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Proposed EQS for Water Framework Directive Annex VIII substances: phenol

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

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Steve Killeen

Head of Science

Use of this report

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

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

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

Executive Summary

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

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

A draft EU Risk Assessment Report (RAR) has been compiled for phenol and the UK is committed to the use of RAR PNECs for the derivation of the WFD Annex X EQSs. Consequently, this report recommends the available RAR PNECs as the corresponding proposed PNECs.

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

Phenol is widely used in manufacturing and process industries. It is a moderately water-soluble substance that is readily biodegraded in the aqueous environment and may also be lost through photodegradation.

Availability of data

A substantial number of laboratory toxicity data are available for nine different freshwater taxonomic groups (algae, crustaceans, fish, amphibians, macrophytes, annelids, insects, rotifers and molluscs). Although the freshwater chronic data are less extensive, the dataset provides coverage of seven of these taxa.

By contrast, saltwater toxicity data are available only for four taxonomic groups (algae, crustaceans, fish and rotifers), with acute data restricted to studies on algae and crustaceans.

There are no field or mesocosm data available for phenol.

Derivation of PNECs

Long-term PNEC for freshwaters

The lowest effect concentrations (2–65 $\mu\text{g l}^{-1}$) were obtained in chronic studies with rainbow trout, *Oncorhynchus mykiss*, and several amphibian species. However, in their review of the data considered for derivation of PNECs, Experts of the Technical Meeting on Existing Substances rejected these data because they were considered outliers and there were no corroborating data or any new evidence to validate them.

Fish appear to be the most sensitive group. The most sensitive fish species was *Cirrhina mrigala* in a reliable 60-day study that resulted in a no observed effect concentration (NOEC) of 77 $\mu\text{g l}^{-1}$. This value is supported by similar concentrations based on reliable studies of the effects of phenol on growth of rainbow trout and common carp. Because representatives of algae, crustaceans and fish are available in the chronic dataset, an assessment factor of 10 applied to this NOEC is recommended, leading to a $\text{PNEC}_{\text{freshwater_lt}}$ of 7.7 $\mu\text{g l}^{-1}$.

This PNEC is four times lower than the existing EQS of 30 $\mu\text{g l}^{-1}$. This reflects new data that have become available since the original EQS was derived: in the absence of chronic data, the existing EQS was based on an assessment factor of 100 applied to an acute LC50 for the water flea, *Ceriodaphnia dubia* (3,100 $\mu\text{g l}^{-1}$).

Short-term PNEC for freshwaters

A wide range of species sensitivities is evident following acute exposures to phenol but, as with chronic data, fish are the most sensitive taxonomic group. The lowest reliable datum, by a considerable margin, is a 96-hour LC50 of 460 $\mu\text{g l}^{-1}$ for guppy (*Poecilia reticulata*). The draft RAR for phenol did not include the guppy study identified above and did not set a short-term PNEC. There is evidence that (a) fish are the most sensitive taxonomic group to phenol and (b) phenol gives rise to a large ratio between acute and chronic toxicity, suggesting the need for a substantial margin between the long-term and short-term PNECs. Together, these indicate that applying a reduced assessment factor (from 100 to 10) to this acute LC50 can be justified as the basis for a PNEC. This results in a $\text{PNEC}_{\text{freshwater_st}}$ of 46 $\mu\text{g l}^{-1}$.

The proposed PNEC is about six times lower than the existing EQS of 300 $\mu\text{g l}^{-1}$. This is entirely a consequence of new data that have become available since the original EQS was derived: the existing EQS was based on an assessment factor of 10 applied to an acute LC50 for the water flea, *Ceriodaphnia dubia* (3,100 $\mu\text{g l}^{-1}$).

Long-term PNEC for saltwaters

Marine taxa appear to share a similar distribution of sensitivities to their freshwater counterparts. Consequently, for the purposes of PNEC derivation, the two datasets may be combined. Support for this approach is provided by separate research into

comparisons of species sensitivities in freshwater and saltwater organisms. This shows similar species sensitivity distributions for acute data (at least in the lower 'tail' of the distribution where the most sensitive species are to be found) and indicates that a PNEC based on freshwater data should protect saltwater species.

In chronic studies with marine species, fish appear to be the most sensitive organisms to long-term exposure to phenol. A reliable 8-day NOEC of $500 \mu\text{g l}^{-1}$ was reported in the grey mullet (*Mugil auratus*), but the lowest NOEC available in the combined freshwater and saltwater database is the same as used for the derivation of the $\text{PNEC}_{\text{freshwater_lt}}$ (60-day NOEC of $77 \mu\text{g l}^{-1}$ for the fish species *Cirrhina mrigala*).

No specific saltwater PNEC was derived in the phenol RAR. According to Annex V of the WFD, the NOEC of $77 \mu\text{g l}^{-1}$ would normally be divided by an assessment factor of 100. However, additional short-term tests are available for freshwater species belonging to relevant groups of annelids and molluscs. Reproduction tests with saltwater and freshwater rotifer species indicate these taxa are not the most sensitive to phenol. It therefore seems unlikely that long-term tests with representatives of these additional taxonomic groups would result in lower chronic toxicity data than those obtained for fish. Consequently, a reduced assessment factor of 10 applied to the fish NOEC of $77 \mu\text{g l}^{-1}$ is justified, resulting in the same PNEC as that in freshwater, i.e. $\text{PNEC}_{\text{saltwater_lt}} = \text{PNEC}_{\text{freshwater_lt}}$ of $7.7 \mu\text{g l}^{-1}$.

The existing EQS of $30 \mu\text{g l}^{-1}$ was 'read across' from the corresponding freshwater EQS.

Short-term PNEC for saltwaters

There are indications that marine crustaceans are particularly sensitive to phenol (acute LC50 values of $260\text{--}710 \mu\text{g l}^{-1}$), but shortcomings in reporting mean these suggestions cannot be validated. There are no reliable short-term data for saltwater fish but, applying the logic outlined above, combining the freshwater and saltwater datasets can be justified. As a result, the lowest acute effect concentration would be the 96-hour LC50 of $460 \mu\text{g l}^{-1}$ for guppy (*Poecilia reticulata*).

Because Annex V does not provide specific guidance on the derivation of short-term PNECs in the marine environment, it is recommended that the $\text{PNEC}_{\text{saltwater_st}}$ be based on an assessment factor of 10 applied to the guppy 96-hour LC50. This factor is justifiable on the assumption that (a) fish are the most sensitive species to both short-term and long-term exposure and (b) the substantial acute to chronic toxicity ratio for fish encourages a substantial margin between short-term and long-term PNECs for phenol. This results in a $\text{PNEC}_{\text{saltwater_st}} = \text{PNEC}_{\text{freshwater_st}}$ of $46 \mu\text{g l}^{-1}$.

The existing EQS of $300 \mu\text{g l}^{-1}$ was also 'read across' from the corresponding freshwater EQS.

PNECs for sediments and secondary poisoning

Since phenol does not partition preferentially into sediment and does not bioaccumulate to any significant extent, there is no justification for deriving sediment PNECs or PNECs to protect against risks of secondary poisoning to mammals and birds.

Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC ($\mu\text{g l}^{-1}$)	Existing EQS ($\mu\text{g l}^{-1}$)
Freshwater/long-term	7.7	30
Freshwater/short-term	46	300
Saltwater/long-term	7.7	30
Saltwater/short-term	46	300

Analysis

The lowest proposed PNEC for phenol is $7.7 \mu\text{g l}^{-1}$. The data quality requirements are that, at a third of the EQS, the total error of measurement should not exceed 50 per cent. Based on this, current analytical methodologies provide detection limits as low as $0.05 \mu\text{g l}^{-1}$, which suggests that they would be adequate for assessing compliance with the proposed PNECs for water.

Implementation issues

The proposed PNECs are recommended for adoption as EQSs without the need for further investigation.

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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 phenol using the methodology described in Annex V of the Directive. There are existing EQSs for phenol, but these are derived using a method not considered to comply with the requirements of Annex V and so cannot be used to derive Annex VIII EQSs.

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

A draft EU Risk Assessment Report (RAR) has been compiled for phenol and the UK is committed to the use of RAR PNECs for the derivation of the WFD Annex X EQSs. Consequently, this report recommends the available RAR PNECs as the corresponding proposed PNECs.

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

This report provides a data sheet for phenol.

1.1 Properties and fate in water

Phenol is widely used in manufacturing and process industries. It is a moderately water-soluble substance that is readily biodegraded in the aqueous environment and may also be lost through photodegradation.

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

² Data quality assessment sheets are provided in Annex 1.

2. Results and observations

2.1 Identity of substance

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

Table 2.1 Substance covered by this report

Name	CAS Number
Phenol	108-95-2

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 [6], and existing EQSs obtained from the literature [75].

Section 2.6 summarises the effects data identified from the literature for phenol. 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	46 $\mu\text{g l}^{-1}$ (see Section 3.1.1)	-	300 $\mu\text{g l}^{-1}$ (MAC)
Freshwater long-term	7.7 $\mu\text{g l}^{-1}$ (see Section 3.1.1)	Insufficient data	30 $\mu\text{g l}^{-1}$ (AA)
Saltwater short-term	46 $\mu\text{g l}^{-1}$ (see Section 3.1.2)	-	300 $\mu\text{g l}^{-1}$ (MAC)
Saltwater long-term	7.7 $\mu\text{g l}^{-1}$ (see Section 3.1.2)	Insufficient data	30 $\mu\text{g l}^{-1}$ (AA)
Freshwater sediment short-term	EQS not required (trigger not met)	-	-
Freshwater sediment long-term	EQS not required (trigger not met)	EQS not required (trigger not met)	-
Saltwater sediment short-term	EQS not required (trigger not met)	-	-
Saltwater sediment long-term	EQS not required (trigger not met)	EQS not required (trigger not met)	-

PNEC	TDG deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater secondary poisoning	EQS not required (trigger not met)	-	-
Saltwater secondary poisoning	EQS not required (trigger not met)	-	-

AA = annual average

AF = assessment factor

MAC = maximum allowable concentration

SSD = species sensitivity distribution

2.3 Hazard classification

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

Table 2.3 Hazard classification

R-phrases and labelling	Reference
Muta. Cat.3; R68-T; R23/24/25-Xn; R48/20/21/22-C; R34	[2]

2.4 Physical and chemical properties

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

Table 2.4 Physical and chemical properties of phenol

Property	Value	Reference
Molecular formula	C ₆ H ₆ O	
Molecular weight	94.11	[1]
Vapour pressure	0.2 hPa at 20°C	[1]
Henry's Law constant	0.022 Pa m ³ /mol at 20°C (calculated from water solubility and vapour pressure). Phenol is only slightly volatile from aqueous solution.	[1]
Solubility in water	84 g at 20°C 67 g at 16°C	[1] [3]
Dissociation constant (pKa)	9.89 at 20°C	[1]

2.5 Environmental fate and partitioning

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

Table 2.5 Environmental fate and partitioning of phenol

Property	Value	Reference
Hydrolytic stability (DT50)	No studies are available in connection with the hydrolytic degradation of phenol. However, no hydrolytic degradation is to be expected due to the chemical structure of the substance.	[1]
Photostability (DT50) (aqueous, sunlight, state pH)	A DT50 of 14 hours ($k_{degOH} = 0.051 \text{ h}^{-1}$) was calculated for photochemical degradation in the atmosphere on the basis of an atmospheric concentration of the OH radicals being $5 \times 10^5 \text{ molecules cm}^{-3}$	[1]
Readily biodegradable	100% degradation within 70 hours in natural waters (pH 5–8 and 22–28°C) Transformation half-lives (hours) in water range from: Distilled: 173 (winter) to 46 (summer) Polluted estuary: 118 (winter) to 43 (summer) Estuary: 94 (winter) to 39 (summer) Estuary dark: 62 (winter) to 28 (summer)	[1] [76] [21]
Degradation in water/sediment: DT50 water DT50 whole system	Water: DT50 14 days ($k = 0.05 \text{ d}^{-1}$) experimentally determined Sediment: DT50 69 days ($k = 0.01 \text{ d}^{-1}$) calculated	[1] [1]
Mineralisation	–	
Bound residue	–	
Distribution in water/sediment systems	The hydrosphere is the target compartment for phenol in the environment. Distribution according to Mackay Level 1 model is 0.8% air, 98.8% water, 0.2% sediment and 0.2% soil.	[1]
Degradation in soil	DT50 7 days ($k = 0.1 \text{ d}^{-1}$) experimentally determined	[1]
Partition coefficient (log Kow)	1.47	[1]
Koc	14–91 l kg^{-1} (reported range in literature) 82.2 l kg^{-1} (calculated according to the TGD on the basis of log Kow 1.47)	[1] [1]
Sediment–water Suspended matter–water	$K_{psed} = 8.278 \text{ l kg}^{-1}$ $K_{psusp} = 8.278 \text{ l kg}^{-1}$	[1] [1]

Property	Value	Reference
Bioconcentration factor (BCF)	The EU Risk Assessment Report (EU RAR) states that phenol has only a low bioaccumulation potential: 17.5 (fish, obtained in a test according to OECD 305E) is used in the EU RAR for assessment.	[1]
	The bioconcentration factors of phenol in various types of aquatic organisms are in general very low (<1–10), although some higher values (up to 2,200) have also been reported. Therefore, phenol is not expected to bioaccumulate significantly.	[3]

DT50 = time taken to degrade by 50%

2.6 Effects data

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

Data collation followed a tiered approach.

Critical data on freshwater and marine organisms were collected from the existing EQS document [75] as well as from the EU Risk Assessment Report (EU RAR) on phenol [1].

Further data published after derivation of the UK EQS were retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database.³

As no data on sediment-dwelling organisms, or mammalian or avian chronic oral toxicity were available in ECOTOX, further databases [4] were searched via the STN portal. Sources used were:

- Hazardous Substances Data Bank (HSDB®) database of the US National Library of Medicine;⁴
- US EPA Integrated Risk Information System (IRIS) database;⁵
- World Health Organization (WHO) *Environmental Health Criteria 161: Phenol* [3].

Toxicity data on phenol concentrations in sediment (mg phenol/kg sediment basis) were not identified.

Toxicity data and other information on the inherent properties of phenol taken from the draft EU RAR have not been subjected to additional quality assessment. These data have been already quality assessed by the authors of the EU RAR and by the 'Technical Meeting on Existing Substances', an international advisory forum of experts from EU Member States, industry and 'green' non-governmental organisations (NGOs). This body was set up to discuss and advise on the risk assessments for existing substances conducted in accordance with Commission Regulation (EC) No. 1488/94. Toxicity tests used in the

³ <http://www.epa.gov/ecotox/>

⁴ <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>

⁵ <http://www.epa.gov/iris/index.html>

context of the EU RAR on phenol were regarded as valid if they were performed according to national or international test guidelines, or if they were sufficiently documented and scientifically acceptable [1].

2.6.1 Toxicity to freshwater organisms

Single species test toxicity data are available for nine different taxonomic groups, i.e. algae, crustaceans, fish, amphibians, macrophytes, annelids, insects, rotifers and molluscs. Short-term acute data (EC50 and LC50 data) are lacking for cyanobacteria and long-term effects no observed effect concentration (NOEC) or EC10 data are not available for insects and annelids.

The search for phenol toxicity data did not yield any field or simulated ecosystem studies describing the effects of phenol on aquatic communities.

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

Figure 2.1 Cumulative distribution function of freshwater long-term data (mg l⁻¹) for phenol

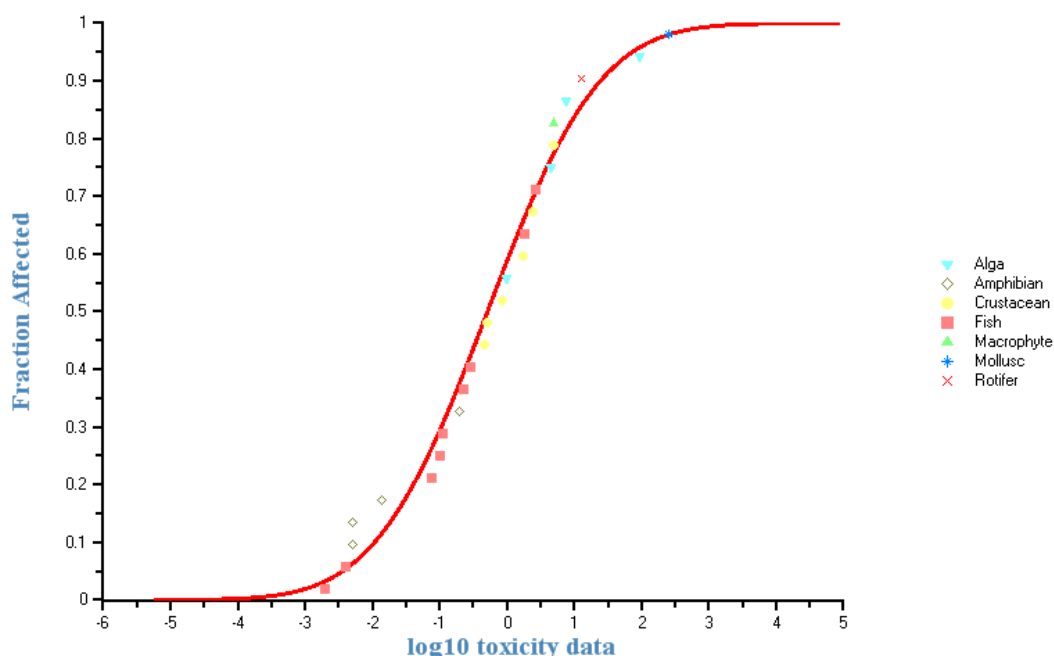


Figure 2.2 Cumulative distribution function of freshwater short-term data (mg l⁻¹) for phenol

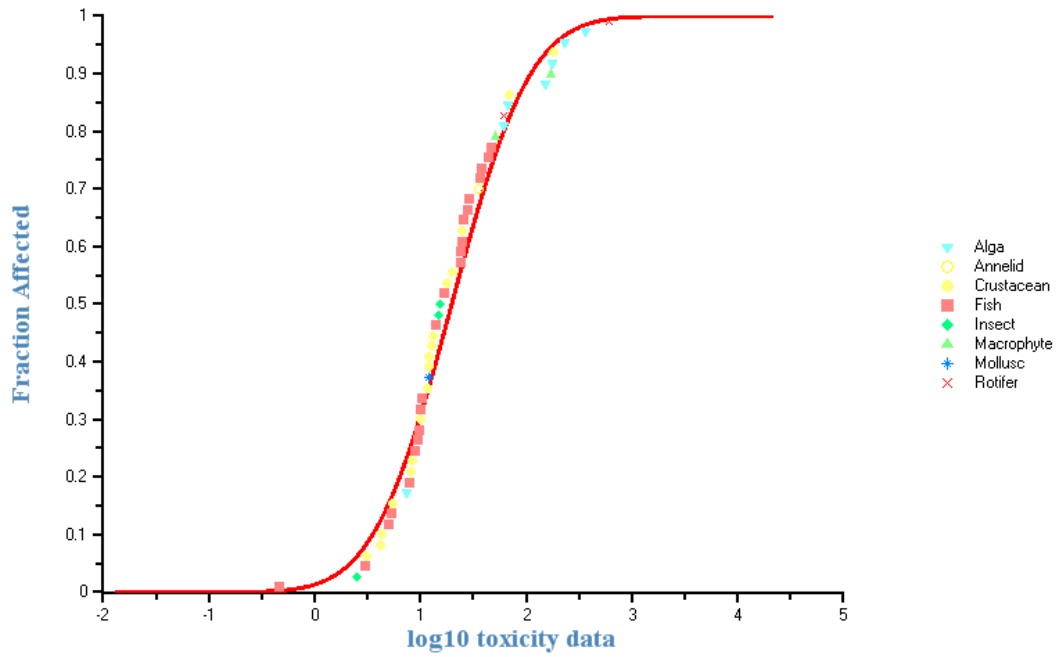


Table 2.6 Long-term aquatic toxicity data for freshwater organisms exposed to phenol

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. ($\mu\text{g l}^{-1}$) ¹	Exposure ²	Toxicant analysis ³	Comments	Reliability index ⁴	Reference
<i>Scenedesmus quadricauda</i>	Green alga	ALG	TGK	GPOP	192 hours	7,500	-	n	TGK = toxic threshold concentration (3% effect compared with control)	RAR	[12]
<i>Selenastrum capricornutum</i>	Green alga	ALG	EC10	GPOP	72 hours	969	s	m	-	2; P	[54]
					48 hours	495					
					24 hours	329					
<i>Microcystis aeruginosa</i>	Cyanobacterium (alga)	CYB (ALG)	TGK	GPOP	192 hours	4,600	-	n	TGK = toxic threshold concentration (3% effect compared with control)	RAR	[12]
<i>Ceriodaphnia dubia</i>	Water flea	CRU	NOEC	MOR	192 hours	840	sr	n	-	RAR; P	[15]
			chronic value	REP	96 days	1,770	-	-	-	RAR	[36]
<i>Daphnia magna</i>	Water flea	CRU	EC10	GRO	16 days	460	sr	n	Animal length as parameter	RAR; P	[19]
<i>Daphnia magna</i>	Water flea	CRU	NOEC	MOR	11 days	500	sr	n	-	RAR	[15]
<i>Daphnia magna</i>	Water flea	CRU	IC10 IC20	REP (PROG)	21 days	2,380 2,910	f	m	-	2; P	[52]
<i>Cirrhina mrigala</i>	larvae 2 day old	FIS	MATC	MOR/ GRO	60 days	77–94	sr	n	NOEC $77 \mu\text{g l}^{-1}$	RAR; P	[56]
<i>Cyprinus carpio</i>	Common carp	FIS	MATC	MOR/ GRO	60 days	110–130	sr	n	NOEC $110 \mu\text{g l}^{-1}$	RAR; P	[55]
<i>Oncorhynchus mykiss</i>	Rainbow trout	FIS	NOEC LOEC	GRO	30 days	100 200	f	m	NOEC derived in RAR	RAR; P	[18]
<i>Oncorhynchus mykiss</i>	Rainbow trout	FIS	EC10	MOR	22–30 days	2	f	m	-	-	[60]
<i>Oncorhynchus mykiss</i>	Rainbow trout	FIS	EC10	MOR	23 days	5	f	m	-	-	[58]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. ($\mu\text{g l}^{-1}$) ¹	Exposure ²	Toxicant analysis ³	Comments	Reliability index ⁴	Reference	
<i>Oryzias latipes</i>	Medaka, high-eyes	FIS	NOEC	GRO	28 days	2,630	-	-	-	-	[27]	
			LOEC	GRO	28 days	5,900						
			MATC	GRO	28 days	3,940						
<i>Pimephales promelas</i>	Fathead minnow	FIS	NOEC LOEC	GRO	30 days	750 2,500	f	m	-	RAR; P	[18]	
<i>Amphimelania holandri</i>	Snail	MOL	-	MOR, HIST	7 days	250,000	sr	n	60% survival (5% mortality during post-exposure period). Degenerative changes of digestive gland	-	[32]	
<i>Brachionus calyciflorus</i>	Rotifer	ROT	NOEC	REP (PROG)	48 hours	12,500	-	-	-	-	[40]	
<i>Lemna minor</i>	Duckweed	MAC	NOEC	GRO	7 days	5,000	-	-	Nominal	RAR	[17]	

¹ The lowest NOECs per taxonomic group are highlighted in bold.

² Exposure: f = flow-through; s = static; sr = static renewal.

³ Toxicant analysis: m = measured; n = nominal.

⁴ The reliability index (RI) is assigned according to the Klimisch Criteria, defined in Annex 1. For data relevant for PNEC derivation, Data Quality Assessment Sheets are available in Annex 1; RAR indicates that the respective study was already quality assessed in the EU RAR on phenol [1] and rated valid; P = published data, a data proforma of the study is available in Annex 2.

ALG = algae; CRU = crustaceans; CYB = cyanobacteria; FIS = fish; MAC = macrophytes; MOL = molluscs; ROT = rotifers

GRO = growth; GPOP = population growth; HIST = histological effects; MOR = mortality; PROG = population rate of growth; REP = reproduction.

MATC = maximum allowable toxicant concentration

LOEC = lowest observed effect concentration

NOEC = no observed effect concentration

EC10 = concentration effective against 10% of the organisms tested

ICx = concentration at which the population effect of the organisms tested is inhibited by X%

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

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. ($\mu\text{g l}^{-1}$) ¹	Exposure ²	Toxicant analysis ³	Comments	Reliability index ⁴	Reference
<i>Chlorella vulgaris</i>		ALG	EC50	GRO	96 hours	370,000	-	n	-	RAR	[45]
<i>Scenedesmus subspicatus</i>	Green alga	ALG	EC50	Chlorophyll	72 hours	229,000	-	-	-	-	[50]
<i>Selenastrum capricornutum</i>	Green alga	ALG	EC50	GRO	8 days	7,500	-	n	-	RAR	[9]
					96 hours	61,100	-	n	-	RAR	[47]
<i>Lumbricus variegatus</i>	Oligochaete worm	ANE	EC50	ITX/IMBL	96 hours	35,600	-	-	-	-	[24]
Amphipoda	Scud order	CRU	EC50	ITX/IMBL	96 hours	8,100	-	-	-	-	[24]
<i>Ceriodaphnia dubia</i>	Water flea	CRU	LC50	MOR	48 hours	3,100	-	-	-	RAR	[38]
			EC50	ITX/IMBL	48 hours	14,000	-	-	-	-	[29]
<i>Daphnia magna</i>	Water flea	CRU	EC50	ITX/IMBL	48 hours	4,200	s	m	-	RAR	[34]
			LC50	MOR	48 hours	8,300	-	-	-	-	[44]
<i>Daphnia obtusa</i>	Water flea	CRU	EC50	ITX/IMBL	48 hours	5,500	-	-	-	-	[42]
<i>Daphnia pulex</i>	Water flea	CRU	EC50	ITX/IMBL	24 hours	17,881	-	-	-	-	[35]
			EC50		48 hours	25,000	-	-	-	-	[51]
<i>Gammarus pulex</i>		CRU	LC50	MOR	96 hours	69,000	f	m	-	RAR	[22]
<i>Oncorhynchus mykiss</i>	Rainbow trout	FIS	LC50	MOR	48 hours	3,000	-	-	-	-	[53]
					96 hours	<u>5,020</u>	sr	-	96-hour GM 8,213 $\mu\text{g l}^{-1}$	RAR	[37]
					96 hours	<u>8,900</u>	f	m	RAR	[18]	
					96 hours	<u>9,700</u>	f	m	RAR	[25]	
					96 hours	<u>10,500</u>	sr	-	RAR	[26]	
<i>Oryzias latipes</i>	Medaka, high-eyes	FIS	LC50	MOR	96 hours	38,300	-	-	-	-	[27]
					48 hours	<u>28,000</u>	-	-	48-hour GM 25,977 $\mu\text{g l}^{-1}$	-	[14]
					48 hours	<u>24,100</u>	-	-	-	-	[41]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. ($\mu\text{g l}^{-1}$) ¹	Exposure ²	Toxicant analysis ³	Comments	Reliability index ⁴	Reference
<i>Pimephales promelas</i>	Fathead minnow	FIS	LC50	MOR	96 hours	<u>27,300</u> <u>24,000</u> <u>24,600</u>	-	-	GM 25,260 $\mu\text{g l}^{-1}$	-	[13]
<i>Poecilia reticulata</i>	Guppy	FIS	LC50	MOR	96 hours	460	sr	n	-	2; P	[43]
<i>Baetis</i> spp.	Mayfly	INS	LC50	MOR	48 hours	1,500	-	-	-	-	[53]
<i>Ichtybotus hudsoni</i>	Mayfly	INS	EC50	ITX/IMBL	96 hours	2,500	s	m	RI 4 because the limited number of individuals available prevented definitive testing. The LC50 is referred to as 'approximate' by the authors.	4	[24]
<i>Sphaerium novaezelandiae</i>	Fingernail clam	MOL	EC50	BEH MOR	96 hours	11,900 243,000	s	m	Effect parameter: self-reburial into sediment within 60 minutes	-	[24]
<i>Lemna gibba</i>	Inflated duckweed	MAC	EC50	REP	7 days	50,819	-	-	-	-	[8]
<i>Brachionus calyciflorus</i>	Rotifer	ROT	EC50	REP	48 hours	62,100	-	-	-	-	[40]

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

² Exposure: f = flow-through; s = static; sr = static renewal.

³ Toxicant analysis: m = measured; n = nominal.

⁴ The reliability index (RI) is assigned according to the Klimisch Criteria, defined in Annex 1. For data relevant for PNEC derivation, Data Quality Assessment Sheets are available in Annex 1; RAR indicates that the respective study was already quality assessed in the EU RAR on phenol [1] and rated valid; P = published data, a data proforma of the study is available in Annex 2.

ALG = algae; ANE = annelids; CRU = crustaceans; FIS = fish; INS = insects; MAC = macrophytes; MOL = molluscs; ROT = rotifers
 BEH = behaviour; GRO = growth; ITX/IMBL = intoxication/immobilisation; MOR = mortality; REP = reproduction
 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

The availability of marine effect data is very poor. Single species toxicity data for marine organisms are available only for four taxonomic groups, i.e. algae, crustaceans, fish and rotifers. No-effect data are available for all of the above mentioned taxa, whereas acute effects data are available only for an alga and some crustacean species.

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

Figure 2.3 Cumulative distribution function of saltwater long-term data (mg l⁻¹) for phenol

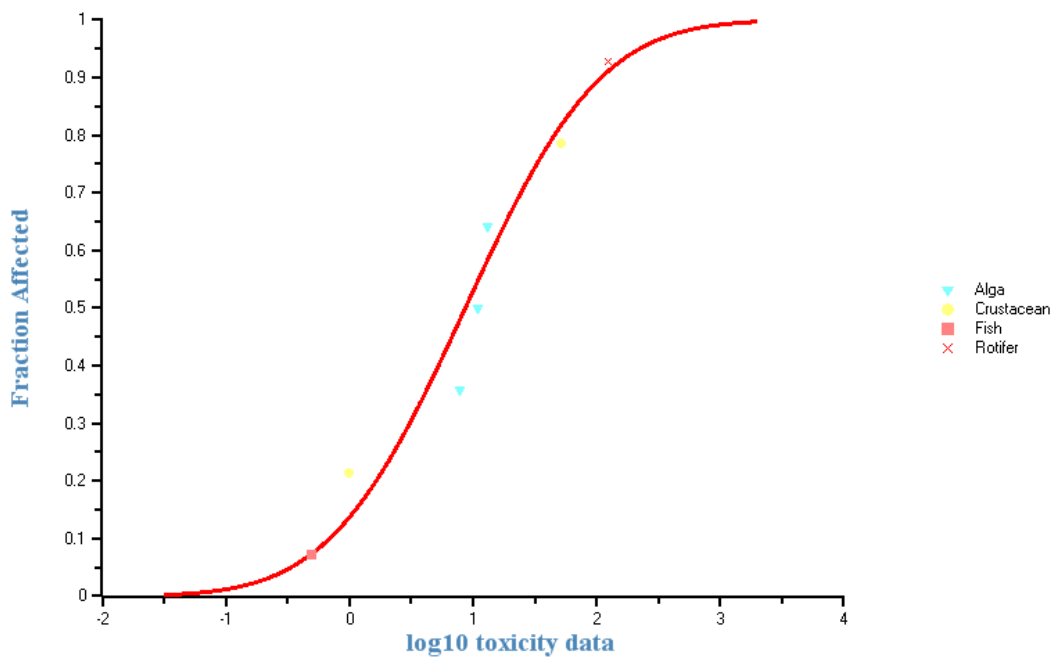


Figure 2.4 Cumulative distribution function of saltwater short-term data (mg l⁻¹) for phenol

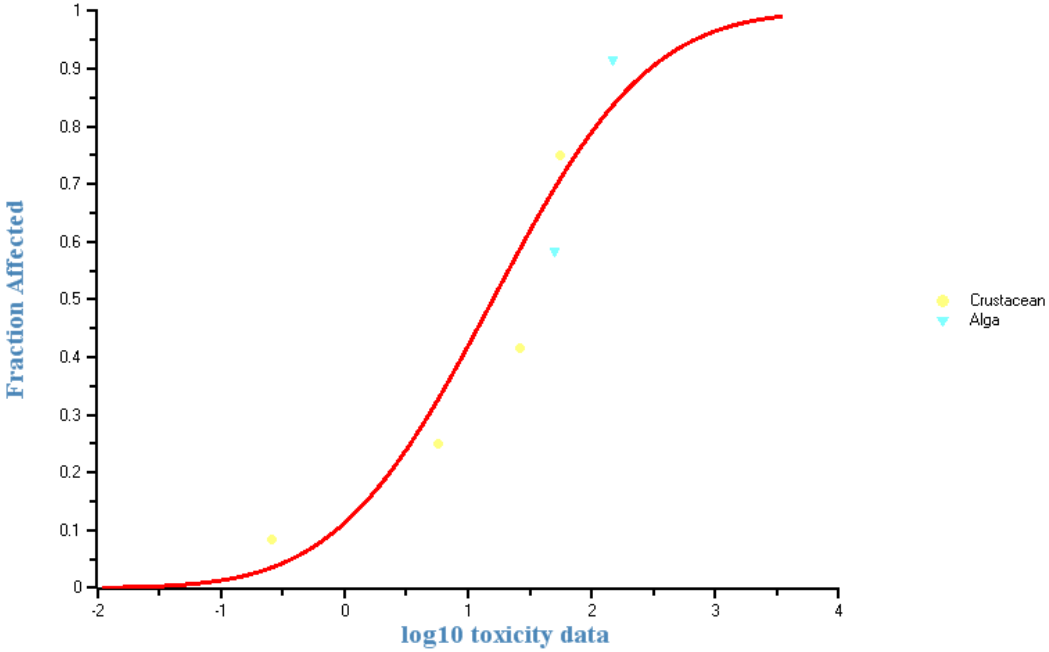


Table 2.8 Long-term aquatic toxicity data for saltwater organisms exposed to phenol

Scientific name	Common name	Taxonomic group	End-point	Effect	Test duration	Conc. ($\mu\text{g l}^{-1}$) ¹	Exposure ²	Toxicant analysis ³	Comments	Reliability index ⁴	Reference
<i>Champia parvula</i>	Red alga	ALG	LOEC MATC	GRO/REP	14 days	7,800 <7,800	sr	n	Growth of tetrasporophytes and number of cystocarps and tetrasporangia produced	2; P	[49]
<i>Gracilaria tenuistipitata</i>	Red alga	ALG	NOEC	GRT	4 days	11,000	-	-	-	-	[23]
<i>Skeletonema costatum</i>	Diatom	ALG	NOEC		120 hours	13,000	-	n	-	RAR	[16]
<i>Balanus amphitrite</i>	Striped barnacle	CRU	NOEC LOEC	BEH	6 days	1,000 10,000	s	n	LOEC already ~50% effect (settlement inhibition)	2; P	[57]
<i>Penaeus japonicus</i>	Kuruma shrimp	CRU	EC10	GRO/BMAS	1 year	52,000	-	-	By means of the regression equation presented in the paper, an EC10 _{biomass} of 52 mg l ⁻¹ can be calculated. Mortality 0% at highest concentration tested (150 mg l ⁻¹).	-	[39]
<i>Mugil auratus</i>	Grey mullet	FIS	NOEC	MOR/BEH	8 days	500	f	m	Fish exposed for 8 days showed no mortality but first signs of neurotoxic symptoms (excited, fast swimming) at 5 mg l ⁻¹ ; at 7.5 mg l ⁻¹ for 8 days 10% mortality and the mentioned signs of neurotoxicity plus sensitivity to light and later depressed activity were observed	2; P	[31]
<i>Brachionus plicatilis</i>	Rotifer	ROT	NOEC	REP	48 hours	12,5000	-	-	-	-	[28]

¹ The lowest NOECs per taxonomic group are highlighted in bold. ² Exposure: f = flow-through; s = static; sr = static renewal. ³ Toxicant analysis: m = measured; n = nominal. ⁴ The reliability index (RI) is assigned according to the Klimisch Criteria, defined in Annex 1. For data relevant for PNEC derivation, Data Quality Assessment Sheets are available in Annex 1; RAR indicates that the respective study was already quality assessed in the EU RAR on phenol [1] and rated valid; P = published data, a data proforma of the study is available in Annex 2.

ALG = algae; CRU = crustaceans; FIS = fish; ROT = rotifers

BEH = behaviour; BMAS = biomass; GRO = growth; GRT = growth rate; MOR = mortality; REP = reproduction

MATC = maximum allowable toxicant concentration

LOEC = lowest observed effect concentration; NOEC = no observed effect concentration

EC10 = concentration effective against 10% of the organisms tested

Table 2.9 Most sensitive short-term aquatic toxicity data for saltwater organisms exposed to phenol

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. ($\mu\text{g l}^{-1}$) ¹	Exposure ²	Toxicant analysis ³	Comments	Reliability index ⁴	Reference
<i>Gracilaria tenuistipitata</i>	Red alga	ALG	EC50	PROG	4 days	149,000	s	-	GM 146,986	-	[23]
						145,000	sr				
<i>Archaeomysis kokuboi</i>	Mysid, juvenile	CRU	LC50	MOR	96 hours	260 560 710	s	-	Salinity 16 ppt Salinity 24 ppt Salinity 32 ppt	4; P	[30]
<i>Artemia</i> sp.	Brine shrimp	CRU	LC50	MOR	48 hours	26,000 38,400 49,400	s	n	Salinity 35 ppt Salinity 25 ppt Salinity 15 ppt	-	[20]
<i>Macrobrachium rosenbergii</i>	Giant river prawn	CRU	LC50	MOR	48 hours	11,830 16,460 19,480	f	-	-	-	[33]
<i>Palaemonetes pugio</i>	Grass shrimp	CRU	LC50	MOR	48 hours 96 hours	11,000 5,800	s	n	-	RAR	[48]

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

² Exposure: f = flow-through; s = static; sr = static renewal.

³ Toxicant analysis: n = nominal.

⁴ The reliability index (RI) is assigned according to the Klimisch Criteria, defined in Annex 1. For data relevant for PNEC derivation, Data Quality Assessment Sheets are available in Annex 1; RAR indicates that the respective study was already quality assessed in the EU RAR on phenol [1] and rated valid; P = published data, a data proforma of the study is available in Annex 2.

ALG = algae; CRU = crustaceans

MOR = mortality; PROG = population rate of growth

EC50 = concentration effective against 50% of the organisms tested

LC50 = concentration lethal to 50% of the organisms tested

ppt = parts per trillion

2.6.3 Toxicity to sediment-dwelling organisms

Toxicity data for phenol concentrations in sediment (mg phenol/kg sediment basis) were not found.

2.6.4 Endocrine-disrupting effects

No data could be located on the effects of phenol on the endocrine system.

2.6.5 Mode of action of phenol

Phenol denatures proteins and is known to disrupt the disulphide bridges of keratin in the skin. *In vitro* studies have shown the formation of the reactive metabolites 4,4'-biphenol and diphenoquinone by neutrophils and activated leukocytes. Both *in vivo* and *in vitro* tests have shown covalent binding of phenol to tissue and plasma protein; some phenol metabolites also bind to proteins, producing coagulation necrosis. The acute lethality of phenol, associated with exposure to high dose concentrations, is commonly attributed to a depressant effect on the central nervous system [5].

2.6.6 Fate and occurrence of relevant metabolites in the aquatic environment

Bacteria play a major role in the degradation of phenol in soil, sediment and water. The number of bacteria capable of utilising phenol is usually a small percentage of the total population present in, for example, a soil sample. However, repeated phenol exposure may result in acclimation.

Phenol may be degraded in its free form as well as after adsorption onto soil or sediment, although the presence of sorbent reduces the rate of biodegradation.

Reported phenol degradation rates suggest rapid aerobic degradation in:

- sewage (typically >90 per cent with an 8-hour retention time);
- soil (typically complete biodegradation in 2–5 days);
- freshwater (typically complete biodegradation in 2–4 days);
- sea water (typically 50 per cent in 9 days).

Anaerobic biodegradation is slower.

Phenol may be completely mineralised by bacteria under aerobic conditions [3] and is considered to be readily biodegradable [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)

The lowest effect values were obtained in tests conducted with *Oncorhynchus mykiss* by Birge and Black and co-workers [58–61]. In these tests, EC10 values in the range of 2–5 $\mu\text{g l}^{-1}$ were calculated for hard water (soft water EC10 = 65 $\mu\text{g l}^{-1}$). In addition, the effect values found in tests conducted by the same authors for several amphibian species are very low (lowest EC10s for exposure 4 days post-hatching = 5–14 $\mu\text{g l}^{-1}$).

Because the effect values found by Birge and Black *et al.* for several organic substances are usually very low compared with those observed by other authors, all the information provided by them was examined carefully, but no plausible reason for the large discrepancies in their data could be found. Nevertheless, it was decided by the EU Experts of the Technical Meeting on Existing Substances that these data were not suitable for the derivation of a PNEC_{aqua}.

In its opinion on the results of the risk assessment on phenol [10], the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) agreed that the data reported by Birge and Black *et al.* are controversial, but criticised the decision of the EU experts to discard them on the grounds that their justification was insufficient. The CSTEE recommended the inclusion of these data in the PNEC derivation unless substantiated scientific evidence was found that the data were invalid or, alternatively, that further information obtained from new testing clarified the true sensitivity of aquatic vertebrates.

This report follows the decision by the Experts of the Technical Meeting on Existing Substances and does not consider the data obtained by Birge and Black *et al.* for PNEC derivation. Reasons for this decision are that neither new toxicity data supporting the findings of Black and Birge *et al.* nor new information on the validity of their data has become available. Furthermore, the decision of the EU experts has been adopted (after the subject was discussed again by a group convening experts from Member States, industry and 'green' NGOs) in the derivation of quality standards for Water Framework Directive Annex X priority substances.

The data and outcomes of the phenol EU RAR [1] have been subject to extensive peer review. The UK is committed to the use of these data for chemical risk assessment purposes and RAR PNECs have also been adopted for the derivation of the Water Framework Directive Annex X EQSs. Consequently, this report adopts the available RAR PNECs as the corresponding proposed PNECs.

3.1.1 PNECs for freshwaters

PNEC accounting for the annual average concentration

Long-term (lt) algal data are available for a number of species. The lowest reliable data are several EC10 (population growth) values for the green alga *Selenastrum capricornutum* [54]. The lowest value obtained was a 24-hour EC10 of 329 µg l⁻¹. Corresponding values of 495 and 969 µg l⁻¹ were reported at 48 and 72 hours, respectively. These data were generated in a static system with measured exposure concentrations.

Crustaceans appear to be of similar sensitivity to algae. The lowest reliable crustacean study reported a 16-day EC10 of 460 µg l⁻¹ for the growth of *Daphnia magna* [19]. This study was based on nominal concentrations, but is regarded as valid by the phenol RAR [1]. Other species of crustacean exhibit similar sensitivity with a 192-hour NOEC of 840 µg l⁻¹ reported in *Ceriodaphnia dubia* [15] in a study classified as valid by the phenol RAR [1].

According to the available long-term data, fish appear to be the most sensitive group (see Table 2.6). Without the data obtained by Birge and Black *et al.* [58–61], the lowest valid NOEC was that reported for *Cirrhina mrigala* [56] (see Table 2.6). Starting with 2-day-old larvae for an exposure period of 60 days, a maximum allowable toxicant concentration (MATC) range of 77–94 µg l⁻¹ was found. The MATC was reported as the range between the NOEC and the LOEC. Hence, the NOEC is 77 µg l⁻¹. This NOEC is supported by a NOEC of 100 µg l⁻¹ for growth of rainbow trout [18] and an MATC of 110–130 µg l⁻¹ for biomass gain of common carp [55]. Both supporting studies were rated as valid by the phenol RAR [1].

The phenol RAR used the following approach to derive the long-term freshwater PNEC. On the basis of the 60-day NOEC of 77 µg l⁻¹ for reduced growth of *Cirrhina mrigala* and given the availability of long-term tests with representatives from algae, crustaceans and fish, the PNEC was derived with an assessment factor of 10:

$$\text{PNEC}_{\text{freshwater_lt}} = 77 \mu\text{g l}^{-1} / \text{AF}(10) = 7.7 \mu\text{g l}^{-1} \text{ phenol}$$

PNEC accounting for transient concentration peaks

Acute short-term (st) toxicity data are available for eight different taxonomic groups, i.e. algae, crustaceans, fish, annelids, molluscs, insects, rotifers and higher plants. Species sensitivities appear to cover a wide range spanning more than two orders of magnitude (Table 2.7). As in the case of long-term effects, fish appear to be the most sensitive species.

There were a number of short-term studies available for freshwater algae. The lowest reported study was an 8-day EC50 (growth) of 7.5 mg l⁻¹ in the green alga *Selenastrum capricornutum* [9]. This study was based on nominal concentrations, but was regarded as valid by the phenol RAR [1]. Shorter exposure periods result in far higher effect concentrations (lower toxicity) with 96-hour EC50s (growth) of 370 and 61.1 mg l⁻¹ reported in *Chlorella vulgaris* and *Selenastrum capricornutum*, respectively [45, 47].

Crustaceans appear to be more sensitive than algae. The lowest reported effect concentration was a 48-hour LC50 of 3.1 mg l⁻¹ for *Ceriodaphnia dubia* [38]. The phenol RAR [1] regarded this study as valid for PNEC derivation. Other species of crustacean

appear to be less sensitive with a 96-hour LC50 of 69 mg l⁻¹ reported for *Gammarus pulex* [22].

The lowest test result considered valid is a 96-hour LC50 of 460 µg l⁻¹ found for guppies (*Poecilia reticulata*) [43]. This test result is six times lower than the next lowest test result for another fish species (rainbow trout, *Oncorhynchus mykiss*, 48-hour LC50 of 3 mg l⁻¹ [53]) and three times lower than the next lowest test result of 1.5 mg l⁻¹ for mortality of the mayfly *Baetis* sp. [53]. This study was based on a semi-static regime (12 hours renewal) using nominal concentrations. However, previous studies by the same authors indicated that during a 12-hour static period, phenols remained within 95 per cent of the nominal concentration when under continuous aeration. The chemical properties of phenol (low volatilisation, little or no hydrolysis) support the theory of limited losses within short timescales. Biodegradation studies indicate half-lives of 28–173 hours for phenols in natural waters [21]. In comparison, biodegradation rates in laboratory waters are likely to be even slower. Consequently, the guppy value is classified as suitable for PNEC derivation.

The phenol RAR [1] did not include the guppy study identified above. It also did not set a short-term PNEC for phenol. The lowest reliable data point identified by the phenol RAR was the 48-hour LC50 of 3.1 mg l⁻¹ l in *Ceriodaphnia dubia*. Based on this datum, a short-term PNEC of 310 µg l⁻¹ [3,100/AF(10)] could be set, given that short-term data are available for the full base set. However, given the availability of the lower guppy data point (*Poecilia reticulata*, 96-hour LC50 of 460 µg l⁻¹) the following PNEC is proposed:

$$\text{PNEC}_{\text{freshwater_lt}} = 460 \mu\text{g l}^{-1}/\text{AF (10)} = 46 \mu\text{g l}^{-1} \text{ phenol}$$

As fish are the most sensitive species in both short-term and long-term exposures, and as data are available for algae, invertebrates and fish, it is recommended that a reduced assessment factor of 10 (instead of 100) be used.

3.1.2 PNECs for saltwaters

The effects dataset for marine species is very small, comprising tests with algae, crustaceans, rotifers and fish species. For short-term data, only E(L)C50 data for one alga species and four crustacean species are available (see Tables 2.8 and 2.9).

The toxicity data for marine taxa do not differ obviously from the range of values obtained for freshwater taxa (see Tables 2.6–2.9). However, the marine database is too small to draw firm conclusions on possible differences. Despite the fact that the bioavailability and the toxicity of phenol in freshwater and saltwater might be (slightly) different due to differences in dissociation and ionisation, the available data suggest that the two datasets could be combined.

This proposal is corroborated by Wheeler *et al.* [11], who investigated the suitability of using acute freshwater data to extrapolate to saltwater effects by examining differences between species sensitivity distributions (SSDs) of freshwater and saltwater species. For phenol, they found differences in the SSDs in the upper tails (i.e. the more tolerant species), whereas greater congruence was seen among the more sensitive species. The HC5 values of the SSDs differed by up to a factor of two, depending on the model fitted [7 or 46], with the saltwater species showing greater sensitivity. The main taxa were present in the datasets, though saltwater annelid and platyhelminth data were lacking.

It can therefore be concluded that, in the case of phenol, freshwater data are likely to be adequately protective of saltwater organisms.

PNEC accounting for the annual average concentration

The marine effects dataset is too small to be used alone for derivation of an annual average PNEC for the marine pelagic community. Moreover, as differences in the sensitivity of freshwater and saltwater species belonging to the same taxonomic groups appear to be small, it is recommended that the freshwater and saltwater datasets are combined (see above).

The lowest available long-term data point for microalgae was a 120-hour NOEC of 13 mg l⁻¹ for the diatom *Skeletonema costatum* [16]. This value was regarded as valid by the phenol RAR [1]. In addition, data are available for saltwater macroalgae that indicate higher sensitivity, with a 14-day MATC (reproduction) of <7.8 mg l⁻¹ in the red alga *Champia parvula* [49].

Saltwater crustaceans appear to be more sensitive than algae, with a 6-day NOEC of 1 mg l⁻¹ for changes in the behaviour of the barnacle *Balanus amphitrite* [57]. Additional crustacean data are available for the shrimp, *Penaeus japonicus*, with a 1-year EC10 of 52 mg l⁻¹ [39].

As with the freshwater environment, fish appear to be the most sensitive organisms to long-term exposure to phenol. An 8-day NOEC (mortality) of 500 µg l⁻¹ was reported in the grey mullet (*Mugil auratus*) [31]. These data were generated in a flow-through study with measured exposure concentrations and are suitable for PNEC derivation.

The lowest NOEC available in the combined freshwater and saltwater database is the same as used for the derivation of the freshwater annual average PNEC (60-day NOEC of 77 µg l⁻¹ for mortality and biomass gain of the fish species *Cirrhina mrigala* [56], see Section 3.1.1). This study was also used in the EU RAR [1] to derive the PNEC_{aqua}. No specific saltwater PNEC was derived in the phenol RAR.

The NOEC of 77 µg l⁻¹ would normally be divided by an assessment factor of 100 according to TGD [6] provisions for marine effects assessment, which are applicable when three long-term tests for freshwater or saltwater species representing the three groups algae, crustaceans and fish are available. However, this standard assessment factor can be reduced to 10 if short-term tests on marine species (e.g. molluscs and echinoderms) are available. Such studies should indicate that these species do not belong to the most sensitive group. It should also be determined, with high probability, that long-term NOECs generated for these marine groups would not be lower than those already obtained.

Additional short-term tests are available for freshwater species belonging to relevant groups of annelids and molluscs. Furthermore, there are reproduction tests with saltwater and freshwater rotifer species available indicating that these groups are not the most sensitive to phenol (see Tables 2.6–2.8). It therefore seems unlikely that long-term tests with representatives of these additional taxonomic groups would result in lower chronic toxicity data than obtained for fish. Consequently, only a reduced assessment factor of 10 need be used to obtain the PNEC_{saltwater_lt} on the basis of the NOEC of 77 µg l⁻¹ found for the fish species *Cirrhina mrigala*:

$$\text{PNEC}_{\text{saltwater_lt}} = \text{PNEC}_{\text{freshwater_lt}} = 77 \mu\text{g l}^{-1}/\text{AF (10)} = 7.7 \mu\text{g l}^{-1} \text{ phenol}$$

PNEC accounting for transient concentration peaks

Data for marine macroalgae indicate low sensitivity under short-term exposures to phenol, with a 4-day EC50 (growth rate) of 149 mg l⁻¹ reported in the red alga *Gracilaria tenuistipitata* [23].

Marine crustaceans appear to be far more sensitive to phenol. If subjected to osmotic stress, juveniles of the saltwater crustacean *Archaeomysis kokuboi* seem to be more sensitive to phenol exposure than the most sensitive freshwater organisms [30], though the sensitivity of this species appears to be less pronounced at 'normal' salinity. The 96-hour LC50 of *Archaeomysis kokuboi* was reported to range from 260 to 710 μg l⁻¹, depending on salinity (16–32 ppt; see Table 2.9). In contrast, the lowest reported acute effect value for freshwater crustaceans is 3.1 mg l⁻¹ (*Ceriodaphnia dubia* 48-hour LC50, see Table 2.7).

It was not possible to locate short-term saltwater data for fish. The lowest LC50 reported for a freshwater species is the 96-hour LC50 of 460 μg l⁻¹ for guppies (*Poecilia reticulata*) [43].

The quality of the study and data reported by Kim and Chin [30] for *Archaeomysis kokuboi* could not be assessed because the main text of the publication is written in Korean with only the abstract in English (the reported data are taken from the abstract). Assuming that the study results are reliable, they show that an additional stressor such as osmotic stress has a marginal effect on the sensitivity of *Archaeomysis kokuboi* in its most sensitive life stage. Therefore, a minimum assessment factor of 10 on the LC50 obtained at normal salinity would cover the risk of additional osmotic stress.

It is therefore recommended that the lowest validated acute test result available for the derivation of the MAC-PNEC saltwater be used. This is the LC50 of 460 μg l⁻¹ reported for 96-hour exposure of *Poecilia reticulata* to phenol.

The TGD does not provide specific guidance on the assessment of acute effects from intermittent releases to marine water bodies. Therefore, it is recommended that the PNEC for short-term exposure to phenol is based on general guidance given in the TGD on effects assessment for intermittent releases (Section 3.3.2 of Part II of the TGD [6]). As fish are the most sensitive species in both short-term and long-term exposures, and as data are available for algae, invertebrates and fish, it is recommended that a reduced assessment factor of 10 (instead of 100) be used:

$$\text{PNEC}_{\text{saltwater_st}} = \text{PNEC}_{\text{freshwater_st}} = 460 \mu\text{g l}^{-1}/\text{AF (10)} = 46 \mu\text{g l}^{-1} \text{ phenol}$$

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

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

3.3 Derivation of existing EQSs

In the 1995 report [75], fish and crustaceans were the most sensitive freshwater taxonomic groups, although the reliability of the more sensitive fish species was questionable. Therefore, the acute 48-hour LC50 of 3.1 mg l⁻¹ for the water flea *Ceriodaphnia dubia* was used to derive the freshwater long-term standard. Owing to the lack of reliable chronic data, an assessment factor of 100 was applied to this datum to give an EQS of 30 µg l⁻¹ expressed as an annual average for the protection of freshwater life.

The same datum was used to derive the freshwater short-term value by applying an assessment factor of 10 to give an EQS of 300 µg l⁻¹ expressed as a maximum allowable concentration.

There were too few data at that time to derive a standard for the protection of saltwater life. However, the acute toxicity of phenol to saltwater organisms appeared to be the same as that reported for freshwater organisms and saltwater fish displayed a similar concentration-dependent pattern of characteristic neurotoxic symptoms as freshwater species. Therefore, a standard in the same range as those for the protection of freshwater life were recommended as sufficient and the freshwater standards were 'read across' for the protection of saltwater life, i.e. 30 µg l⁻¹ expressed as an annual average and 300 µg l⁻¹ expressed as a maximum allowable concentration.

3.4 Derivation of PNECs for sediment

The derivation of specific PNECs for the protection of benthic communities in freshwater and saltwater environments is not necessary because the trigger value (log K_{ow} ≥ 3) is not met in the case of phenol.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

Phenol is readily absorbed through all routes, such as the lungs, intact and abraded skin, and the gastrointestinal tract of both humans and animals. It has moderate acute toxicity for mammals. Oral LD50 values in rodents range from 300 to 600 mg phenol/kg body weight [3].

Toxic effects in rat kidney have been reported to occur at oral dose levels of 40 mg/kg per day or more. Liver toxicity was evident in rats administered at least 100 mg/kg per day. In a limited 14-day study in rats, an oral no observed adverse effect level (NOAEL) of 12 mg/kg per day was reported, based on kidney effects [3]. In this study, miosis (an iris response to light) was inhibited at 4 mg/kg per day. However, the significance of this finding is not clear. Some biological changes were reported to occur in the intestinal mucosa and kidneys of mice at dose levels below 1 mg/kg per day, though this finding is of uncertain toxicological significance [3].

There are no adequate studies on the reproductive toxicity of phenol, although phenol has been identified as a developmental toxicant in studies with rats and mice. In two multiple dose rat studies, NOAEL values of 40 mg/kg per day [the lowest observed adverse effect level (LOAEL) was 53 mg/kg per day] and 60 mg/kg per day (the LOAEL was 120 mg/kg per day) have been reported [3]. In the mouse, the NOAEL was 140 mg/kg per day (the LOAEL was 280 mg/kg per day) [3].

The majority of bacterial mutagenicity tests have given negative results [3]. Mutations, chromosomal damage and DNA effects have been observed in mammalian cells *in vitro*. Induction of micro-nuclei in bone marrow cells of mice has been observed in some studies. No micronuclei were observed in mice studies at lower doses [3].

Two carcinogenicity studies have been conducted with male and female rats and mice receiving phenol in their drinking water [3]. Malignancies (e.g. C-cell thyroid carcinoma, leukaemia) were seen only in low-dose male rats. No adequate dermal or inhalation carcinogenicity studies have been conducted [3]. Two-stage carcinogenicity studies have shown that phenol, applied repeatedly to mouse skin, has promoting activity [3].

No avian toxicity data were found for phenol.

3.5.2 PNECs for secondary poisoning of predators

The derivation of specific PNECs for the protection of predators against secondary poisoning by phenol is not necessary because the trigger values, i.e. bioconcentration factor (BCF) ≥ 100 or biomagnification factor (BMF) > 1 , are not met. In addition, the substance will be completely metabolised or excreted if taken up orally by vertebrates. The bioaccumulation potential of phenol is considered to be low [1, 3].

4. Analysis and monitoring

The accuracy and sensitivity of phenol determination in environmental samples depends on:

- sample preconcentration and pretreatment
- the analytical method employed.

The two preconcentration methods commonly used for phenols in water are:

- adsorption on XAD resin
- adsorption on activated carbon.

Both can give low recoveries [74] owing to the high solubility of phenol. Solvent extraction at acidic pH with subsequent solvent concentration also provides low recoveries for phenol, with up to 60 per cent loss from evaporation [70].

Consequently, acetylation with subsequent solvent extraction [72] is considered a more reliable method of pretreatment, prior to separation by gas chromatography (GC) [64, 71, 72] and flame ionisation detection (FID) or mass spectrometry (MS).

For spectrophotometric methods, the following limits of detection (LODs) are achievable:

- $\sim 1 \mu\text{g l}^{-1}$ in drinking water using GC-FID [62];
- $0.14 \mu\text{g l}^{-1}$ in water and wastewater using high performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection [65];
- $0.05 \mu\text{g l}^{-1}$ in water using GC-MS [63];
- $1.5\text{--}10 \mu\text{g l}^{-1}$ in groundwater [66, 68, 69].

Solid phase extraction GC-MS is also reported to be a sensitive method for water samples, providing an LOD of $0.58 \mu\text{g l}^{-1}$ [67].

HPLC separation with electrochemical detection may provide the best sensitivity for phenol quantification in biological samples, although GC-FID may be a more versatile method if other non-ionic pollutants have to be quantified.

The advantages and disadvantages of different methods available for the quantification of phenol and metabolites in biological and environmental samples are discussed by Tesarova and Packova [73]. Limits of detection of approximately 0.3 mg/kg have been reported for these media [70, 74].

The lowest proposed PNEC derived for phenol is $7.7 \mu\text{g l}^{-1}$. To provide adequate precision and accuracy, the data quality requirements are that, at a third of the EQS, the total error of measurement should not exceed 50 per cent. From the literature, it can be seen that analytical methodologies provide detection limits as low as $0.05 \mu\text{g l}^{-1}$, which suggests that current analytical methodologies offer adequate performance for the analysis of phenol for compliance with the derived PNECs for water.

5. Conclusions

5.1 Availability of data

A substantial number of laboratory toxicity data are available for nine different freshwater taxonomic groups (algae, crustaceans, fish, cyanobacteria, higher plants, annelids, insects, rotifers and molluscs). Although the freshwater chronic data are less extensive, the dataset provides coverage of seven of these taxa.

By contrast, saltwater toxicity data are available only for four taxonomic groups (algae, crustaceans, fish and rotifers), with acute data restricted to studies on algae and crustaceans.

There are no field or mesocosm data available for phenol.

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 no effect concentrations (2–65 $\mu\text{g l}^{-1}$) were obtained in chronic studies with rainbow trout, *Oncorhynchus mykiss*, and several amphibian species. However, in their review of the data considered for derivation of PNECs, Experts of the Technical Meeting on Existing Substances rejected these data because they were considered outliers and there were no corroborating data or any new evidence to validate them.

Fish appear to be the most sensitive group. The most sensitive fish species was *Cirrhina mrigala* in a reliable 60-day study that resulted in a NOEC of 77 $\mu\text{g l}^{-1}$. This value is supported by similar concentrations based on reliable studies of the effects of phenol on growth of rainbow trout and common carp. Because representatives of algae, crustaceans and fish are available in the chronic dataset, an assessment factor of 10 applied to this NOEC is recommended, leading to a PNEC_{freshwater_lt} of 7.7 $\mu\text{g l}^{-1}$.

This PNEC is four times lower than the existing EQS of 30 $\mu\text{g l}^{-1}$. This reflects new data that have become available since the original EQS was derived: in the absence of chronic data, the existing EQS was based on an assessment factor of 100 applied to an acute LC50 for the water flea, *Ceriodaphnia dubia* (3.1 mg l^{-1}).

5.2.2 Short-term PNEC for freshwaters

A wide range of species sensitivities is evident following acute exposures to phenol but, as with chronic data, fish are the most sensitive taxonomic group. The lowest reliable datum, by a considerable margin, is a 96-hour LC50 of 460 $\mu\text{g l}^{-1}$ for guppy (*Poecilia reticulata*). The draft RAR for phenol did not include the guppy study identified above and did not set a short-term PNEC. There is evidence that (a) fish are the most sensitive taxonomic group to phenol and (b) phenol gives rise to a large ratio between acute and chronic toxicity, suggesting the need for a substantial margin between the long-term and short-term PNECs.

Together, these indicate that applying a reduced assessment factor (from 100 to 10) to this acute LC50 can be justified as the basis for a PNEC. This gives a $\text{PNEC}_{\text{freshwater_st}}$ of $46 \mu\text{g l}^{-1}$.

The proposed PNEC is about five times lower than the existing EQS of $300 \mu\text{g l}^{-1}$. This is entirely a consequence of new data that have become available since the original EQS was derived: the existing EQS was based on an assessment factor of 10 applied to an acute LC50 for the water flea, *Ceriodaphnia dubia* (3.1 mg l^{-1}).

5.2.3 Long-term PNEC for saltwaters

Marine taxa appear to share a similar distribution of sensitivities to their freshwater counterparts. Consequently, for the purposes of PNEC derivation, the two datasets may be combined. Support for this approach is provided by separate research into comparisons of species sensitivities in freshwater and saltwater organisms. This shows similar species sensitivity distributions for acute data (at least in the lower 'tail' of the distribution where the most sensitive species are to be found) and indicates that a PNEC based on freshwater data should protect saltwater species.

In chronic studies with marine species, fish appear to be the most sensitive organisms to long-term exposure to phenol. A reliable 8-day NOEC of $500 \mu\text{g l}^{-1}$ was reported in the grey mullet (*Mugil auratus*), but the lowest NOEC available in the combined freshwater and saltwater database is the same as used for the derivation of the $\text{PNEC}_{\text{freshwater_lt}}$ (60-day NOEC of $77 \mu\text{g l}^{-1}$ in the fish species *Cirrhina mrigala*).

No specific saltwater PNEC was derived in the phenol RAR. According to Annex V of the WFD, the NOEC of $77 \mu\text{g l}^{-1}$ would normally be divided by an assessment factor of 100. However, additional short-term tests are available for freshwater species belonging to relevant groups of annelids and molluscs. Reproduction tests with saltwater and freshwater rotifer species indicate these taxa are not the most sensitive to phenol. It therefore seems unlikely that long-term tests with representatives of these additional taxonomic groups would result in lower chronic toxicity data than those obtained for fish. Consequently, a reduced assessment factor of 10 applied to the fish NOEC of $77 \mu\text{g l}^{-1}$ is justified, resulting in the same PNEC as that in freshwater, i.e. $\text{PNEC}_{\text{saltwater_lt}} = \text{PNEC}_{\text{freshwater_lt}}$ of $7.7 \mu\text{g l}^{-1}$.

The existing EQS of $30 \mu\text{g l}^{-1}$ was 'read across' from the corresponding freshwater EQS.

5.2.4 Short-term PNEC for saltwaters

There are indications that marine crustaceans are particularly sensitive to phenol (acute LC50 values of $260\text{--}710 \mu\text{g l}^{-1}$), but shortcomings in reporting mean these suggestions cannot be validated. There are no reliable short-term data for saltwater fish but, applying the logic outlined above, combining the freshwater and saltwater datasets can be justified. As a result, the lowest acute effect concentration would be the 96-hour LC50 of $460 \mu\text{g l}^{-1}$ for guppy (*Poecilia reticulata*).

Because Annex V does not provide specific guidance on the derivation of short-term PNECs in the marine environment, it is recommended that the $\text{PNEC}_{\text{saltwater_st}}$ be based on an assessment factor of 10 applied to the guppy 96-hour LC50. This factor is justifiable on the assumption that (a) fish are the most sensitive species to both short-term and long-term exposure and (b) the substantial acute to chronic toxicity ratio for fish

encourages a substantial margin between short-term and long-term PNECs for phenol. This results in a $PNEC_{\text{saltwater_st}} = PNEC_{\text{freshwater_st}}$ of $46 \mu\text{g l}^{-1}$.

The existing EQS of $300 \mu\text{g l}^{-1}$ was also 'read across' from the corresponding freshwater EQS.

5.2.5 PNEC for sediments and secondary poisoning

Since phenol does not partition preferentially into sediment and does not bioaccumulate to any significant extent, there is no justification for deriving sediment PNECs or PNECs to protect against risks of secondary poisoning to mammals and birds.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC ($\mu\text{g l}^{-1}$)	Existing EQS ($\mu\text{g l}^{-1}$)
Freshwater/long-term	7.7	30
Freshwater/short-term	46	300
Saltwater/long-term	7.7	30
Saltwater/short-term	46	300

5.3 Analysis

The lowest proposed PNEC for phenol is $7.7 \mu\text{g l}^{-1}$. The data quality requirements are that, at a third of the EQS, the total error of measurement should not exceed 50 per cent. Based on this, current analytical methodologies provide detection limits as low as $0.05 \mu\text{g l}^{-1}$, which suggests that they would be adequate for assessing compliance with the proposed PNECs for water.

5.4 Implementation issues

The proposed PNECs are recommended for adoption as EQSs without the need for further investigation.

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List of abbreviations

AA	annual average
AF	assessment factor
BCF	bioconcentration factor
CAS	Chemical Abstracts Service
EC50	concentration effective against 50% of the organisms tested
ECx	concentration effective against X% of the organisms tested
ELS	early life stage
EQS	Environmental Quality Standard
FID	flame ionisation detection
GC	gas chromatography
GLP	Good Laboratory Practice (OECD)
HPLC	high performance liquid chromatography
HSDB	Hazardous Substances Data Bank
ICx	concentration at which the population effect of the organisms tested is inhibited by X%
IRIS	Integrated Risk Information System
IUPAC	International Union of Pure and Applied Chemistry
LC50	concentration lethal to 50% of the organisms tested
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOEC	lowest observed effect concentration
lt	long term
MAC	maximum allowable concentration
MATC	maximum allowable toxicant concentration
MS	mass spectrometry
NGO	non-governmental organisation
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PNEC	predicted no-effect concentration
ppt	parts per trillion
RAR	Risk Assessment Report
SEPA	Scottish Environment Protection Agency
SNIFFER	Scotland & Northern Ireland Forum for Environmental Research

SSD	species sensitivity distribution
st	short term
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 reference number (see References & Bibliography).

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

Table A1 Klimisch Criteria*

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

* Klimisch H-J, Andreae M and Tillmann U, 1997 *A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data*. Regulatory Toxicology and Pharmacology, **25**, 1–5.

** OECD Principles of Good Laboratory Practice (GLP). See:

http://www.oecd.org/departement/0,2688,en_2649_34381_1_1_1_1_1.00.html

Reference number	18
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Information on the test species	
Test species used	<i>Oncorhynchus mykiss</i> <i>Pimephales promelas</i>
Source of the test organisms	State of Wyoming Game and Fish Department
Holding conditions prior to test	<i>O. mykiss</i> : newly fertilised eggs (5 hours post-fertilisation) were incubated in well water (11°C) until initiation of experiment <i>P. promelas</i> : newly fertilised eggs from a fathead minnow brook stock maintained at 25±°C.
Life stage of the test species used	<i>O. mykiss</i> : eyed eggs <i>P. promelas</i> : embryo-larvae

Information on the test design	
Methodology used	
Form of the test substance	Phenol
Source of the test substance	Not stated
Type and source of the exposure medium	Laboratory well water
Test concentrations used	Seven plus control
Number of replicates per concentration	<i>O. mykiss</i> : not stated <i>P. promelas</i> : 10–13
Number of organisms per replicate	<i>O. mykiss</i> : 100 <i>P. promelas</i> : 50
Nature of test system (static, semi-static or flow through, duration, feeding)	Flow-through; feeding
Measurement of exposure concentrations	Yes, analysis in freshly prepared stock solutions used for dilution.
Measurement of water quality parameters	pH, temperature, dissolved oxygen, free CO ₂ , hardness, conductivity
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference number	30
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Information on the test species	
Test species used	<i>Archaeomysis kokuboi</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Adults, juveniles

Information on the test design	
Methodology used	Static bioassay
Form of the test substance	Phenol
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Six plus control
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow through, duration, feeding)	Static test system, 96-hour exposure of test organisms
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Only the abstract of the paper could be read and evaluated. The main text is in Korean.

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

Reference number	31
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Information on the test species	
Test species used	<i>Mugil auratus</i>
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Fish 22–25 cm long and weighing ~125 g

Information on the test design	
Methodology used	Fish test
Form of the test substance	Phenol
Source of the test substance	Not stated
Type and source of the exposure medium	Test were carried out in aerated 200-litre aquaria in continuously flowing sea water (about 1,000 litres per day) at constant temperature ($12\pm 0.5^{\circ}\text{C}$) and a salinity of 37.5 ± 0.3 ppt. Phenol was added with a dosing apparatus and the toxicant concentration in the aquaria was analysed twice per day.
Test concentrations used	Five (0.5 , 5 , 7.5 , 10 and 25 mg l^{-1}) plus control
Number of replicates per concentration	Not stated
Number of organisms per replicate	5
Nature of test system (static, semi-static or flow through, duration, feeding)	Flow-through, 8 days; feeding of test animals not mentioned
Measurement of exposure concentrations	Yes, twice per day
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good – moderate (description of test conditions and history of the test animals a bit poor; behavioural abnormalities: no standard test parameter and difficult to quantify).

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference number	43
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Information on the test species	
Test species used	<i>Poecilia reticula</i>
Source of the test organisms	Laboratory stock culture
Holding conditions prior to test	Guppies were reared in an aquarium room with a photoperiod of 16 hours light/8 hours dark. The pH of water was maintained at 6.5–7.5 and the temperature at 26±1°C. Commercial dry food (Vitacraft®) was used for feeding.
Life stage of the test species used	Individuals weighting 40–60 mg were used for testing.

Information on the test design	
Methodology used	Acute fish test
Form of the test substance	Phenol (p.a.)
Source of the test substance	Merck
Type and source of the exposure medium	Dechlorinated local tap water, which contained 25–30 ppm Ca and 3.5–5.5 ppm Mg (corresponding to a total hardness of 80–100 ppm as CaCO ₃). pH was adjusted from the original value of 8 to the desired value using HCl and NaHCO ₃ . Dissolved oxygen remained >90% of saturation.
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow through, duration, feeding)	5-litre glass jars; static renewal (replacement of 80% of test medium every 12 hours); no feeding during test
Measurement of exposure concentrations	Preceding analyses indicated that, during the 12-hour static period, the concentration of any compound tested did not decline by more than 5% of the initial value despite constant aeration.
Measurement of water quality parameters	pH, temperature, dissolved oxygen
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated

Overall comment on quality	Good. In the paper, the test organisms and test procedure are not described in detail but reference is made to another publication from which these details were taken.*
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Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

* Saarikoski J and Viluksela M, 1981 *Influence of pH on the toxicity of substituted phenols to fish*. Archives of Environmental Contamination and Toxicology, **10**, 747–753.

Reference number	49
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Information on the test species	
Test species used	<i>Champia parvula</i>
Source of the test organisms	Unialgal stock cultures of males, females and tetrasporophytes
Holding conditions prior to test	Unialgal stock cultures of males, females and tetrasporophytes were maintained in 1000 ml aerated Erlenmeyer flasks in a growth medium partially described in the paper or in references cited. The medium contained nitrate-N, dihydrogenphosphate, iron ⁺ in the form of chloride salt and vitamins (e.g. B12, biotin and thiamine) in filtered sea water. The flasks were illuminated with 75–80 $\mu\text{E m}^{-2} \text{s}^{-1}$ for 16 hours/day with cool white fluorescent light. Temperature was 22–24°C and salinity 30 ppt. Media were changed once each week.
Life stage of the test species used	2–3 mm branch tips of the life history stage tested (females, tetrasporophytes)

Information on the test design	
Methodology used	Algae test
Form of the test substance	Phenol dissolved in deionised water
Source of the test substance	Not stated
Type and source of the exposure medium	Test medium had ¼ strength of the medium used for the stock cultures. To 400 ml of test medium in screw-capped 500 ml Erlenmeyer flasks, 150 mg l ⁻¹ sodium bicarbonate was added. Flasks were shaken at 100 rpm. Media were changed on days 7 and 11 of a test. All other conditions were the same as for the stock cultures (see holding conditions prior to test). Tests lasted 11–14 days.
Test concentrations used	Six plus control (dilution factor 0.6)
Number of replicates per concentration	Only stated that replicate flasks were used but not the number. However, two complete test series were run.
Number of organisms per replicate	Five branch tips of 2–3 mm length per life history stage (females, tetrasporophytes) tested (a 1-cm male branch producing spermatia was added to the female tips).
Nature of test system (static, semi-static or flow through, duration, feeding)	Semi-static (replacement of test media on days 7 and 11 for exposures lasting 11–14 days). Plants were exposed in the growth medium described.
Measurement of exposure concentrations	No, test results refer to nominal concentrations

Measurement of water quality parameters	Test media checked before beginning of the test, but not during the test.
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good (however, no analysis of exposure concentrations despite relatively long change intervals for the media).

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference number	52
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Information on the test species	
Test species used	<i>Daphnia magna</i>
Source of the test organisms	Laboratory culture of <i>D. magna</i> obtained from the Department of Biology, University of Ljubljana.
Holding conditions prior to test	The daphnia were cultured at 21±1°C in 3-litre glass aquaria. Illumination 12 hours per day with 1,800 lux. Replacement of water once per week. Start with 20 daphnids per aquarium. Daphnids were fed a diet of dry yeast and the algae <i>Scenedesmus subspicatus</i> every Monday, Wednesday and Friday.
Life stage of the test species used	Neonates about 24 hours old

Information on the test design	
Methodology used	Daphnia reproduction test similarly to the test design reported by OECD 1982*
Form of the test substance	Phenol
Source of the test substance	Not stated
Type and source of the exposure medium	Clean surface water having a total hardness of 241 mg l ⁻¹ CaCO ₃ , an alkalinity of 236 mg l ⁻¹ CaCO ₃ and a pH of 8.3.
Test concentrations used	Four plus control
Number of replicates per concentration	4
Number of organisms per replicate	5
Nature of test system (static, semi-static or flow through, duration, feeding)	Flow-through; feeding with <i>Scenedesmus subspicatus</i> three times a week at 2.5 mg dry weight per litre test medium.
Measurement of exposure concentrations	Yes – analysis in freshly prepared test solutions
Measurement of water quality parameters	pH, temperature, dissolved oxygen
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	good

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

* Organisation for Economic Co-operation and Development (OECD), 1982 *Daphnia magna*, reproduction test. TG 202B. Paris: OECD.

Reference number	54
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Information on the test species	
Test species used	<i>Selenastrum capricornutum</i> (CCAP 278/4)
Source of the test organisms	Culture Collection of Algae and Protozoa (CCAP), Natural Environment Research Council, Cambridge, UK
Holding conditions prior to test	Semi-continuously grown in a growth cabinet at 23±2°C in synthetic algal nutrient medium (reference for composition given). Continuous light at 300 µmol photon m ² s ⁻¹ was supplied by cool white fluorescent tubes.
Life stage of the test species used	Cells in the exponential growth phase

Information on the test design	
Methodology used	Alga test
Form of the test substance	Phenol
Source of the test substance	Not stated
Type and source of the exposure medium	Synthetic algal nutrient medium (reference for composition given), buffered, pH 7.5±0.5.
Test concentrations used	0 (control) , 0.001, 0.01, 1, 10, 100 mg l ⁻¹
Number of replicates per concentration	Not stated
Number of organisms per replicate	10 ⁴ ml ⁻¹
Nature of test system (static, semi-static or flow through, duration, feeding)	Static; algae grown in nutrient solution and at 300 µmol photon m ² s ⁻¹ .
Measurement of exposure concentrations	Yes – samples taken immediately after addition of toxicant.
Measurement of water quality parameters	pH, temperature
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Overall comment on quality	Good, but reference to nominal concentrations

Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

Reference number	57
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Information on the test species	
Test species used	<i>Balanus amphitrite</i>
Source of the test organisms	Barnacles were collected from Tolo Harbour, Hong Kong
Holding conditions prior to test	Barnacle brood sacks containing mature nauplii were dissected and nauplii released in filtered sea water. Naupliar larvae actively swimming towards light were collected and transferred to a 5-litre beaker at a density of 1,000 nauplii per litre sea water. The culture was aerated and maintained at 27°C on a 15 hours light: 9 hours dark photoperiod. Sea water was changed every day and the larvae fed with the diatom <i>Skeletonema costatum</i> at 2 million cells per ml. The larvae developed through six naupliar stages into free swimming, non-feeding cyprid larvae within 6 days. The cyprids were collected within 24 hours after molting and used for testing.
Life stage of the test species used	Cyprid larvae <24 hours after molting

Information on the test design	
Methodology used	Barnacle settlement inhibition test
Form of the test substance	Phenol
Source of the test substance	Not stated
Type and source of the exposure medium	Filtered sea water (27±1°C, salinity 32 ppt, pH 7.8 and dissolved oxygen 7±1 mg l ⁻¹).
Test concentrations used	Six (0.1, 0.5, 1.0, 10, 50 and 100 mg l ⁻¹) plus control (0 mg l ⁻¹)
Number of replicates per concentration	3
Number of organisms per replicate	100
Nature of test system (static, semi-static or flow through, duration, feeding)	100 ml exposure medium in 150 ml beaker, a black opaque polystyrene slide served as substrate for settlement) static, no feeding, exposure for 6 days
Measurement of exposure concentrations	no
Measurement of water quality parameters	temperature, salinity, pH, dissolved oxygen
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated

Overall comment on quality	Good – moderate (no analysis of toxicant concentration in test vessels despite relatively long exposure time; behavioural abnormalities: no standard endpoint).
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Reliability of study	Reliable
Relevance of study	Relevant
Klimisch Code	2

ANNEX 2 Data sheets: water column data

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

Reference	15
CAS RN	108-95-2
Chemical	Phenol
Chemical species	
Organism (common name)	Two water flea species
Organism (scientific name)	<i>Ceriodaphnia dubia</i> <i>Daphnia magna</i>
Life stage (e.g. egg, embryo, ELS, adult)	Juveniles <12 hours old
Exposure regime (e.g. static, renewal, etc.)	Static–renewal (exposure medium replaced every other day)
Test method	
Analysis (measured or nominal)	Nominal
Temperature	25±2°C
Hardness	<i>C. dubia</i> : 90–110 mg l ⁻¹ as CaCO ₃ <i>D. magna</i> : 160–180 mg l ⁻¹ as CaCO ₃
pH	8.2 ± 0.2
Salinity	Lake Huron water, sterilised, aerated and adjusted to hardness
Exposure duration	<i>C. dubia</i> : 7–10 days <i>D. magna</i> : 9–11 days
Endpoint (e.g. NOEC, EC50)	NOEC, LC50
Effect (e.g. reproduction, survival, growth)	Survival, total progeny, adult weight
Concentration	<i>C. dubia</i> : 8-day NOEC 840 µg l ⁻¹ ; 96-hour LC50 20 mg l ⁻¹ ; 8-day LC50 9 mg l ⁻¹ <i>D. magna</i> : 11-day NOEC 500 µg l ⁻¹ ; 96-hour LC50 13 mg l ⁻¹ ; 11-day LC50 4 mg l ⁻¹
Initial quality assessment (e.g. good, moderate, poor)	Good – moderate, no analysis of test concentration, semi-static test. The test was quality assessed in the course of the EU risk assessment on phenol and rated valid.
Comments	

Reference	18
CAS RN	108-95-2
Chemical	Phenol
Chemical species	
Organism (common name)	Rainbow trout Fathead minnow
Organism (scientific name)	<i>Oncorhynchus mykiss</i> <i>Pimephales promelas</i>
Life stage (e.g. egg, embryo, ELS, adult)	<i>O. mykiss</i> : eyed eggs <i>P. promelas</i> : embryo-larvae
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	
Analysis (measured or nominal)	Measured, analysis weekly in freshly prepared stock solutions used to be diluted with the test medium
Temperature	<i>O. mykiss</i> : 11°C <i>P. promelas</i> : 25°C
Hardness	<i>O. mykiss</i> : 582 mg l ⁻¹ CaCO ₃ <i>P. promelas</i> : 704 mg l ⁻¹ as CaCO ₃
pH	<i>O. mykiss</i> : 7.8. <i>P. promelas</i> : 8.1
Salinity	Freshwater
Exposure duration	60 days
Endpoint (e.g. NOEC, EC50)	NOEC, LOEC
Effect (e.g. reproduction, survival, growth)	Mortality, growth
Concentration	<i>O. mykiss</i> : LOEC weight, survival 0.2 mg l ⁻¹ (weight reduction compared with the control <20%, mean weight at 0.2 mg l ⁻¹ 1.4 g compared with 1.57 at control). <i>P. promelas</i> : NOEC weight 0.75 mg l ⁻¹ , LOEC 2.5 mg l ⁻¹
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

Reference	19
CAS RN	108-95-2
Chemical	Phenol
Chemical species	
Organism (common name)	Water flea
Organism (scientific name)	<i>Daphnia magna</i>
Life stage (e.g. egg, embryo, ELS, adult)	Juveniles <24 hours old
Exposure regime (e.g. static, renewal, etc.)	Static–renewal (exposure medium replaced every Monday, Wednesday and Friday)
Test method	
Analysis (measured or nominal)	Nominal
Temperature	19±1°C
Hardness	Not stated (but in line with the provisions for Dutch Standard Water)
pH	Not stated (but in line with the provisions for Dutch Standard Water)
Salinity	Dutch Standard Water (reference given)
Exposure duration	16 days
Endpoint (e.g. NOEC, EC50)	NOEC, EC10, EC50
Effect (e.g. reproduction, survival, growth)	Reproduction; growth (body length from top of head to the end of the tail)
Concentration	EC50 reproduction: 10 mg l ⁻¹ ; EC10 growth: 0.46 mg l ⁻¹ ; NOEC growth: 0.16 mg l ⁻¹
Initial quality assessment (e.g. good, moderate, poor)	Good – moderate, no analysis of test concentration, semi-static test. The test was quality assessed in the course of the EU risk assessment on phenol and rated valid.
Comments	Tests conducted according to Dutch standard guideline NEN 6502

Reference	30
CAS RN	108-95-2
Chemical	Phenol
Chemical species	
Organism (common name)	Mysid
Organism (scientific name)	<i>Archaeomysis kokuboi</i>
Life stage (e.g. egg, embryo, ELS, adult)	Juveniles or adults
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	Static bioassay
Analysis (measured or nominal)	Not stated
Temperature	25°C
Hardness	Not stated (sea water)
pH	Not stated
Salinity	16, 24 or 32 ppt
Exposure duration	96 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	<u>Juveniles:</u> 16 ppt salinity: 260 µg l ⁻¹ 24 ppt salinity: 560 µg l ⁻¹ 32 ppt salinity: 710 µg l ⁻¹ <u>Adults:</u> 16 ppt salinity: 1,490 µg l ⁻¹ 24 ppt salinity: 2,710 µg l ⁻¹ 32 ppt salinity: 4,530 µg l ⁻¹
Initial quality assessment (e.g. good, moderate, poor)	Not assignable. Only the abstract of the paper could be read and evaluated. The main text written in Korean.
Comments	

Reference	31
CAS RN	108-95-2
Chemical	Phenol
Chemical species	
Organism (common name)	Grey mullet
Organism (scientific name)	<i>Mugil auratus</i>
Life stage (e.g. egg, embryo, ELS, adult)	Fish 22–25 cm long and about 125 g in weight
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	Fish test
Analysis (measured or nominal)	Measured twice per day
Temperature	12±0.5°C
Hardness	Sea water
pH	Sea water
Salinity	37.5±0.3 ppt
Exposure duration	8 days
Endpoint (e.g. NOEC, EC50)	
Effect (e.g. reproduction, survival, growth)	Behavioural abnormalities Mortality
Concentration	At 5 mg l ⁻¹ , fish exposed for 8 days showed no mortality but first signs of neurotoxic symptoms (excited, fast swimming). At 7.5 mg l ⁻¹ for 8 days, 10% mortality and mentioned signs of neurotoxicity plus sensitivity to light and later depressed activity were observed. From these observations, a LOEC of 5 mg l ⁻¹ and a NOEC of 0.5 mg l ⁻¹ could be inferred.
Initial quality assessment (e.g. good, moderate, poor)	Good – moderate (description of test conditions and history of the test animals a bit poor, behavioural abnormalities: no standard test parameter and difficult to quantify).
Comments	

Reference	43
CAS RN	108-95-2
Chemical	Phenol
Chemical species	Phenol (p.a.)
Organism (common name)	Guppy
Organism (scientific name)	<i>Poecilia reticula</i>
Life stage (e.g. egg, embryo, ELS, adult)	Individuals weighting 40–60 mg
Exposure regime (e.g. static, renewal, etc.)	Static–renewal (replacement of 80% of test medium every 12 hours)
Test method	Acute fish test
Analysis (measured or nominal)	Preceding analyses indicated that during the 12-hour static period the concentration of any compound tested did not decline by more than 5% of the initial value despite constant aeration.
Temperature	26±1°C
Hardness	80–100 ppm as CaCO ₃
pH	7
Salinity	Freshwater
Exposure duration	96 hours
Endpoint (e.g. NOEC, EC50)	LC50
Effect (e.g. reproduction, survival, growth)	Mortality
Concentration	log 1/LC50 given in the publication: 0.34 ≈ 457 µg l ⁻¹
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	In the paper, test organisms and test procedure are not described in detail but reference is made to another publication by the same authors from which the respective details were taken: Saarikoski J and Viluksela M, 1981 <i>Influence of pH on the toxicity of substituted phenols to fish</i> . Archives of Environmental Contamination and Toxicology, 10 , 747–753.

Reference	49
CAS RN	108-95-2
Chemical	Phenol
Chemical species	
Organism (common name)	Red alga
Organism (scientific name)	<i>Champia parvula</i>
Life stage (e.g. egg, embryo, ELS, adult)	2–3 mm branch tips of the life history stage tested (females, tetrasporophytes)
Exposure regime (e.g. static, renewal, etc.)	Static–renewal; replacement of test media on days 7 and 11
Test method	Alga test
Analysis (measured or nominal)	Nominal
Temperature	24–26°C
Hardness	Not stated (sea water)
pH	Not stated (sea water)
Salinity	30 ppt
Exposure duration	11–14 days
Endpoint (e.g. NOEC, EC50)	Chronic values
Effect (e.g. reproduction, survival, growth)	<ol style="list-style-type: none"> 1. Vegetative growth of the exposed branch tips. 2a. Production of cystocarps as evidence for sexual reproduction (if females were exposed along with a male branch tip) 2b. Production of tetrasporangia (site of meiosis) if tetrasporophytes were exposed 3. Number of cystocarps or tetrasporangia produced
Concentration	<ol style="list-style-type: none"> 1. Growth: female 21,600 µg l⁻¹; tetrasporophyte 7,800 µg l⁻¹* 2. Production of: cystocarps: 21,600 µg l⁻¹, tetrasporangia 60,000 µg l⁻¹ 3. Significant reduction in number of: cystocarps 7,800 µg l⁻¹*, tetrasporangia 7,800 µg l⁻¹* <p>* lowest concentration tested The reported overall result is a MATC <7,800 µg l⁻¹.</p>
Initial quality assessment (e.g. good, moderate, poor)	Good (however, no analysis of exposure concentrations despite relatively long change intervals of the media).
Comments	

Reference	52
CAS RN	108-95-2
Chemical	Phenol
Chemical species	
Organism (common name)	Water flea
Organism (scientific name)	<i>Daphnia magna</i>
Life stage (e.g. egg, embryo, ELS, adult)	Neonates about 24 hours old
Exposure regime (e.g. static, renewal, etc.)	Flow-through
Test method	Daphnia reproduction test similarly to the test design reported by OECD 1982*
Analysis (measured or nominal)	Measured, analysis in freshly prepared test solutions
Temperature	21±1°C
Hardness	241 mg l ⁻¹ CaCO ₃
pH	8.3
Salinity	Freshwater
Exposure duration	21 days
Endpoint (e.g. NOEC, EC50)	IC10, IC20, IC25
Effect (e.g. reproduction, survival, growth)	Mortality, reproduction
Concentration	IC10 2.38 mg l ⁻¹ ; IC20 2.91 mg l ⁻¹ ; IC25 3.18 mg l ⁻¹
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	

* Organisation for Economic Co-operation and Development (OECD), 1982 *Daphnia magna*, reproduction test. TG 202B. Paris: OECD.

Reference	54
CAS RN	108-95-2
Chemical	Phenol
Chemical species	
Organism (common name)	Green algae
Organism (scientific name)	<i>Selenastrum capricornutum</i>
Life stage (e.g. egg, embryo, ELS, adult)	Cells in the exponential growth phase
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	Alga test
Analysis (measured or nominal)	Measured concentrations
Temperature	23±2°C
Hardness	Not reported
pH	7.5±0.5
Salinity	Synthetic algal growth medium
Exposure duration	24, 48, 72 hours
Endpoint (e.g. NOEC, EC50)	EC10
Effect (e.g. reproduction, survival, growth)	Growth (cell numbers), chlorophyll a content
Concentration	EC10 growth: 72 hours: 0.969 mg l ⁻¹ 48 hours: 0.495 mg l ⁻¹ 24 hours: 0.329 mg l ⁻¹
Initial quality assessment (e.g. good, moderate, poor)	Good
Comments	Decrease in toxicity by time – presumably result of algal adaptation

Reference	55
CAS RN	108-95-2
Chemical	Phenol
Chemical species	Technical phenol, Thomas Baker & Co. (Eng.)
Organism (common name)	Carp
Organism (scientific name)	<i>Cyprinus carpio</i>
Life stage (e.g. egg, embryo, ELS, adult)	2-day-old larvae procured from a fish hatchery, acclimatised for 24 hours prior to testing.
Exposure regime (e.g. static, renewal, etc.)	Static–renewal (exposure medium replaced daily)
Test method	
Analysis (measured or nominal)	Nominal
Temperature	20–23.2°C
Hardness	60–88 mg l ⁻¹
pH	7.2
Salinity	Freshwater
Exposure duration	60 days
Endpoint (e.g. NOEC, EC50)	MATC
Effect (e.g. reproduction, survival, growth)	Growth of standing fish crop
Concentration	MATC 60 days: 110–130 µg l ⁻¹
Initial quality assessment (e.g. good, moderate, poor)	Good – moderate, no analysis of test concentration, semi-static test. The test was quality assessed in the course of the EU risk assessment on phenol and rated valid.
Comments	

Reference	56
CAS RN	108-95-2
Chemical	Phenol
Chemical species	Technical phenol, Thomas Baker & Co. (Eng.)
Organism (common name)	Asian cyprinid fish
Organism (scientific name)	<i>Cirrhina mrigala</i>
Life stage (e.g. egg, embryo, ELS, adult)	Larvae size 4.5±0.4 mm, weight 51.0±3.0 mg
Exposure regime (e.g. static, renewal, etc.)	Static-renewal (exposure medium siphoned daily)
Test method	
Analysis (measured or nominal)	Nominal
Temperature	21–25°C
Hardness	70–74 mg l ⁻¹
pH	7.2–7.4
Salinity	Freshwater
Exposure duration	60 days
Endpoint (e.g. NOEC, EC50)	MATC
Effect (e.g. reproduction, survival, growth)	Growth of standing fish crop
Concentration	MATC 60 days: 76.8–93.5 µg l ⁻¹
Initial quality assessment (e.g. good, moderate, poor)	Good – moderate, no analysis of test concentration, semi-static test. The test was quality assessed in the course of the EU risk assessment on phenol and rated valid.
Comments	

Reference	57
CAS RN	108-95-2
Chemical	Phenol
Chemical species	
Organism (common name)	Barnacle
Organism (scientific name)	<i>Balanus amphitrite</i>
Life stage (e.g. egg, embryo, ELS, adult)	Cyprid larvae <24 hours after molting
Exposure regime (e.g. static, renewal, etc.)	Static
Test method	Barnacle settlement inhibition test
Analysis (measured or nominal)	Nominal
Temperature	27±1°C
Hardness	Not stated (sea water)
pH	7.8
Salinity	32 ppt
Exposure duration	6 days
Endpoint (e.g. NOEC, EC50)	NOEC LOEC
Effect (e.g. reproduction, survival, growth)	Significant reduction in settlement percentage of barnacle larvae
Concentration	NOEC: 1 mg l ⁻¹ LOEC 10 mg l ⁻¹ (already ~50% settlement inhibition compared with the controls)
Initial quality assessment (e.g. good, moderate, poor)	Good – moderate (no analysis of toxicant concentration in test vessels despite relatively long exposure time; behavioural abnormalities: no standard endpoint.
Comments	

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