates the results of this study and PNECs are re-visited using the more complete dataset. It should be noted that no additional review of any other data/literature that may have been published since the original 2007 report has been made.

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 2,4-dichlorophenol using the methodology described in Annex V of the Directive. There are existing EQSs for 2,4-dichlorophenol, 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 2,4-dichlorophenol, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data. Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V.

This report is an update of report Number SCHO0407BLVR-E-E 'Proposed EQSs for Water Directive Annex VIII substances: 2,4-dichlorophenol' produced in 2007 as part of a programme of work commissioned by the UK Technical Advisory Group (UKTAG) to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). The original report proposed PNECs derived according to the Annex V methodology but because of a lack of certain data, large assessment factors were used in their derivation. This led to the UKTAG concluding that the values were unsuitable for use as EQSs since they were subject to excessive uncertainty, but that this uncertainty may be reduced by appropriate additional ecotoxicity testing (SP Report 2007). Consequently an ecotoxicity study on the alga *Pseudokirchneriella subcapitata* (Environment Agency 2008) was commissioned with the aim of reducing the data gap, assessment factors and ultimately the uncertainty in the PNEC values. This report incorporates the results of this study and PNECs are re-visited using the more complete dataset. It should be noted that no additional review of any other data/literature that may have been published since the original 2007 report has been made.

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

Dichlorophenol compounds are considered to act as polar narcotics in fish, with the toxicity exhibited being characterised by a convulsant action where the loss of reaction to external stimuli and/or loss of equilibrium are detected.

The pKa (pH at which an acid compound is 50 per cent dissociated) of 7.89 for 2,4-dichlorophenol indicates that, at the pH range characterising most physiological and environmental conditions (typically pH 7–8), these compounds will exist predominately in the more toxicologically active undissociated form. 2,4-Dichlorophenol is not

expected to persist in the water column when the substance is released to the aquatic compartment. It is expected to volatilise from water surfaces and is rapidly degraded by exposure to ultraviolet light. 2,4-Dichlorophenol is not expected to persist in the soil when the substance is released to the terrestrial compartment due to a low adsorption to organic matter (based on a log Koc of 2.54) and the processes of volatilisation and degradation.

Availability of data

Long-term laboratory data are available for five different freshwater taxonomic groups including algae, crustaceans, fish, insects and macrophytes. Freshwater short-term toxicity data are available for four taxonomic groups including algae, crustaceans, fish and protozoa. The limited dataset means that it is not possible to discern which taxonomic group is most sensitive to 2,4-dichlorophenol. For marine organisms, single species short-term toxicity data are available for three different taxonomic groups (algae, crustaceans and fish).No long term toxicity data was located for saltwaters. Laboratory data are supplemented by saltwater mesocosm data which show effects of 2,4-dichlorophenol on algae and crustaceans.

There is currently no definitive data that demonstrates that 2,4-dichlorophenol causes endocrine-mediated effects in aquatic organisms.

Derivation of PNECs

Long-term PNEC for freshwaters

The lowest valid reported long-term toxicity value for 'standard' ecotoxicological endpoints (e.g. growth, reproduction and mortality) is an an 85 day NOEC of 100 µg l^{-1} for effects on the survival of early life stage rainbow trout *Oncorhynchus mykiss*. The same study also reported an 85-day LOEC of 100 µg l^{-1} for effects on the growth (as wet weight) of fry of the rainbow trout *Oncorhynchus mykiss* at the 4 weeks post swim-up stage. There was a 40% reduction in the wet weight of fry at this concentration and because the effect level is greater than 20% the TGD approach cannot be used to derive a NOEC value from the LOEC A lower NOEC value of 0.1 µg l^{-1} for effects of 2,4-dichlorophenol on the net spinning behaviour of larvae of the trichopterna *Hydropsyche slossonae* was reported, but there were concerns about the validity of these data. Since reliable long-term NOECs are available for algae, invertebrates (including crustaceans and insects) and fish, an assessment factor of 10 could be applied to the lowest valid toxicity value resulting in a $PNEC_{freshwater\_lt}$ of 10 µg l^{-1}.

However, in order to be protective of the effects of 2,4-dichlorophenol observed on the growth of rainbow trout *Oncorhynchus mykiss* at 100 µg l^{-1}, it is proposed that the chronic algal and invertebrate dataset is used to derive the PNEC, The lowest reliable value for this dataset is a 21-day NOEC of 210 µg l^{-1} for reproductive effects in the water flea *Daphnia magna*. By applying an assessment factor of 50 (for NOECs from two taxonomic groups) the resulting value would be 4.2 µg l^{-1} 2,4-dichlorophenol.

This value provides a margin of safety against the observed effects of 2,4-dichlorophenol on the growth of rainbow trout at 100 µg l^{-1}.

This value is lower than the existing EQS of 20 µg l^{-1}. This was derived by applying an assessment factor of 10 (to account for extrapolation to a no-effects concentration and possible interspecies differences in sensitivity) to the lowest chronic effects concentration (i.e. the 85-day LOEC for larvae of rainbow trout *Oncorhynchus mykiss* of 180 µg l^{-1} from the same study as considered above).

Short-term PNEC for freshwaters

Reliable short-term data are available for algal, invertebrate and fish species. The lowest valid short-term data is considered to be a 48-hour EC50 of 1,400 µg l^{-1} for effects on the mobility of the cladoceran copepod *Daphnia magna.* Since there is an acceptable short-term toxicity database for freshwater organisms, an assessment factor of 10 has been applied resulting in a $PNEC_{freshwater\_st}$ of 140 µg l^{-1}.

The value is the same as the current EQS of 140 µg l^{-1}. This was derived by applying the same assessment factor (of 10) to the same data point (48-hour EC50 of 1,400 µg l^{-1} for effects on the mobility of the cladoceran copepod *Daphnia magna*).

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. It is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation.

The lowest valid reported long-term toxicity value for 'standard' ecotoxicological endpoints (e.g. growth, reproduction and mortality) is a 85-day NOEC of 100 µg l^{-1} for effects on the survival of early life stages of rainbow trout *Oncorhynchus mykiss*. Reliable long-term NOECs are available for freshwater algae, invertebrates and fish but no toxicity data are available for marine taxa such as echinoderms and molluscs. Given that there is limited data for these marine taxa, a total assessment factor of 100 (including a factor of 10 to account for the greater uncertainty due to the limited data for marine taxa) could be applied to the lowest valid toxicity value resulting in a $PNEC_{saltwater\_lt}$ of 1.0 µg l^{-1}.

However, in order to be protective of the effects of 2,4-dichlorophenol observed on the growth of rainbow trout *Oncorhynchus mykiss* at 100 µg l^{-1}, it is proposed that the chronic algal and invertebrate dataset is used to derive the PNEC, The lowest reliable value for this dataset is a 21-day NOEC of 210 µg l^{-1} for reproductive effects in the water flea *Daphnia magna*. By applying a total assessment factor of 500 (for NOECs from two taxonomic groups and accounting for the greater uncertainty due to the limited data for marine taxa) the resulting value would be 0.42 µg l^{-1} 2,4-dichlorophenol.

This value is lower than the existing EQS of 20 µg l^{-1}, which was 'read across' from the freshwater long-term value.

Short-term PNEC for saltwaters

The lowest valid short-term toxicity value is a 72-hour EC50 value of 600 µg l^{-1} for effects on the growth of the diatom *Phaeodactylum tricornutum.* Lower short-term toxicity values have been reported in studies that did not meet OECD principles of Good Laboratory Practice, but these are considered to be unreliable due to the absence of measured concentration data. Reliable short-term L(E)C50s are available for freshwater algae, invertebrates and fish but no toxicity data are available for marine taxa such as echinoderms and molluscs. Given that there is limited data for these marine taxa, a total assessment factor of 100 (including a factor of 10 to account for the greater uncertainty due to the limited data for marine taxa) should be applied to the lowest valid toxicity value resulting in a $PNEC_{saltwater\_lt}$ of 6.0 µg l^{-1}.

This value is lower than the existing EQS of 140 µg l^{-1}, which was 'read across' from the freshwater short-term value.

PNECs for sediments

Since the log Kow of 2,4-dichlorophenol is >3, the derivation of PNECs for the protection of benthic organisms is required. An extensive literature search for data on the toxicity of 2,4-dichlorophenol to sediment-dwelling organisms did not identify any

relevant studies. As a result it is not possible to derive a $PNEC_{sediment}$ based on experimental toxicity data.

PNECs for secondary poisoning

Bioconcentration data – as bioconcentration factor (BCF) values – for 2,4-dichlorophenol for aquatic organisms are generally low with values for fish ranging from 3.8 to 100 at neutral pH. Higher BCFs of 282–980 have been reported for one taxonomic group, leeches, although this has been attributed to a deficiency in these organisms of the enzyme necessary for the metabolism of chlorophenols. Hence, the trigger EU Technical Guidance Document BCF 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	4.2	20
Freshwater/short-term	140	140
Saltwater/long-term	0.42	20
Saltwater/short-term	6.0	140
Sediment	Insufficient data	–
Secondary poisoning	Not required	–

Analysis

The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing gas chromatography/mass spectrometry (GC-MS), which are capable of achieving detection limits as low as 2–5 ng l^{-1}, should offer adequate performance to analyse for 2,4-dichlorophenol.

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 sensitive enough to assess compliance with the proposed PNECs in receiving waters.
- The freshwater long term and short term PNECs are not subject to excessive uncertainty with assessment factors of 10 being applied to derive the PNECs. These PNECs are therefore suitable for use. In relation to the saltwater PNECs larger assessment factors have been applied which reflects the higher level of uncertainty. This uncertainty could be reduced by undertaking additional ecotoxicity testing for marine organisms.

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 2,4-dichlorophenol using the methodology described in Annex V of the Directive. There are existing EQSs for 2,4-dichlorophenol, 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 2,4-dichlorophenol, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data.[2] Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V of the WFD. This report is an update of report Number SCHO0407BLVR-E-E 'Proposed EQSs for Water Directive Annex VIII substances: 2,4-dichlorophenol' produced in 2007 as part of a programme of work commissioned by the UK Technical Advisory Group (UKTAG) to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). The original report proposed PNECs derived according to the Annex V methodology but because of a lack of certain data, large assessment factors were used in their derivation. This led to the UKTAG concluding that the values were unsuitable for use as EQSs since they were subject to excessive uncertainty, but that this uncertainty may be reduced by appropriate additional ecotoxicity testing (SP Report 2007). Consequently an ecotoxicity study on the alga *Pseudokirchneriella subcapitata* (Environment Agency 2008) was commissioned with the aim of reducing the data gap, assessment factors and ultimately the uncertainty in the PNEC values. This report incorporates the results of this study and PNECs are re-visited using the more complete dataset. It should be noted that no additional review of any other data/literature that may have been published since the original 2007 report has been made.

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

Dichlorophenol compounds are considered to act as polar narcotics in fish, with the toxicity exhibited being characterised by a convulsant action where the loss of reaction to external stimuli and/or loss of equilibrium are detected.

The pKa (pH at which an acid compound is 50 per cent dissociated) of 7.89 for 2,4-dichlorophenol indicates that, at the pH range characterising most physiological and environmental conditions (typically pH 7–8), these compounds will exist predominately in the more toxicologically active undissociated form. 2,4-Dichlorophenol is not expected to persist in the water column when the substance is released to the aquatic compartment. It is expected to volatilise from water surfaces and is rapidly degraded by exposure to ultraviolet light. 2,4-Dichlorophenol is not expected to persist in the soil when the substance is released to the

[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.

terrestrial compartment due to a low adsorption to organic matter (based on a log Koc of 2.54) and the processes of volatilisation and degradation.

2 Results and observations

2.1 Identity of substance

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

Table 2.1 Species covered by this report

Name	CAS Number
2,4-dichlorophenol	120-83-2

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 2,4-dichlorophenol. 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	140 µg l^{-1}	–	140 µg l^{-1} (MAC)
Freshwater long-term	4.2 µg l^{-1}	Insufficient data	20 µg l^{-1} (AA)
Saltwater short-term	6.0 µg l^{-1}	–	140 µg l^{-1} (MAC – T)
Saltwater long-term	0.42 µg l^{-1}	Insufficient data	20 µg l^{-1} (AA – T)
Sediment	Insufficient data	Insufficient data	–
Secondary poisoning	Not required	–	–

AA = annual average
AF = assessment factor
MAC = maximum allowable concentration
SSD = species sensitivity distribution
T = tentative

2.3 Hazard classification

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

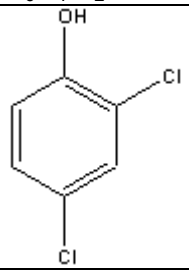
Table 2.3 Hazard classification

R-phrases and labelling	Reference
R22, 24, 34, 51/53 S1/2, 26, 36/37/39, 45, 61	ECB 2006

2.4 Physical and chemical properties

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

Table 2.4 Physical and chemical properties of 2,4-dichlorophenol

Property	Value	Reference
CAS number	120-83-2	ECB 2006
Substance name	2,4-dichlorophenol, 1-hydroxy-2,4-dichlorobenzene	ECB 2006
Molecular formula	$C_6H_4Cl_2O$	ECB 2006
Molecular structure		Chemfinder 2006
Molecular weight	163.0	ECB 2006
Colour/form	Colourless needle-like crystals or white solid	ECB 2006
Odour	Strong medicinal type odour	ECB 2006
Melting point (°C)	45 (at 760 nm)	WHO 1989
Boiling point (°C)	210 (at 760 nm)	WHO 1989
Vapour pressure	0.12 mmHg at 25°C	HSBD 2006
Density/specific gravity	d60/25 = 1.383	HSDB 2006
Henry's Law constant	5.5×10^{-6} atm-m^3/mol	HSBD 2006
Solubility	4.5 g l^{-1} in water at 20°C Soluble in benzene, carbon tetrachloride, ethanol and diethyl ether	WHO 1989

Chlorophenols are weak acids in aqueous solution and one of the major factors affecting their environmental transport, degradation and toxicity is the degree to which the compounds are dissolved in natural waters. Under acidic conditions, chlorophenols exist primarily in the toxic molecular (undissociated) form while, under basic conditions, the less toxic dissociated form predominates (HSDB 2006). The pKa (pH at which an acid compound is 50 per cent dissociated) of 7.89 for 2,4-dichlorophenol indicates that, at the pH range characterising most physiological and environmental conditions (typically pH 7– 8), these compounds will exist predominately in the more toxicologically active undissociated form. Furthermore, as pH decreases the proportion of molecules in the undissociated state will increase further, leading to yet higher activity as shown by parameters such as increased adsorption to suspended solids and sediment, and greater toxicity.

2.5 Environmental fate and partitioning

Table 2.5 summarises the information obtained from the literature on the environmental fate and partitioning of 2,4-dichlorophenol.

Table 2.5 Environmental fate and partitioning of 2,4-dichlorophenol

Property	Value	Reference
Abiotic fate	Photolysis and, potentially, volatilisation are the main routes of non-biological degradation. Hydrolysis is not expected to be an important fate process.	HSDB 2006
Speciation	An important factor determining the environmental distribution of the chlorophenols is ionisation; the degree of dissociation of chlorophenols into phenolate and hydronium ions will depend on the pH of the aqueous medium. With a pKa of 7.89, a substantial fraction, sometimes the majority, will be undissociated.	Serjeant and Dempsey 1979
Hydrolytic stability	Hydrolysis is not expected to occur due to the lack of hydrolysable functional groups.	HSDB 2006
Photostability	Photolysis appears to be the main abiotic route of degradation. Photolysis of 2,4-dichlorophenol in water under ultraviolet light is rapid, with DT50 values being in the range of 2–30 minutes depending on water pH. Vapour-phase 2,4-dichlorophenol is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals, which generally leads to the formation of catechol and other hydroxybenzenes.	Aly and Faust 1964, Meylan and Howard 1993
Volatilisation	The Henry's Law constant for 2,4-dichlorophenol (5.5×10^{-6} atm-m^3/mol) suggests that the substance is expected to volatilise from water surfaces.	Lyman *et al.* 1990
Distribution in water/sediment systems (active substance)	There is evidence of adsorption of the substance to sediments of aquatic ecosystems. The affinity for organic carbon in aquatic sediments decreases with increasing degree of dissociation and hence is stronger at acidic pH than under neutral/basic conditions.	Johnson *et al.* 1985
Degradation in soil	2,4-Dichlorophenol undergoes rapid biodegradation in soils.	Baker *et al.* 1980
Biodegradation	2,4-Dichlorophenol was not biodegradable in a ready biodegradability test (0% loss in 28 days), but was inherently biodegradable in a modified Zahn–Wellens test (98% loss in 120 hours).	IUCLID 2002
Octanol–water partition coefficient (log Kow)	3.06–3.25 at 20°C	HSDB 2006
Organic carbon partition coefficient (log Koc)	2.42–2.82	HSDB 2006
Bioaccumulation BCF	The vast majority of aquatic organisms do not readily accumulate 2,4-dichlorophenol to high levels.	Suntio *et al.* 1988, Shiu *et al.*

Property	Value	Reference
	Bioconcentration factors (BCFs) are not generally high, with the majority for fish ranging from 3.8–100 at neutral pH, with depuration half-lives in the order of hours to days. However, higher BCFs of 282–980 have been reported for one taxonomic group, leeches, although this has been attributed to a deficiency in these organisms of the enzyme necessary for the metabolism of chlorophenols.	1994

The pKa value of 7.89 for 2,4-dichlorophenol means it will exist in water (and sediment or soil) in a partially dissociated state, which may affect its transport and reactivity in these media.

Its Henry's Law constant of 5.5×10^{-6} atm-m^3/mol indicates that 2,4-dichlorophenol is expected to volatilise from water surfaces; based on this value, the volatilisation half-life from a model river (1 m deep with a flow of 1 m sec^{-1} and a wind velocity of 3 m sec^{-1}) is estimated as approximately 9 days (Lyman *et al.* 1990). The estimated volatilisation half-life from a model lake (1 m deep with a flow of 0.05 m sec^{-1} and a wind velocity of 0.5 m sec^{-1}) is approximately 67 days (Lyman *et al.* 1990).

Baker *et al.* (1980) found that, at 0–20°C, 39–84 per cent of the initial 2,4-dichlorophenol present in stream water was degraded during 40 days of aerobic incubation, while 35–60 per cent was degraded in sterile stream water controls over 40 days. Aly and Faust (1964, cited in US EPA 1980) found that 2,4-dichlorophenol in distilled water was rapidly degraded by exposure to ultraviolet light, with the rate of photolysis decreasing as the water pH decreased. A 50 per cent reduction in 2,4-dichlorophenol concentration under ultraviolet light was observed after 2, 5 and 34 minutes at water pH values of 9, 7 and 4 respectively. As a result, 2,4-dichlorophenol is not expected to persist in the water column when the substance is released to the aquatic compartment.

Koc values of 263 for 2,4-dichlorophenol in lake sediment and 661 in river sediment indicate that it is expected to have low to moderate mobility in soil and that volatilisation could be greatly attenuated by adsorption to suspended solids and sediments in water (HSDB 2006).

Volatilisation of 2,4-dichlorophenol from moist soil surfaces is expected to be important based on the estimated Henry's Law constant (Yalkowsky and Dannenfelser 1992). 2,4-Dichlorophenol is not expected to volatilise from dry soil surfaces based on the vapour pressure of 0.12 mm-Hg (Shiu *et al.* 1994). In highly alkaline soils (pH 10), it will exist primarily in the ionised form; it has been observed to be poorly adsorbed to soil under such conditions, since non-dissociated 2,4-dichlorophenol is expected to undergo more adsorption than the ionised form (Johnson *et al.* 1985). Baker *et al.* (1980) found that, at 0–4°C, 79–82 per cent of the initial concentration of 2,4-dichlorophenol in a clay loam soil was degraded during 12–14 days of aerobic incubation, while 0–1 per cent was degraded in the sterile soil controls. In the same study at 0–20°C, 43–73 per cent of the initial concentration of 2,4-dichlorophenol in a stream sediment system was degraded during 15–30 days of aerobic incubation, while 21 per cent was degraded in sterile sediment controls over 30 days. As a result, 2,4-dichlorophenol is not expected to persist in the soil when the substance is released to the terrestrial compartment.

2.6 Effects data

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

Data collation followed a tiered approach. First, critical freshwater and saltwater data were compiled from existing EQS documents. Further data published after derivation of the current UK EQS were then retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database[3] and sources such as ScienceDirect®.[4]

2.6.1 Toxicity to freshwater organisms

Freshwater toxicity data are available for various taxonomic groups including algae, invertebrates and fish, as required for the application of the approach specified in the EU Technical Guidance Document (ECB 2003).

Single species long-term toxicity data on the effects of 2,4-dichlorophenol (which acts as a polar narcotic) on freshwater organisms are available for five taxonomic groups, i.e. algae, crustaceans, fish, insects and macrophytes. Single species short-term toxicity data for freshwater organisms are available for four taxonomic groups, i.e. algae, crustaceans, fish and protozoa.

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for 2,4-dichlorophenol 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 (SSDs) and have not been used to set the 2,4-dichlorophenol PNECs. The lowest critical freshwater data for 2,4-dichlorophenol are presented in Tables 2.6 (for long-term data) and 2.7 (for short-term data).

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

Figure 2.1 Cumulative distribution function of freshwater long-term data (µg l^{-1}) for 2,4-dichlorophenol

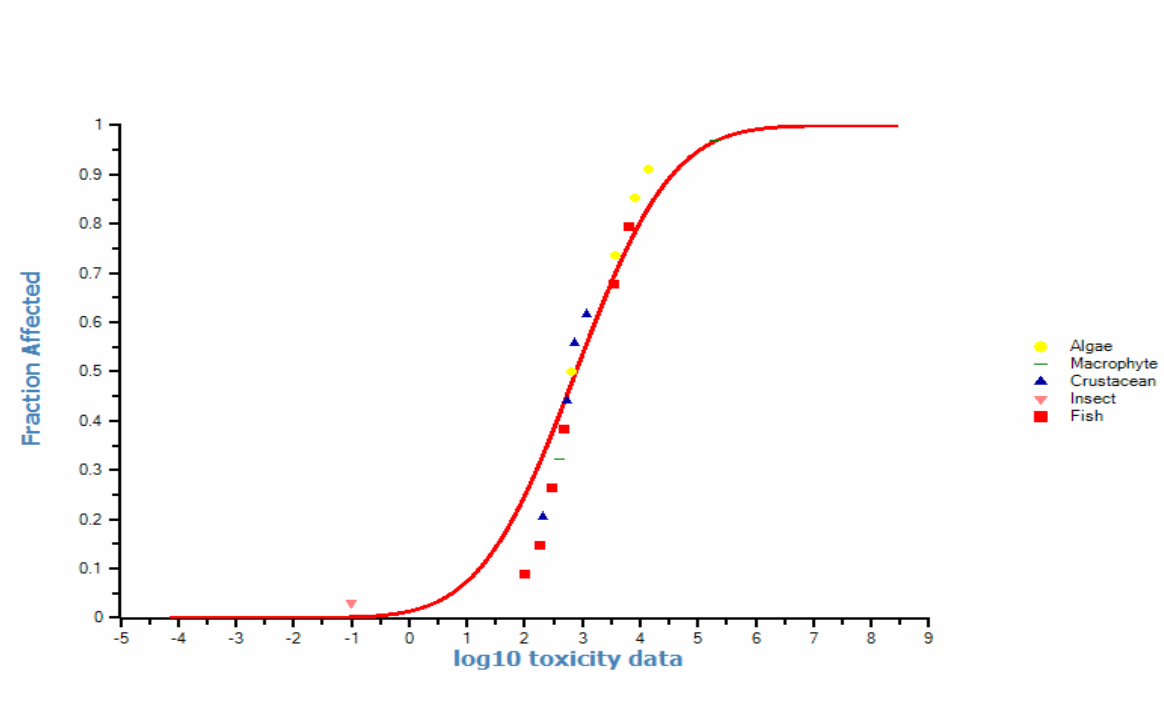


Figure 2.2 Cumulative distribution function of freshwater short-term data (µg l^{-1}) for 2,4-dichlorophenol

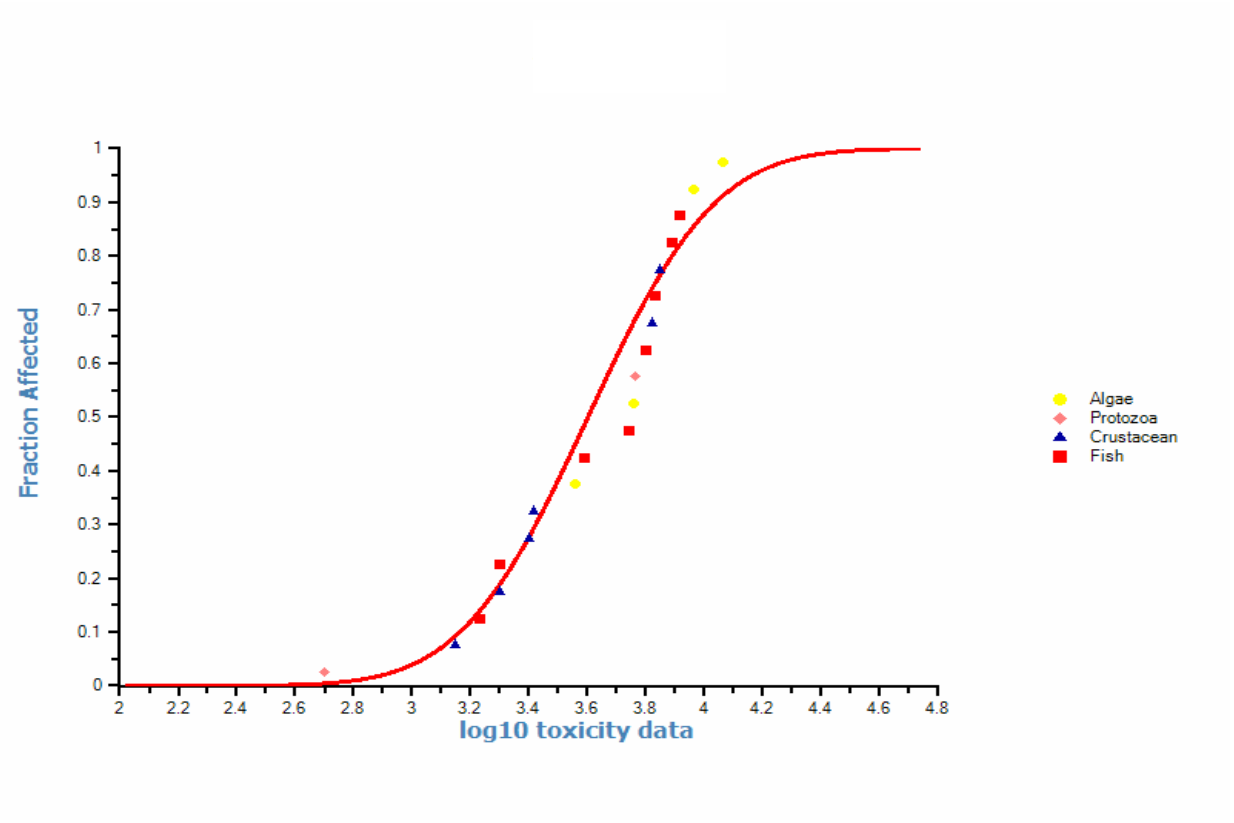


Table 2.6 Most sensitive long-term aquatic toxicity data for freshwater organisms exposed to 2,4-dichlorophenol

Scientific name	Common name	Taxonomic group	Test duration (days)	Effect	End-point	Conc. (µg l^{-1})	Expo-sure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Algae and macrophytes											
Pseudokirchneriella subcapitata	Green algae	ALG	3	Growth (growth rate and biomass)	NOEC	640	s	y	22±2°C	2	Environment Agency (2008)
Scenedesmus quadricauda	Green algae	ALG	7	Growth	MATC	3,600	s	n	27°C	3	Bringmann and Kuhn 1980
Lemna gibba	Duckweed	MAC	10	Growth	EC10	410	s	y	27.8°C	2	Ensley *et al.* 1994
Invertebrates											
Daphnia magna	Water flea	CRU	21	Reproduction	NOEC	210	ss	y	25°C; pH 7.0	2	Kuhn *et al.* 1989b
Hydropsyche slossonae	Larvae	INS	20	Behaviour	NOEC	0.1	ss	n	15±1°C; pH 6.9±0.2	3	Tessier *et al.* 2000
Fish											
Oncorhynchus mykiss	Rainbow trout	FIS	85	Growth/ development	LOEC	100	f	y	9.6–9.8°C; pH 7.9	2	Hodson *et al.* 1991
Oncorhynchus mykiss	Rainbow trout	FIS	85	Mortality	NOEC	100	f	y	9.6–9.8°C; pH 7.9	2	Hodson *et al.* 1991

* 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; INS = insects; MAC = macrophytes
MATC = maximum allowable toxicant concentration
EC10 = concentration effective against 10% of the organisms tested
NOEC = no observed effect concentration

Table 2.7 Most sensitive short-term aquatic toxicity data for freshwater organisms exposed to 2,4-dichlorophenol

Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. (µg l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Algae											
Chlorella vulgaris	Green algae	ALG	EC50	Growth	96	9,200	s	n	21±1°C	3	Shigeoka *et al.* 1988
Pseudokirchneriella subcapitata	Green algae	ALG	EC50	Growth (growth rate)	72	5,700	s	y	22±2°C	2	Environment Agency (2008)
				Growth (biomass)	72	1,200	s	y	22±2°C	2	
Scenedesmus subspicatus	Green algae	ALG	EC50	Growth	48	11,500	s	n	24±1°C	2	Kuhn and Pattard 1990
Selenastrum capricornutum	Green algae	ALG	EC50	Growth	96	14,000	s	n	21±1°C	3	Shigeoka *et al.* 1988
Protozoa											
Entosiphon sulcatum	Flagellate euglenoid	PRO	MATC	Population growth rate	72	500	s	n	25°C	3	Bringmann and Kuhn 1980
Invertebrates											
Daphnia magna	Water flea (<24 hours)	CRU	EC50	Immobilisation	48	1,400	s	n	20°C; pH 8.0±0.2	2	Kuhn *et al.* 1989a
Fish											
Lepomis machrochirus	Bluegill sunfish	FIS	LC50	Mortality	96	2,000	s	n	21–23°C; pH 6.5–7.9	3	Buccafusco *et al.* 1981
Salmo trutta	Brown trout (4.5 g)	FIS	LC50	Mortality	24	1,700	s	n	5°C	3	Hattula *et al.* 1981

* See Annex 1.
[1] Exposure: s = static.
[2] Toxicant analysis: n = nominal.
ALG = algae; CRU = crustaceans; FIS = fish; PRO = protozoans
MATC = maximum allowable toxicant concentration
EC50 = concentration effective against 50% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested

2.6.2 Toxicity to saltwater organisms

Single species short-term toxicity data on the effects of 2,4-dichlorophenol on marine organisms are available for three taxonomic groups, i.e. algae, crustaceans and fish. These represent the base set required for the use of the approach specified in the EU Technical Guidance Document (ECB 2003).

Diagrammatic representation of the available short-term saltwater data (cumulative distribution functions) for 2,4-dichlorophenol 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 the 2,4-dichlorophenol PNECs. The lowest critical short-term toxicity data for marine species is summarised in Table 2.8.

No single species long-term toxicity data on the effects of 2,4-dichlorophenol on marine organisms have been located.

Figure 2.3 Cumulative distribution function of saltwater short-term data (µg l^{-1}) for 2,4-dichlorophenol

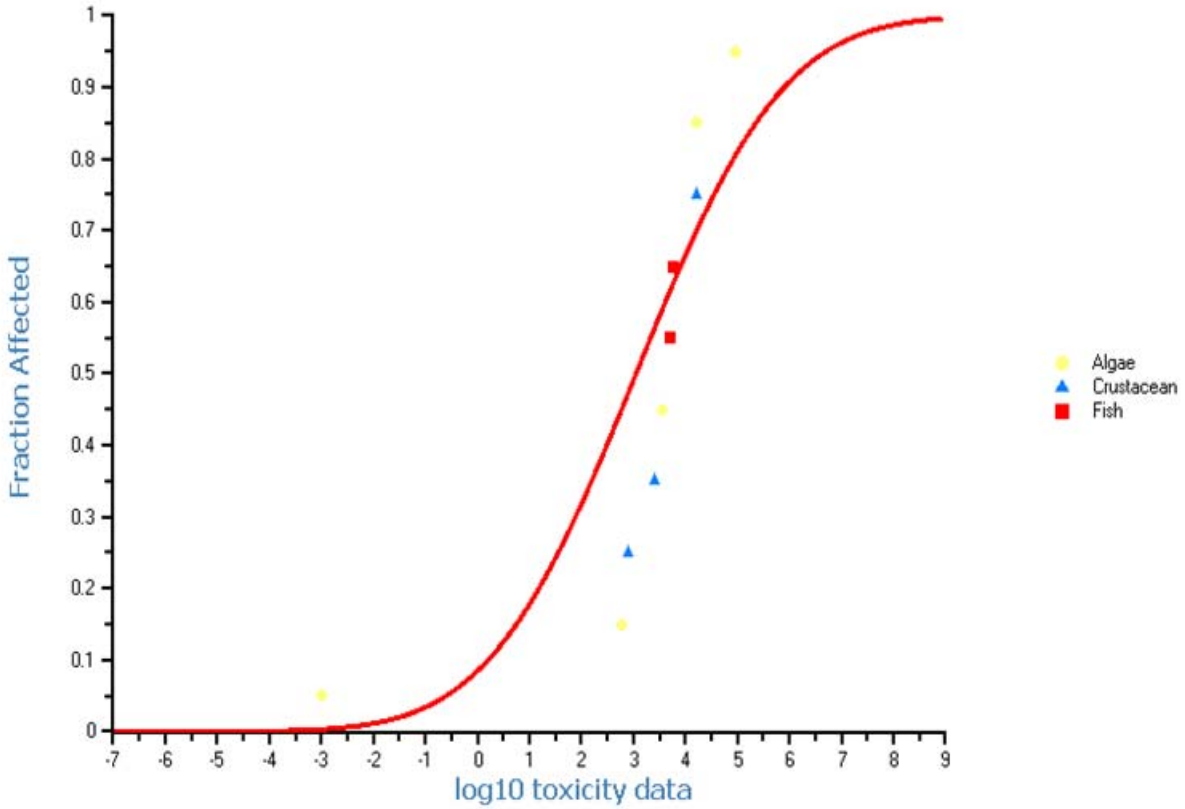


Table 2.8 Most sensitive short-term aquatic toxicity data for saltwater organisms exposed to 2,4-dichlorophenol

Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration (hours)	Conc. (µg l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Algae											
Phaeodactylum tricornutum	Diatom	ALG	EC50	Growth	72	600	s	y	15±1°C; Salinity 28‰	2	Kusk and Nyholm 1992
Phyllospora comosa	Brown algae	ALG	LOEC	Mortality	96	0.001	s	n	15±1°C Salinity 34±2‰	3	Burridge *et al.* 1995
Invertebrates											
Allorchestes compressa	Amphipod	CRU	LOEC	Mortality	96	800	s	n	20±1°C; Salinity 34±2‰	3	Burridge *et al.* 1995
Fish											
Solea solea	Sole (45 g)	FIS	LC50	Mortality	96	5,130	ss	y	6°C; pH 8.0; Salinity 22‰	2	Smith *et al.* 1994

* See Annex 1.
[1] Exposure: s = static; ss = semi-static.
[2] Toxicant analysis: y = measured; n = nominal.
ALG = algae; CRU = crustaceans; FIS = fish
LOEC = lowest observed effect concentration
EC50 = concentration effective against 50% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested

2.6.3 Toxicity to sediment dwelling organisms

Although 2,4-dichlorophenol has a low log Kow of 3.06–3.25 (see Section 2.5), it is known to sorb to a limited degree to organic matter. An extensive literature search for data on the toxicity of 2,4-diclorophenol to sediment-dwelling organisms did not identify any relevant studies. As a result it is not possible to derive a $PNEC_{sediment}$ based on experimental toxicity data.

2.6.4 Endocrine-disrupting effects

A comprehensive assessment of the potentially endocrine disrupting properties of 2,4-dichlorophenol was carried out as part of a European Commission (EU 2002), which evaluated 12 candidate substances for which there was scientific evidence of endocrine disruption or potential endocrine disruption, but which were not restricted and were not being addressed under existing community legislation. The study concluded that, in terms of endocrine mediated effects on aquatic organisms:

> 'The available aquatic effects data shows that the threshold exposure concentrations above which effects on reproduction in invertebrates and fish are observed is slightly lower or similar to the threshold level for general toxic effects (i.e. lethality and/or growth) in these species. However, there is generally no data in the reported studies which indicate whether the observed effects on reproduction are endocrine mediated. Indeed in invertebrates there is limited knowledge of the endocrinology of many taxonomic groups and it uncertain whether reproductive processes are modulated by oestrogens or androgens.'

2.6.5 Mode of action of 2,4-dichlorophenol

Dichlorophenol compounds are considered to act as polar narcotics in fish (Ahlborg and Thunberg 1980, Schultz 1987, Bryant and Schultz 1994, van Wezel *et al.* 1995). The toxicity exhibited by polar narcotics is characterised by a convulsant action where the loss of reaction to external stimuli and/or loss of equilibrium are detected. This differs from non-polar narcotic effects, which can be considered to represent 'minimum toxicity' caused by accumulation of compounds in the hydrophobic phases of an organism such as storage lipids and membrane lipids (van Wezel *et al.* 1995). Furthermore, quantitative structure–activity relationship (QSAR) analyses have shown that chlorophenols with two or more substituents, such as 2,4-dichlorophenol, uncouple oxidative phosphorylation in mitochondria (Penttinen 1995).

In contrast, QSAR studies in saltwater crustaceans have shown that the toxicity of chlorophenols in these organisms is characterised by a non-specific narcotic mode of action (Smith *et al.* 1994).

2.7 Mesocosm and field studies

2.7.1 Freshwater mesocosm and field studies

No information on the effects of 2,4-dichlorophenol on freshwater organisms from mesocosm and field studies was located.

2.7.2 Saltwater mesocosm and field studies

Kuiper and Hanstveit (1984) investigated the fate and effects of 2,4-dichlorophenol added to North Sea coastal plankton communities enclosed by large plastic bags in three experiments of 4–6 weeks duration. The experiments were conducted with 2,4-dichlorophenol involving exposure concentrations of 100 and 1,000 µg l^{-1} in the first (May–June 1978), and 300 and 1,000 µg l^{-1} in the second (August–September 1978) and third (August–September 1980) studies. Duplicate controls were used in each study.

At the start of each experiment, the bags were simultaneously filled with 1.5 m^3 of natural seawater collected a few miles offshore. The bags were anchored near a raft in the harbour of Den Helder, the Netherlands. During the experiment, the development of the phytoplankton, zooplankton, and bacteria were measured, as was a set of physicochemical parameters including nutrients (ammonia, nitrate, nitrite and phosphate), pH, light, temperature and concentrations of 2,4-dichlorophenol in water and sediment. Chlorophyll concentrations, primary productivity, particle volume distribution and species composition were measured to follow the development of the phytoplankton. Zooplankton organisms were counted, and their biomass and production were estimated.

Addition of 100 µg l^{-1} 2,4-dichlorophenol was found to have no or a slight inhibitory effect on the phytoplankton growth rate, but had no marked effects on zooplankton density and composition. There was a “threshold” of effect on these indices at 300 µg l^{-1}. Exposure to 1,000 µg l^{-1} 2,4-dichlorophenol inhibited the phytoplankton growth rate and changed the species composition; it also influenced zooplankton density and composition. Based on these studies, the no observed effect concentration (NOEC) for effects on phytoplankton and zooplankton communities is approximately 100 µg l^{-1}.

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). Therefore, the EU TGD assessment factor (AF) method can be applied. The taxonomic groups for which long-term toxicity tests are available (algae, crustaceans, fish, insects and macrophytes) are limited and it is not possible to determine which taxonomic group is the most sensitive.

The lowest long-term toxicity value for freshwater organisms is an extremely sensitive 20-day NOEC of 0.1 µg l^{-1} for the effects of 2,4-dichlorophenol on the net-spinning behaviour of larvae of the trichopteran *Hydropsyche slossonae* (Tessier *et al.* 2000). At higher exposure concentrations, two distinct abnormalities were observed which could affect the ability of organisms to capture food. The first was a distortion of the midline meshes (termed 'midline' anomaly), where the normal diamond-shape structure was disrupted and the meshes were separated by extra strands. The second was 'chaotic net' aberration, where the nets were highly irregular without any real structure or well-defined areas. The 'midline' anomaly was the first to be observed and the authors stated that this reflected the physiological stress associated with the polar narcotic effects of 2,4-dichlorophenol (see Section 2.4). However, this aberration is followed by a more significant increase in the 'chaotic' net anomaly, which the authors considered to characterise the effects of uncoupling of oxidative phosphorylation.

The study involved a replicated design and a semi-static exposure regime, and was carried out in a dynamic flow system. The exposure concentrations were replaced every day but there was no analytical confirmation of the actual concentrations. As a result, there are issues with the reliability of the study. With regard to the ecological significance of the endpoint, there is no clear evidence that links the change in the net-spinning behaviour of this taxonomic group with resulting population effects. However, a theoretical link can be made between aberrant net quality, a reduced ability to capture food and detrimental physiological effects.

The lowest valid reported long-term toxicity value for 'standard' ecotoxicological endpoints (e.g. growth, reproduction and mortality) is an 85-day LOEC of 100 µg l^{-1} for effects on the growth (as wet weight) of fry of the rainbow trout *Oncorhynchus mykiss* at the 4 weeks post swim-up stage (Hodson *et al.* 1991). The study involved a flow-through design and occasional measurement of exposure concentrations. There was a 40% reduction in the wet weight of fry at this concentration and because the effect level is greater than 20% the TGD approach cannot be used to derive a NOEC value from the LOEC. However, the study also reported a NOEC of 100 µg l^{-1} for mortality effects on the exposed rainbow trout.

..

The rainbow trout data is supported by a 21-day NOEC of 210 µg l^{-1} for reproductive effects in the water flea *Daphnia magna* (Kuhn *et al.* 1989b). This study involved a semi-static design with replacement of the test solutions every 48 hours and measurement of the exposure concentrations.

The lowest long-term toxicity value for algae or macrophytes is a 10-day EC10 of 410 µg l^{-1} for effects of 2,4-dichlorophenol on the growth of duckweed *Lemna gibba* (Ensley *et al.* 1994). However, this study adopted a static exposure regime and accompanying fate and behaviour studies indicated that the 2,4-dichlorophenol was converted into 2,4-dichlorophenyl-β-D-glucopyranoside.

The lowest long-term toxicity value for algae in the original dataset was a 144-hour toxicity threshold – considered equivalent to a maximum allowable toxicant concentration (MATC) of 3,600 µg l^{-1} for effects on the growth of the green alga *Scenedesmus quadricauda* (Bringmann and Kuhn 1980). The description of the methodology in this study is limited and, given the absence of measured data on the exposure concentrations, it was not considered appropriate to use the data to derive the PNEC. Therefore, the Environment Agency commissioned a study of the effects of 2,4-dichlorophenol on *Pseudokirchneriella subcapitata (*Environment Agency 2008*).* The 72-hour study was carried out to OECD Guideline 201 and involved analytical confirmation of the exposure concentrations. The study reported 72-hour NOEC values of 640 µg l^{-1} for effects on growth as measured using both growth rate and biomass endpoints.

Overall, on the basis of the available long-term data for freshwater organisms, algae and macrophytes appear to be slightly less sensitive to 2,4-dichlorophenol than invertebrates and fish. The lowest reliable NOEC is the value of 100 µg $l^{-1}$for mortality in the rainbow trout after 85 days exposure and given that reliable chronic values are also available for algae and invertebrates it is proposed that an assessment factor of 10 is applied. Deriving the $PNEC_{freshwater\_lt}$ on the basis of the 85 day NOEC for effects on the survival of early life stage rainbow trout *Oncorhynchus mykiss ,* the resulting value would be:

$PNEC_{freshwater\_lt}$ = 100 µg l^{-1}/AF (10) = 10 µg l^{-1} 2,4-dichlorophenol

This value provides a margin of safety against the observed effects of 2,4-dichlorophenol on the growth of rainbow trout at 100 µg l^{-1}.

However, given the absence of a NOEC for the effects of 2,4-dichlorophenol on the growth of rainbow trout it is appropriate to consider other approaches for the derivation of a long-term freshwater PNEC including:

1. The use of an alternative chronic toxicity value (and assessment factor) to derive the PNEC
2. The use of acute data to derive the PNEC

If it is not considered appropriate to use the data from the Hodson *et al* . (1991) rainbow trout study in the derivation of the PNEC then the PNEC could be developed using the available long-term algal and invertebrate data, with the chronic fish toxicity data being used in a supporting role. The lowest reliable value from this dataset is a 21-day NOEC of 210 µg l^{-1} for reproductive effects in the water flea *Daphnia magna.* By applying an assessment factor of 50 (for NOECs from two taxonomic groups) the resulting value would be:

$PNEC_{freshwater\_lt}$ = 210 µg l^{-1}/AF (50) = 4.2 µg l^{-1} 2,4-dichlorophenol

The TGD also proposes the derivation of the PNEC from acute data with an AF of 100 if acute effect data are available that are lower than the lowest long-term NOEC. However, the lowest short-term value is a 48-hour EC50 value of 1,400 µg l^{-1} for effects on the mobility of the

cladoceran copepod *Daphnia magna* which is higher than the lowest long-term NOEC. Therefore, this approach is not deemed to be appropriate for the derivation of the PNEC.

Based on the review of the available data and to provide an adequate level of protection it is proposed that the PNEC of 4.2µg l^{-1} derived using chronic algal and invertebrate data is applied as the long-term value. This value provides a margin of safety against the observed effects of 2,4-dichlorophenol on the growth of rainbow trout at 100 µg l^{-1}.

PNEC accounting for transient concentration peaks

Single species short-term toxicity data are available for four different taxonomic groups, i.e. algae, crustaceans, fish and protozoa. The lowest reported short-term acute toxicity values for traditional endpoints (e.g. growth and mortality) is a 72-hour MATC of 500 µg l^{-1} for effects on the growth of the flagellate euglenoid protozoan *Entosiphon sulcatum* (Bringmann and Kuhn 1980). The description of the methodology used in the study is limited and, given the absence of measured data on the exposure concentrations, it is not considered appropriate to use the data to derive the PNEC.

The lowest valid short-term data is considered to be a 48-hour EC50 of 1,400 µg l^{-1} for effects on the mobility of the cladoceran copepod *Daphnia magna* (Kuhn *et al.* 1989a). This study was carried out according to German DIN Standard 38412, Part II (*Daphnia* short-time test). There was no confirmation of the exposure concentrations, but the stock solutions used were apparently analysed.

The lowest short-term toxicity values for algae in the original dataset are 96-hour EC50 values of 9,200 and 14,000 µg l^{-1} for effects on the growth of *Chlorella vulgaris* and *Selenastrum capricornutum* respectively (Shigeoka *et al.* 1988). These values are supported by a 48-hour EC50 value of 11,500 µg l^{-1} for effects on the growth of *Scenedesmus subspicatus* (Kuhn and Pattard 1990). In both these studies, there was no analytical confirmation of the exposure concentrations, though the methods used are well described. The study on the effects of 2,4-dichlorophenol on *Scenedesmus* growth is considered to be of greater validity because it followed a draft German DIN standard and the main validity criterion for the test was achieved (i.e. >16 times increase in cell numbers in the controls). Subsequently the Environment Agency commissioned a study of the effects of 2,4-dichlorophenol on *Pseudokirchneriella subcapitata (*Environment Agency 2008*).* The 72-hour study was carried out to OECD Guideline 201 and involved analytical confirmation of the exposure concentrations. The study reported 72-hour EC50 values of 5,700 µg l^{-1} for effects on growth as measured using the growth rate endpoint and 1,200 µg l^{-1} for effects on growth as measured using the biomass endpoint.

The lowest short-term toxicity values for fish are a 24-hour LC50 of 1,700 µg l^{-1} for mortality of brown trout *Salmo trutta* (Hattula *et al.* 1981) and a 96-hour LC50 of 2,000 µg l^{-1} for mortality of bluegill sunfish *Lepomis macrochirus* (Buccafusco *et al.* 1981). In both these studies, there was no analytical confirmation of the exposure concentrations and the methods used are not particularly well described.

Given the available data, the $PNEC_{freshwater\_st}$ should be derived on the basis of the 48-hour EC50 value of 1,400 µg l^{-1} for effects on the mobility of the cladoceran copepod *Daphnia magna* (Kuhn *et al.* 1989a). Based on the guidance given in the TGD on effects assessment for intermittent releases (Section 3.3.2 of Part II of the TGD document) and because there is an acceptable short-term toxicity database for freshwater organisms, an assessment factor of 10 rather than 100 can be applied resulting in the following values:

$PNEC_{freshwater\_st}$ = 1400 µg l^{-1}/AF (10) = 140 µg l^{-1} 2,4-dichlorophenol

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 (algae, crustacea and fish). However, no long-term data were located.

The available short-term toxicity data of the marine taxa show some differences from the range of values obtained for comparative 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 limited available data, it is proposed that:

- the TGD approach of using freshwater data within the marine effect assessment is used;
- suggested freshwater PNECs 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 relating to 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. Therefore, it is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation (see Section 3.1.1), an approach consistent with that described in the EU 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 and molluscs. Given that there is limited data for these marine taxa, a total assessment factor of 100 (including a factor of 10 to account for the greater uncertainty due to the limited data for marine taxa) should be applied to the lowest valid toxicity value.

Deriving the $PNEC_{saltwater\_lt}$ on the basis of the lowest reliable chronic value (i.e. an 85-day NOEC for effects on the survival), the resulting value would be:

$PNEC_{saltwater\_lt}$ = 1000 µg l^{-1}/AF (100) = 1.0 µg l^{-1} 2,4-dichlorophenol

However, in order to be protective of the effects of 2,4-dichlorophenol observed on the growth of rainbow trout *Oncorhynchus mykiss* at 100 µg l^{-1}, it is proposed that the chronic algal and invertebrate dataset is used to derive the PNEC, The lowest reliable value for this dataset is a 21-day NOEC of 210 µg l^{-1} for reproductive effects in the water flea *Daphnia magna*. By applying a total assessment factor of 500 (for NOECs from two taxonomic groups and accounting for the greater uncertainty due to the limited data for marine taxa) the resulting value would be:

$PNEC_{freshwater\_lt}$ = 210 µg l^{-1}/AF (500) = 0.42 µg l^{-1} 2,4-dichlorophenol

PNEC accounting for transient concentration peaks

Single species short-term toxicity data referring to marine organisms are available for three different taxonomic groups, i.e. algae, crustaceans and fish, with algae being the most sensitive group.

The lowest short-term toxicity value reported for saltwater organisms is a 96-hour LOEC of 0.001 µg l^{-1} for effects of 2,4-dichlorophenol on the survival of fertilised zygotes of the brown alga *Phyllospora comosa* (Burridge *et al.* 1995). The study involved a static design and there was no

measurement of exposure concentrations, which means the data should not be used for the PNEC derivation.

Kusk and Nyholm (1992) reported a 72-hour EC50 value of 600 µg l^{-1} for effects on the growth of the diatom *Phaeodactylum tricornutum*. In the test, the exposure concentrations were analysed at the end of the test and generally deviated <10 per cent from the nominal concentrations.

The lowest short-term toxicity values for invertebrates and fish are a 96-hour LC50 of 800 µg l^{-1} for effects on the survival of the amphipod *Allorchetes compressa* (Burridge *et al.* 1995) and a 96-hour LC50 of 5130 µg l^{-1} for effects on the survival of sole *Solea solea* (Smith *et al.* 1994). The Burridge *et al.* (1995) study involved a semi-static design, but there was no measurement of the exposure concentrations. The Smith *et al.* (1994) study involved a semi-static design and incorporated measurements of exposure concentrations, which indicated these were within 80 per cent of nominal values.

No data were located for exclusively marine taxa such as echinoderms. However, data are available from a saltwater mesocosm study.

The TGD does not provide specific guidance for assessment of short-term effects of intermittent releases to marine water bodies. Therefore, it is suggested that the PNEC accounting for effects following short-term exposure to 2,4-dichlorophenol is calculated 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 TGD). Given that there is limited data for these marine taxa, a total assessment factor of 100 (including a factor of 10 to account for the greater uncertainty due to the limited data for marine taxa) should be applied to the lowest valid toxicity value

Deriving the $PNEC_{saltwater\_st}$ on the basis of the 72-hour EC50 of 600 µg l^{-1} for effects on the growth of the diatom *Phaeodactylum tricornutum* and an assessment factor of 100, the resulting value would be:

$PNEC_{saltwater\_st}$ = 600 µg l^{-1}/AF (100) = 6.0 µg l^{-1} 2,4-dichlorophenol

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

There are insufficient data to construct an SSD based upon long-term exposure data.

3.3 Derivation of existing EQSs

The derivation of the existing proposed EQSs for 2,4-dichlorophenol is described by Grimwood and Mascarenhas (1997) in a report published by the Environment Agency.

In freshwaters, the proposed annual average (AA) was derived by applying a safety factor of 10 (to account for extrapolation to a no-effects concentration and possible interspecies differences in sensitivity) to the lowest chronic effects concentration (i.e. the 85-day LOEC for mortality of larvae of rainbow trout *Oncorhynchus mykiss* of 180 µg l^{-1}) resulting in an EQS of around 20 µg l^{-1}.

The proposed freshwater maximum allowable concentration (MAC) for 2,4-dichlorophenol was based on the lowest reliable 48-hour EC50 of 1,400 µg l^{-1} reported in a laboratory study for the water flea *Daphnia magna*. Given the large dataset available and evidence of a small effect to no-effects ratio, a factor of 10 was applied to this value resulting in an EQS of 140 µg l^{-1} expressed as a MAC.

Given the limited database on the toxicity of 2,4-dichlorophenol to saltwater organisms (including the absence of reliable long-term toxicity data), it was proposed that the EQSs set for the protection of freshwater life should also be adopted as tentative values for the protection of saltwater life. This was justified by the fact that the available data on the toxicity, fate and behaviour of 2,4-dichlorophenol was similar under both freshwater and saltwater conditions.

3.4 Derivation of PNECs for sediment

Since the log Kow of 2,4-dichlorophenol is >3 (see Section 2.5), the derivation of PNECs for the protection of benthic organisms is required. An extensive literature search for data on the toxicity of 2,4-dichlorophenol to sediment-dwelling organisms did not identify any relevant studies. As a result, it is not possible to derive a $PNEC_{sediment}$ based on experimental toxicity data.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

Mammalian and avian toxicity data were taken from WHO *Guidelines for Drinking Water Quality* (1996) and the study on the potential endocrine-disrupting properties of 2,4-dichlorophenol prepared for the European Commission (EC 2002) (see Section 2.6.4). However, it is important to recognise that the dataset on 2,4-dichlorophenol is limited.

The metabolism and distribution of 2,4-dichlorophenol in mammals has been investigated in both *in vitro* and *in vivo* studies. In a metabolism study in isolated rat liver, 2,4-dichlorophenol was reported to be conjugated into its glucuronide or metabolised into dichloromethoxyphenols (Somani *et al.* 1984). In an *in vitro* study on the human P450 3A4-mediated metabolism of 2,4-dichlorophenol, thin-layer chromatography detected 2-chloro-1,4-dihydroxybenzene, 2-chloro-1,4-benzoquinone and 1,2,4-trihydroxybenzene (Mehmood *et al.* 1997).

Somani and Khalique (1982) administered 2,4-dichlorophenol to male Sprague–Dawley (SD) rats (250–300 g) by a single intravenous injection at 10 mg/kg. They found the test substance was transferred rapidly into a glucuronide conjugate or other conjugates (though no description was given regarding the nature of the conjugated substances). The half-lives of 2,4-dichlorophenol and its metabolites in the brain, liver, kidney and plasma were found to be 4–30 minutes. Within 10–15 minutes after administration, 2,4-dichlorophenol and its conjugates were detected in the brain, liver, kidney and plasma while 2,4-dichlorophenol alone was detected in the adipose tissues. One hour after administration, 76 per cent of the total administered dose was detected in the kidney, with maximal concentration in the renal tissues of 17.7 mg/kg (kidney weight).

In rabbits, 2,4-dichlorophenol is excreted mainly as its glucuronide conjugate but some fraction (≤16 per cent) of the administered dose was reported to be converted into its sulphate conjugate (HSDB 2006). In calves, it is reported that the entire amount of 2,4-dichlorophenol (20 g per calf) administered was excreted within 24 hours after administration (HSDB 2006).

2,4-Dichlorophenol is of moderate acute oral toxicity to mammals with median lethal dose (LD50) values in rats ranging from 580 to 4,500 mg/kg body weight/day (Kobayashi *et al.* 1972) and in mice ranging from 1,276 to 1,630 mg/kg body weight/day (Kobayashi *et al.* 1972, Borzelleca *et al.* 1985).

Table 3.1 summarises a number of short-term and long-term studies on the effects of 2,4-dichlorophenol following oral exposure.

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

Study and result	Details
Sub-chronic toxicity to mammals	
Kobayashi *et al.* 1972 **Sub-chronic NOAEL = 100 mg/kg/day**	ICR mice (seven males per group) were exposed to 2,4-dichlorophenol concentrations of 0, 0.02, 0.05, 0.1 and 0.2% (corresponding to 18, 45, 100 and 230 mg/kg/day) in the diet in a six-month dosed feed study. In the study, the relative liver weight decreased in the 230 mg/kg/day group, with hepatocellular swelling in one, small round cell infiltration in the interstitium in two, and thinning of the adrenal cortex in two males.
NTP 1989 **Sub-chronic NOAEL = 1,000 mg/kg/day 10,000 ppm in males or 500 mg/kg/day 5,000 ppm in females**	F344 rats (10 rats per sex per group) were exposed to 2,4-dichlorophenol concentrations of 0, 2,500, 5,000, 10,000, 20,000 and 40,000 parts per million (ppm) in the diet in a 13-week dosed feed study. Atrophy of the bone marrow and marked decreases in the erythrocytes and myelocytes were observed in six out of 10 females in the 10,000 ppm group, and all rats in the 20,000 ppm or higher groups. In addition, a suppression of the body weight (bw) gain, hunchback posture, rough hair coat and a decrease in the food consumption in the 40,000 ppm group was observed.
NTP 1989 **Sub-chronic NOAEL = <700 mg/kg/day <2,500 ppm**	B6C3F1 mice (10 mice per sex per group) were exposed to 2,4-dichlorophenol concentrations of 0, 2,500, 5,000, 10,000, 20,000 and 40,000 ppm in the diet in a 13-week dosed feed study. Exposure caused rough hair coat in both sexes and appearance of multinuclear hepatocytes in males at 10,000 ppm or above, suppression of the body weight gain and a decrease in the food consumption at 20,000 ppm, with cellular necrosis in all males and death of all mice within 3 weeks. Epithelial necrosis of the urinary tubules occurred at 40,000 ppm.
Chronic toxicity to mammals	
NTP 1989 **NOAEL = 210 mg/kg/day 5,000 ppm**	F344 rats were exposed to 2,4-dichlorophenol concentrations of 0, 5,000 and 10,000 ppm (corresponding to 0, 210 and 400 mg/kg/day) for females in the diet in a two-year dosed feed study. Exposure had no effect on the survival rate at any dose levels, but caused a suppression of body weight gain in both sexes in the high dose group. In males, the incidence of diffuse degeneration of the respiratory epithelium tended to increase with increasing doses of 2,4-dichlorophenol (control

Study and result	Details
	group: 35 out of 45; 5,000 ppm group: 38 out of 48; 10,000 ppm group: 42 out of 46).
NTP 1989 **NOAEL = 800 mg/kg/day 5,000 ppm in males**	B6C3F1 mice (50 mice per sex per group) were exposed to 2,4-dichlorophenol concentrations of 0, 5,000, and 10,000 ppm (males: 0, 800 and 1,300 mg/kg/day; females: 0, 430 and 820 mg/kg/day) in the diet in a two-year dosed feed study. The body weight gain was suppressed in the 10,000 ppm group and the incidence of multinuclear hepatocytes increased dose-dependently in males (control group: 11 out of 50; 5,000 ppm group: 33 out of 49; 10,000 ppm group: 42 out of 48)
Effects on reproduction of mammals	
Exon *et al.* 1984, Exon and Koller 1985 **NOEL = 50 mg/kg/day 3,000 ppm**	SD rats (3-week-old females, 10 females per group) were exposed to 2,4-dichlorophenol in drinking water at concentrations of 0, 3, 30, and 300 ppm (corresponding to 0, 0.5, 5 and 50 mg/kg/day) in a one generation study. Administration was continued during mating with untreated males at the age of 13 weeks, through gestation and lactation periods until weaning. The offspring were weaned at the age of three weeks and treated with 2,4-dichlorophenol in drinking water until the age of 12 weeks. Exposure of pregnant female rats to levels of 3 to 300 ppm 2,4-dichlorophenol had no significant effects on reproduction parameters such as conception, litter size and weight, number of still-born pups, or survival to weaning. Although there was a dose-dependent trend toward an increase in body weight at weaning age, this effect was probably more related to a non-significant smaller litter size and was not evident after 6 weeks of age. The number of still-born pups also tended to be higher, but not significantly, in 2,4-dichlorophenol litters.
Borzelleca *et al.* 1985 **NOEL = 50 mg/kg/day**	CD-1 mice were exposed to 0, 50, 150 and 500 mg/kg bw/day via drinking water in a one generation study. An absolute control (de-ionised water) and a vehicle control (10% Emulphor) were used. 2,4-Dichlorophenol was administered in water containing 21% Emulphor (intended to enhance the solubility and palatability of the substance). Administration occurred in males and females for 90 days prior to mating, then during mating and the gestation period. After 90 days, 10 males and 10 females per treatment were mated. The test was terminated 18 days after mating when all females were sacrificed. No significant effects were observed on any

Study and result	Details
	reproductive parameters at any test dose. The only effect observed was an increase in the resorption rate at 150 mg/kg bw/day, but this change was not statistically significant.
Effects on development of mammals – no data located	
Embryotoxicity and teratogenicity	
Rodwell *et al.* 1989 **NOEL = 750 mg/kg/day**	F344 rats (34 females) were exposed to doses of 0, 200, 375 and 750 mg 2,4-dichlorophenol/kg per day (in corn oil) on gestation days 6–15. Dams were caesarean-sectioned on gestation day 20. In the 200 mg/kg/day or higher groups, a suppression of the body weight gain and soiling of the external genitalia were observed in dams. In the 750 mg/kg/day group, the maternal toxicities included alopecia, abnormal respiratory sound, adhesion of a blood-like substance around the eye, nostrils and mouth and death (four out of 34 dams), and delayed ossification of the sternebrae and vertebral arches were observed in foetuses. It was concluded that 2,4-dichlorophenol has no teratogenic potential, but caused delayed foetal development secondary to the maternal toxicities at 750 mg/kg/day.
Neurotoxicity to mammals – no studies were available on the neurotoxicity of 2,4-dichlorophenol to mammals	
Endocrine disruption	
A comprehensive assessment of the endocrine disrupting properties of 2,4-dichlorophenol was carried out as part of a European Commission study EC 2002 see Section 2.6.4. This study found that, in terms of endocrine-disrupting effects of 2,4-dichlorophenol on humans: • *In vivo* studies including the rodent uterotrophic and Hershberger assays did not indicate that 2,4-dichlorophenol caused sex hormone receptor-mediated endocrine disrupting effects at the dose tested CERI 2001. • A study on the effects on *in vitro* fertilisation in mice showed no effects on sperm motility or fertilisation rate Seyler *et al.* 1984 • *In vitro* data for 2,4-dichlorophenol relates only to assays assessing oestrogenic mechanisms of action in mammalian cells and tissues. The data indicate an absence of induction of oestrogen-sensitive gene products and limited binding of 2,4-dichlorophenol to the human oestrogen receptor Jones *et al.* 1998, Körner *et al.* 1998. Exposure of human mammary tumour cells to 2,4-dichlorophenol results in a weak induction of cell proliferation Jobling *et al.* 1995	
Sub-chronic and chronic toxicity to birds – no studies were available on the sub-chronic and/or chronic toxicity of 2,4-dichlorophenol.	
Effects on reproduction to birds – no studies were available on the effects of 2,4-dichlorophenol on reproduction in birds.	

NOAEL = no observed adverse effect level
NOEL = no observed effect level

3.5.2 PNECs for secondary poisoning of predators

Bioconcentration data (as BCF values) for 2,4-dichlorophenol for the majority of aquatic organisms are low, with values for fish ranging from 3.8 to 100 at neutral pH. Hence the trigger of BCF values >100 is not met and the derivation of PNECs for secondary poisoning of predators is not required.

Shumway and Palensky (1973) reported that 2,4-dichlorophenol causes tainting in largemouth bass (*Micropteus salmoida*) at 0.4 µg l^{-1} and rainbow trout (*Onchorhynchus mykiss*) at 1.0 µg l^{-1}.

4 Analysis and monitoring

Analytical methods for the determination of 2,4-dichlorophenol published between 1988 and 1997 are discussed by Grimwood and Mascarenhas (1997). A number of recent studies on the analysis of chlorophenols (including 2,4-dichlorophenol) are discussed below, indicating the approach taken and the limit of detection.

Carabias-Martinez *et al.* (2004) reported on a sensitive analytical method based on liquid chromatography–electrospray ionisation mass spectrometry (LC-ESI-MS) developed for the detection of a range of organic compounds (including 2,4-dichlorophenol) in water. Acidification of the LC mobile phase was necessary to achieve good separation. However, post-column addition of base was also required to raise the sample pH, which aided the ionisation process for substances such as 2,4-dichlorophenol with higher pKa values. The use of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) diluted in methanol proved to be the most efficient post-column reagent for enhancing the MS signal and resulted in limits of detection for the compounds investigated in the low $\mu g\ l^{-1}$ range. For water samples, an extraction and preconcentration step using a solid-phase extraction technique (with the Oasis HLB polymeric sorbent) was advocated, which resulted in recoveries of 70–110 per cent for most of the compounds investigated.

Kawaguchi *et al.* (2004) described a method for the analysis of a range of organic chemicals (including 2,4-dichlorophenol) which involved the use of stir-bar sorptive extraction (SBSE) with *in situ* derivatisation followed by thermal desorption/gas chromatography/mass spectrometry (TD-GC-MS) analysis. In this method, a polydimethylsiloxane-coated stir bar and acetic acid anhydride derivatisation reagents were added to 10 ml of water sample before stirring for 10–180 minutes at room temperature (25^{o}C) in a headspace vial. The optimum time for SBSE with *in situ* derivatisation was 90 minutes. The extract was then analysed by TD-GC-MS. The detection limit for 2,4-dichlorophenol was 0.002 $\mu g\ l^{-1}$ and the correlation coefficient was 0.99. For 2,4-dichlorophenol, the method showed linearity over the concentration range 0.01 to 1.0 $\mu g\ l^{-1}$. The average recoveries for the different compounds in river water samples were ≥93.9 per cent (relative standard deviation (RSD) <7.2 per cent).

Li *et al.* (2004) reported a simple and rapid solid-phase microextraction (SPME) method using a 5,11,17,23-tetra-*tert*-butyl-25,27-diethoxy-26,28-dihydroxycalix[4] arene [C[4]/hydroxyl-terminated silicone oil (HO-TSO)] sol-gel coated novel fibre for the direct analysis of four chlorophenols (including 2,4-dichlorophenol). Optimal extraction procedures such as extraction temperature, time and pH were described. The linear range for each chlorophenol was three or four orders of magnitude and detection limits (S/N = 3) ranged from 0.005 to 0.276 $\mu g\ l^{-1}$. RSD (n = 6) values ranged from 0.72 to 6.8 per cent. Recoveries for the chlorophenols in river water samples ranged from 86.3 to 97.4 per cent.

Suliman *et al.* (2006) described a simple, sensitive and rapid reverse-phase high performance liquid chromatography (RP-HPLC) method for the detection of a range of phenols in water. This method involves the use of coumarin-6-sulfonyl chloride as a fluorescence-labelling reagent that reacts with phenols within 20 minutes under mild conditions (ambient temperature, pH 9.0). The resulting sulphonates can be separated by RP-HPLC employing fluorescence detection at λ_{ex} = 360 nm and λ_{em} = 460 nm. Detection limits in the range 0.1–0.9 $\mu g\ l^{-1}$ were obtained for the phenols studied (including mono-, di- and trichlorophenols) and calibration curves for 2,3-dichlorophenol and 3,5-dichlorophenol were linear in the range 3–100 $\mu g\ l^{-1}$.

The proposed PNECs are in the range 0.5–140 $\mu g\ l^{-1}$. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current newer analytical methodologies (non-standard) capable of achieving detection limits as low as 2-5 ng l^{-1}, offer adequate performance to analyse for 2,4-dichlorophenol.

5 Conclusions

5.1 Availability of data

Long-term laboratory data are available for five different freshwater taxonomic groups including algae, crustaceans, fish, insects and macrophytes. Freshwater short-term toxicity data are available for four taxonomic groups including algae, crustaceans, fish and protozoa. The limited dataset means that it is not possible to discern which taxonomic group is most sensitive to 2,4-dichlorophenol. For marine organisms, single species short-term toxicity data are available for three different taxonomic groups (algae, crustaceans and fish). However, no long-term toxicity data are available for taxa largely or exclusively found in saltwaters. Laboratory data are supplemented by saltwater mesocosm data which show effects of 2,4-dichlorophenol on algae and crustaceans.

There is currently no definitive data that demonstrates that 2,4-dichlorophenol causes endocrine-mediated effects in aquatic organisms.

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 reported long-term toxicity value for 'standard' ecotoxicological endpoints (e.g. growth, reproduction and mortality) is an 85 day NOEC of 100 µg l^{-1} for effects on the survival of early life stage rainbow trout *Oncorhynchus mykiss.* The same study also reported a LOEC of 100 µg l^{-1} for effects on the growth (as wet weight) of fry of the rainbow trout *Oncorhynchus mykiss* at the 4 weeks post swim-up stage. There was a 40% reduction in the wet weight of fry at this concentration and because the effect level is greater than 20% the TGD approach cannot be used to derive a NOEC value from the LOEC. A lower NOEC value of 0.1 µg l^{-1} for effects of 2,4-dichlorophenol on the net spinning behaviour of larvae of the trichopterna *Hydropysche slossonae* was reported, but there were concerns about the validity of these data. Since reliable long-term NOECs are available for algae , invertebrates (including crustaceans and insects) and fish, an assessment factor of 10 could be applied to the lowest valid toxicity value resulting in a $PNEC_{freshwater\_lt}$ of 10 µg l^{-1}.

However, in order to be protective of the effects of 2,4-dichlorophenol observed on the growth of rainbow trout *Oncorhynchus mykiss* at 100 µg l^{-1}, it is proposed that the chronic algal and invertebrate dataset is used to derive the PNEC, The lowest reliable value for this dataset is a 21-day NOEC of 210 µg l^{-1} for reproductive effects in the water flea *Daphnia magna*. By applying an assessment factor of 50 (for NOECs from two taxonomic groups) the resulting value would be:

$PNEC_{freshwater\_lt}$ = 210 µg l^{-1}/AF (50) = 4.2 µg l^{-1} 2,4-dichlorophenol

This value provides a margin of safety against the observed effects of 2,4-dichlorophenol on the growth of rainbow trout at 100 µg l^{-1}.

This value is lower than the existing EQS of 20 µg l^{-1}. This was derived by applying a safety factor of 10 (to account for extrapolation to a no-effects concentration and possible interspecies differences in sensitivity) to the lowest chronic effects concentration (i.e. the 85-day LOEC for mortality of larvae of rainbow trout *Oncorhynchus mykiss* of 180 µg l^{-1} from the same study as considered above).

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 data is considered to be a 48-hour EC50 of 1,400 µg l^{-1} for effects on the mobility of the cladoceran copepod *Daphnia magna.* Since there is an acceptable short-term toxicity database for freshwater organisms, an assessment factor of 10 has been applied resulting in a $PNEC_{freshwater\_st}$ of 140 µg l^{-1}.

The value is the same as the current EQS of 140 µg l^{-1}. This was derived by applying the same safety factor to the same data point.

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. It is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation.

The lowest valid reported long-term toxicity value for 'standard' ecotoxicological endpoints (e.g. growth, reproduction and mortality) is a 85-day NOEC of 100 µg l^{-1} for effects on the survival of early life stage rainbow trout *Oncorhynchus mykiss* Reliable long-term NOECs are available for freshwater algae, invertebrates and fish but no toxicity data are available for marine taxa such as echinoderms and molluscs. Given that there is limited data for these marine taxa, a total assessment factor of 100 (including a factor of 10 to account for the greater uncertainty due to the limited data for marine taxa) could be applied to the lowest valid toxicity value resulting in a $PNEC_{saltwater\_lt}$ of 1.0 µg l^{-1}.

However, in order to be protective of the effects of 2,4-dichlorophenol observed on the growth of rainbow trout *Oncorhynchus mykiss* at 100 µg l^{-1}, it is proposed that the chronic algal and invertebrate dataset is used to derive the PNEC, The lowest reliable value for this dataset is a 21-day NOEC of 210 µg l^{-1} for reproductive effects in the water flea *Daphnia magna*. By applying a total assessment factor of 500 (for NOECs from two taxonomic groups and accounting for the greater uncertainty due to the limited data for marine taxa) the resulting value would be 0.42 µg l^{-1} 2,4-dichlorophenol.

This value is lower than the existing EQS of 20 µg l^{-1}, which was 'read across' from the freshwater long-term value.

5.2.4 Short-term PNEC for saltwaters

The lowest valid short-term toxicity value is a 72-hour EC50 value of 600 µg l^{-1} for effects on the growth of the diatom *Phaeodactylum tricornutum.* Lower short-term toxicity values have been reported in studies that did not meet OECD principles of Good Laboratory Practice, but these are considered to be unreliable due to the absence of measured concentration data. Reliable short-term L(E)C50s are available for freshwater algae, invertebrates and fish but no toxicity data are available for marine

taxa such as echinoderms and molluscs. Given that there is limited data for these marine taxa, a total assessment factor of 100 (including a factor of 10 to account for the greater uncertainty due to the limited data for marine taxa) should be applied to the lowest valid toxicity value resulting in a $PNEC_{saltwater\_lt}$ of 6.0 µg l^{-1}.

This value is lower than the existing EQS of 140 µg l^{-1}, which was 'read across' from the freshwater short-term value.

5.2.5 PNEC for sediments

Since the log Kow of 2,4-dichlorophenol is >3, the derivation of PNECs for the protection of benthic organisms is required. An extensive literature search for data on the toxicity of 2,4-dichlorophenol to sediment-dwelling organisms did not identify any relevant studies. As a result it is not possible to derive a $PNEC_{sediment}$ based on experimental toxicity data.

5.2.6 PNEC for secondary poisoning

Bioconcentration data (as BCF values) for 2,4-dichlorophenol for aquatic organisms are generally low with values for fish ranging from 3.8 to 100 at neutral pH. Higher BCFs of 282–980 have been reported for one taxonomic group, leeches, although this has been attributed to a deficiency in these organisms of the enzyme necessary for the metabolism of chlorophenols. Hence, the trigger EU Technical Guidance Document BCF of 100 is not exceeded and the derivation of a PNEC in whole fish for secondary poisoning of predators is not required.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1})	Existing EQS (µg l^{-1})
Freshwater/long-term	4.2	20
Freshwater/short-term	140	140
Saltwater/long-term	0.42	20
Saltwater/short-term	6.0	140
Sediment	Insufficient data	–
Secondary poisoning	Not required	–

5.3 Analysis

The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing GC-MS, which are capable of achieving detection limits as low as 2–5 ng l^{-1}, should offer adequate performance to analyse for 2,4-dichlorophenol.

5.4 Implementation issues

1. The high lipophilicity of 2,4-dichlorophenol leads it to partition out of the water column onto sediments and biota. However, the lack of reliable sediment toxicity

data precludes the derivation of a $PNEC_{sediment}$. Consequently, the generation of freshwater and saltwater sediment toxicity data is necessary to set a standard for this compartment.

2. The freshwater long term and short term PNECs are not subject to excessive uncertainty with assessment factors of 10 being applied to derive the PNECs. These PNECs are therefore suitable for use. In relation to the saltwater PNECs larger assessment factors have been applied which reflects the higher level of uncertainty. This uncertainty could be reduced by undertaking additional ecotoxicity testing for marine organisms.

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List of abbreviations

AA	annual average
AF	assessment factor
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstracts Service
EC	European Commission
EC50	concentration effective against 50% of the organisms tested
EHC	Environmental Health Criteria
EQS	Environmental Quality Standard
GLP	Good Laboratory Practice (OECD)
IUPAC	International Union of Pure and Applied Chemistry
LC50	concentration lethal to 50% of the organisms tested
LC-ESI-MS	liquid chromatography/electrospray ionisation mass spectrometry
LOEC	lowest observed effect concentration
lt	long term
MAC	maximum allowable concentration
MATC	maximum allowable toxicant 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
QSAR	quantitative structure–activity relationship
RP-HPLC	reverse-phase high performance liquid chromatography
SBSE	stir-bar sorptive extraction
SPME	solid-phase microextraction
SSD	species sensitivity distribution
st	short term
TD-GC-MS	thermal desorption/gas chromatography/mass spectrometry
TGD	Technical Guidance Document
UKTAG	UK Technical Advisory Group
US EPA	US Environmental Protection Agency
WFD	Water Framework Directive
WHO	World Health Organization

ANNEX 1 Data quality assessment sheets

Identified and ordered by alphabetical order of references.

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

Table A1 Klimisch Criteria*

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

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

Reference	Bringmann and Kuhn 1980

Information on the test species	
Test species used	Green alga (*Scenedesmus quadricauda*) Flagellate euglenoid (*Entosulphon sulcatum)*
Source of the test organisms	In-house stock cultures
Holding conditions prior to test	Inoculated in nutrient media
Life stage of the test species used	Growth phase

Information on the test design	
Methodology used	The method is described to a limited extent in the paper.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Nutrient media
Test concentrations used	Not stated
Number of replicates per concentration	*Scenedesmus*: four (three inoculated, one un-inoculated) *Entosiphon*: two
Number of organisms per replicate	Not relevant
Nature of test system (Static, semi-static or flow through, duration, feeding)	*Scenedesmus*: static, 144 hours, no feeding *Entosiphon*: static, 72 hours, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	The study is not well described and the exposure concentrations used were not measured.

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

Reference	Buccafusco *et al.* 1981

Information on the test species	
Test species used	Bluegill sunfish (*Lepomis macrochirus*)
Source of the test organisms	Commercial fish suppliers in the USA
Holding conditions prior to test	Flow-through system receiving well water with daily feeding
Life stage of the test species used	Young of the year (0.32–1.2 g)

Information on the test design	
Methodology used	The method is reasonably well described in the paper.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Reconstituted US EPA soft water
Test concentrations used	Not stated
Number of replicates per concentration	1
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow through, duration, feeding)	Static, 96 hours, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Yes (pH, temperature and dissolved oxygen)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	The study is reasonably well described but the exposure concentrations used were not measured.

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

Reference	Burridge *et al.* 1995

Information on the test species	
Test species used	Brown alga (*Phyllospora comosa*) Amphipod (*Allorchestes compressa*)
Source of the test organisms	*P. comosa* – mature specimens from ocean beaches at Sorrento, Victoria. *A. compressa* – laboratory cultures
Holding conditions prior to test	*P. comosa* – tests initiated immediately on return to laboratory *A. compressa* – semi-static seawater holding system (of 34‰) and fed *ad libitum* with seagrass
Life stage of the test species used	*P. comosa* – 1- and 7-day-old fertilised zygotes *A. compressa* – 7, 10 and 12 mm length organisms

Information on the test design	
Methodology used	The method used is reasonably well described in the paper.
Form of the test substance	Analytical standard (99% purity)
Source of the test substance	Aldrich
Type and source of the exposure medium	Filtered seawater
Test concentrations used	Control and at least five exposure concentrations (actual concentrations not stated)
Number of replicates per concentration	*P. comosa* – 4 *A. compressa* – 3
Number of organisms per replicate	*P. comosa* – 25 *A. compressa* – 10
Nature of test system (Static, semi-static or flow through, duration, feeding)	*P. comosa* – static, 96 hours *A. compressa* - static, 96 hours, no feeding
Measurement of exposure concentrations	The exposure concentrations were not measured.
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
Comments	The study is reasonably well described but exposure concentrations were not measured.

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

Reference	Ensley *et al.* 1994

Information on the test species	
Test species used	Duckweed (*Lemna gibba*)
Source of the test organisms	Not stated
Holding conditions prior to test	Sterile culture media
Life stage of the test species used	Growth phase

Information on the test design	
Methodology used	The method is described in the paper.
Form of the test substance	Not stated
Source of the test substance	Aldrich
Type and source of the exposure medium	Nutrient media
Test concentrations used	0 (control), 408, 994, 1,956, 3,912 and 9,780 µg l^{-1}
Number of replicates per concentration	10
Number of organisms per replicate	Not stated
Nature of test system (Static, semi-static or flow through, duration, feeding)	Static, 10 days, no feeding
Measurement of exposure concentrations	Loss of exposure concentrations was measured in a separate study.
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
Comments	A static exposure regime was used and the exposure concentrations used were not measured directly.

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

Reference	Environment Agency 2008

Information on the test species	
Test species used	*Pseudokirchneriella subcapitata*
Source of the test organisms	In house cultures
Holding conditions prior to test	Nutrient media (ATCC 22662)
Life stage of the test species used	Growth phase

Information on the test design	
Methodology used	The method is well described in the report.
Form of the test substance	Analytical material (99% purity)
Source of the test substance	Sigma-Aldrich, Dorset, UK
Type and source of the exposure medium	Nutrient media
Test concentrations used	0 (control), 95, 190, 380, 760, 1500, 3000, 6000 and 12000 µg l^{-1} (nominal concentrations)
Number of replicates per concentration	Six (for controls) and three (for treatments)
Number of organisms per replicate	Initial starting density = 5 x 10^3 cells/ml
Nature of test system (Static, semi-static or flow through, duration, feeding)	Static, 72 hours, no feeding
Measurement of exposure concentrations	The test concentrations were analysed at the beginning and end of the test (measured values were 68 to 88% of nominal concentrations).
Measurement of water quality parameters	Yes (pH and temperature)
Test validity criteria satisfied	Yes (127 times increase in controls)
Water quality criteria satisfied	Yes
Study conducted to GLP	The study was carried out to the principles of GLP
Comments	The study was well conducted, is of good quality and the exposure concentrations used were measured.

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

Reference	Hattula *et al.* 1981

Information on the test species	
Test species used	Brown trout (*Salmo trutta*)
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	4.5 g fish

Information on the test design	
Methodology used	The method is not well described in the paper.
Form of the test substance	97% 2,4-dichlorophenol
Source of the test substance	Fluka AG
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	1
Number of organisms per replicate	5
Nature of test system (Static, semi-static or flow through, duration, feeding)	Static, 24 hours, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	Limited numbers of test organisms were used and the exposure concentrations used were not measured.

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

Reference	Hodson *et al*. 1991

Information on the test species	
Test species used	Rainbow trout (*Oncorhynchus mykiss*)
Source of the test organisms	Fertilised eggs were obtained from a commercial supplier in Ontario.
Holding conditions prior to test	Fertilised eggs were used immediately on return to the laboratory.
Life stage of the test species used	Test initiated with fertilised eggs and ended with freely swimming fry.

Information on the test design	
Methodology used	The method used is well described.
Form of the test substance	Highly purifed standard
Source of the test substance	Eastman Kodak
Type and source of the exposure medium	Lake Ontario water as a dechlorinated municipal supply (hardness = 135 mg l^{-1} $CaCO_3$)
Test concentrations used	0 (control), 100, 180, 320, 560 and 1,000 µg l^{-1}
Number of replicates per concentration	3
Number of organisms per replicate	200–300 eggs
Nature of test system (Static, semi-static or flow through, duration, feeding)	Flow-through (161–171 ml min^{-1}), 85 days, gradual feeding with salmonid starter diet when the yolk of fish was almost completely used
Measurement of exposure concentrations	Limited monitoring indicated that measured concentrations were close to nominal.
Measurement of water quality parameters	Yes (pH, temperature, dissolved oxygen, conductivity and hardness)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	The study was well conducted and is of good quality and the exposure concentrations used were measured.

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

Reference	Kuhn and Pattard 1990

Information on the test species	
Test species used	*Scenedesmus subspicatus*
Source of the test organisms	In house cultures
Holding conditions prior to test	In accordance with the procedure practised by the institute
Life stage of the test species used	Growth phase

Information on the test design	
Methodology used	The method is well described in the paper (German DIN 38 412 Draft Standard).
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Growth media
Test concentrations used	Control, 320–40,000 µg l^{-1}
Number of replicates per concentration	Eight (four inoculated and four uninoculated)
Number of organisms per replicate	Initial cell density is 10,000 cells ml^{-1}
Nature of test system (Static, semi-static or flow through, duration, feeding)	Static, 48 hours, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Yes (>16 times increase in cell numbers in the controls)
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	The study was well conducted but the exposure concentrations used were not measured.

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

Reference	Kuhn *et al.* 1989a

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	In house cultures
Holding conditions prior to test	In accordance with the procedure practised by the institute
Life stage of the test species used	<24-hour-old neonates

Information on the test design	
Methodology used	The method (DIN 38412, Part II – Daphnia short-term test) is reasonably well described in the paper.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Reconstituted water
Test concentrations used	Not stated
Number of replicates per concentration	2
Number of organisms per replicate	10
Nature of test system (Static, semi-static or flow through, duration, feeding)	Static, 48 hours, no feeding
Measurement of exposure concentrations	There was analytical confirmation of the stock solutions.
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
Comments	The study is reasonably well described in the paper and was carried out to a standardised method. The exposure concentrations used were not measured but there was confirmation of the stock solutions.

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

Reference	Kuhn *et al.* 1989b

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	In house cultures of IRCHA strain
Holding conditions prior to test	In accordance with the procedure practised by the institute since 1978 (described in the paper)
Life stage of the test species used	<24 hour old neonates

Information on the test design	
Methodology used	The method used is well described in the paper.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Synthetic German Institute of Standardisation freshwater
Test concentrations used	0 (control), 80–10,000 µg l^{-1}
Number of replicates per concentration	20
Number of organisms per replicate	1
Nature of test system (Static, semi-static or flow through, duration, feeding)	Semi-static exposure regime with replacement of solutions every Monday, Wednesday and Friday Test duration was 21 days. Feeding occurred on the same days as test solution replacement.
Measurement of exposure concentrations	The test concentrations were measured with samples being taken twice during the test period (on the 7th days and between the 16th and 21st day).
Measurement of water quality parameters	Water quality parameters (pH, temperature and dissolved oxygen) were measured on each of the test.
Test validity criteria satisfied	Yes (the mean reproduction rate per parent animal after 21 days was 88.8 offspring and parent animal mortality was 7.1%).
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	The study was well conducted and the exposure concentrations used were measured.

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

Reference	Kusk and Nyholm 1992

Information on the test species	
Test species used	*Phaeodactylum tricornutum*
Source of the test organisms	In house cultures
Holding conditions prior to test	Nutrient media
Life stage of the test species used	Growth phase

Information on the test design	
Methodology used	The method is well described in the paper.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Nutrient media
Test concentrations used	Not stated
Number of replicates per concentration	3
Number of organisms per replicate	Not relevant
Nature of test system (Static, semi-static or flow through, duration, feeding)	Static, 72 hours, no feeding
Measurement of exposure concentrations	The test concentrations were analysed at the end of the test.
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
Comments	The study was well conducted, is of good quality and the exposure concentrations used were measured.

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

Reference	Shigeoka *et al.* 1988

Information on the test species	
Test species used	*Chlorella vulgaris* *Selenastrum capricornutum*
Source of the test organisms	In house cultures
Holding conditions prior to test	Nutrient media
Life stage of the test species used	Growth phase

Information on the test design	
Methodology used	The method is reasonably well described in the paper.
Form of the test substance	Reagent grade
Source of the test substance	Tokyo Kasei Kogyo, Tokyo
Type and source of the exposure medium	Nutrient media
Test concentrations used	*Chlorella*: control, 5,000–60,000 µg l^{-1} *Selenastrum*: control, 2,000–10,000 µg l^{-1}
Number of replicates per concentration	six (three inoculated and three uninoculated)
Number of organisms per replicate	Not relevant
Nature of test system (Static, semi-static or flow through, duration, feeding)	Static, 96 hours, no feeding
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	The study is reasonably well described but the exposure concentrations used were measured.

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

Reference	Smith *et al.* 1994

Information on the test species	
Test species used	Copepod *(Tisbe battaglia)* Flounder (*Platichthys flesus*) Sole (*Solea solea*)
Source of the test organisms	Copepod – in-house cultures Fish – netting around UK power station cooling water screens
Holding conditions prior to test	Copepod – in-house cultures Fish – recirculating aquaria at 22‰ and 6°C
Life stage of the test species used	Copepods – 6-day-old copepodides Flounder (56.0±2.5 g), sole (45.0±2.5 g)

Information on the test design	
Methodology used	The methods used are well described in the paper.
Form of the test substance	Analytical grade standards (98–99% purity)
Source of the test substance	Aldrich
Type and source of the exposure medium	Copepods – filtered natural seawater of 30‰ Flounder and sole – filtered natural seawater of 5 and 22‰, respectively
Test concentrations used	Copepods and fish – control and five experimental concentrations
Number of replicates per concentration	Copepods – not clear Fish – 2
Number of organisms per replicate	Copepods – not clear Fish – 12
Nature of test system (Static, semi-static or flow through, duration, feeding)	Copepods – static, 24 hours, no feeding Fish – semi-static with replacement after 48 hours, 96 hours, no feeding
Measurement of exposure concentrations	Copepods – not measured Fish – measured every 12 hours
Measurement of water quality parameters	Copepods and fish – pH, temperature and dissolved oxygen
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	The study was well conducted and the exposure concentrations used were measured in the fish studies.

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

Reference	Tessier *et al.* 2000

Information on the test species	
Test species used	*Hydropsyche slossonae*
Source of the test organisms	Collected from the Becancour River in southern Quebec, Canada.
Holding conditions prior to test	40-litre aquaria supplied with flowing reconstituted soft water (40 mg l^{-1} $CaCO_3$)
Life stage of the test species used	Fourth instar larvae

Information on the test design	
Methodology used	The methodology is well described in the paper.
Form of the test substance	99% 2,4-dichlorophenol stock solution
Source of the test substance	Aldrich
Type and source of the exposure medium	Reconstituted soft water (40 mg l^{-1} $CaCO_3$)
Test concentrations used	0 (control), 0.1, 1.0, 10, 25 and 50 µg l^{-1}
Number of replicates per concentration	One aquarium per concentration
Number of organisms per replicate	70 larvae
Nature of test system (Static, semi-static or flow through, duration, feeding)	Flowing system with a current velocity of 10–20 cm s^{-1} with water changed every 4 hours for the first 24 hours and subsequent changes every 24 hours. The test duration was 20 days with larvae fed 1.0 g of frozen *Artemia* sp. per aquarium twice a week.
Measurement of exposure concentrations	No measurement of exposure concentrations
Measurement of water quality parameters	Water quality parameters (pH, temperature, dissolved oxygen and conductivity) were measured throughout the test.
Test validity criteria satisfied	Not appropriate, but only 4 and 9% mortality occurred in the control and exposure concentrations over the 20 day exposure period.
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	The study was well conducted but the exposure concentrations used were not measured. A semi-static regime should have assisted with the maintenance of the concentrations.

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