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Proposed EQS for Water Framework Directive Annex VIII substances: toluene

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SNIFFER
SCOTLAND & NORTHERN IRELAND FORUM FOR ENVIRONMENTAL RESEARCH

WFD UK TAG
Water Framework Directive
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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 toluene using the methodology described in Annex V of the Directive. There are existing EQSs for toluene, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs

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

An EU Risk Assessment Report (RAR) has been published for toluene 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
Toluene is widely used in manufacturing and process industries. It has low solubility in water and volatilisation is expected to be an important fate process. While it is readily biodegradable at high concentrations in water, toluene exhibits a reduced degradation rate at lower concentrations.

Availability of data
Acute toxicity data are available for eight different freshwater taxonomic groups (algae, crustaceans, fish, amphibians, molluscs, insects, rotifers and protozoans). The freshwater chronic dataset is less extensive, providing coverage of only the first three of these taxa.

The saltwater toxicity data are very limited and available only for four taxonomic groups (algae, crustaceans, fish and rotifers). Chronic toxicity marine studies are again restricted to algae, crustaceans, and fish. There are no field or mesocosm data available for toluene.

Derivation of PNECs

Long-term PNEC for freshwaters

Fish, crustaceans and algae appear to be of similar sensitivity. The lowest valid long-term datum reported was a no observed effect concentration (NOEC) of 0.74 mg l^{-1} for the water flea *Ceriodaphnia dubia* from a 7-day reproduction study. This value was supported by similar concentrations obtained from studies of *Daphnia magna*. Because good quality chronic datasets are available for algae, crustaceans and fish, an assessment factor of 10 is recommended, resulting in a $PNEC_{freshwater\_lt}$ of 74 µg l^{-1}.

This PNEC is 1.5 times higher than the existing EQS of 50 µg 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 coho salmon, *Oncorhynchus kisutch* (5.5 mg l^{-1}).

Short-term PNEC for freshwaters

Data for the acute exposure of eight taxonomic groups to toluene showed crustaceans and fish to be the most sensitive species. This is similar to the cases observed for chronic toxicity.

The lowest effect concentration was a 48-hour LC50 of 3.78 mg l^{-1} for *Ceriodaphnia dubia*. As crustaceans are the most sensitive species with respect to long- and short-term exposure, and because toluene acts non-specifically by narcosis, a reduced assessment factor (from 100 to 10) is recommended to extrapolate from the 50 per cent acute effect level to a short-term no-effect level. This results in $PNEC_{freshwater\_st}$ of 380 µg l^{-1}.

The proposed PNEC is 1.5 times lower than the existing EQS of 500 µg l^{-1}. This is a consequence of new more sensitive 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 coho salmon, *Oncorhynchus kisutch* (5.5 mg l^{-1}).

Long-term PNEC for saltwaters

The available data for marine species are too limited to be used as the basis for PNEC derivation. Because there are no apparent differences in the sensitivity of freshwater and saltwater species belonging to the same taxonomic groups, the freshwater and saltwater data are combined for PNEC derivation.

The lowest NOEC available in the combined freshwater and saltwater dataset was the same as that used for the derivation of the freshwater long-term PNEC (7-day NOEC of 0.74 mg l^{-1} for a reproduction study of *Ceriodaphnia dubia*).

According to Annex V of the Water Framework Directive, the NOEC of 0.74 mg l^{-1} would normally be divided by an assessment factor of 100. However, in the combined datasets, additional short-term tests are available for molluscs, rotifers

and protozoa. These organisms do not belong to the most sensitive groups, though given the non-specific mode of action of toluene, it seems unlikely that long-term tests with representatives of these additional taxonomic groups would result in lower chronic toxicity than that obtained for crustaceans. Consequently, a reduced assessment factor of 10 applied to the *Ceriodaphnia dubia* NOEC of 0.74 mg l^{-1} is recommended, resulting in the same PNEC as that for freshwater, i.e. $PNEC_{saltwater\_lt}$ = $PNEC_{freshwater\_lt}$ of 74 µg l^{-1}.

The proposed PNEC is approximately two times higher than the existing tentative EQS of 40 µg l^{-1}. This reflects new data that have become available since the original EQS was derived and the use of the combined freshwater and saltwater dataset. The existing EQS was based on an assessment factor of 100 applied to an acute LC50 for bay shrimp, *Crangon franciscorum* (3.7 mg l^{-1}).

Short-term PNEC for saltwaters

A slightly larger dataset is available for short-term saltwater exposures with toluene. Crustaceans are the most sensitive taxonomic group for both marine and freshwater species with the lowest valid acute effects being nearly identical (LC50 of 3.78 mg l^{-1} for the freshwater crustacean *Ceriodaphnia dubia* and LC50 of 3.70 mg l^{-1} for the marine crustacean *Crangon franciscorum*). As a result, the saltwater data is used for the derivation of the short-term PNEC.

As crustaceans are the most sensitive species with respect to long- and short-term exposure, and because toluene acts nonspecifically by narcosis, a reduced assessment factor (from 100 to 10) is recommended to extrapolate from the 50 per cent acute effect level to the short-term no-effect level. This results in a $PNEC_{saltwater\_st}$ of 370 µg l^{-1}.

The proposed PNEC is slightly lower than the existing EQS of 400 µg l^{-1}. It has the same basis, but differs slightly because the existing EQS was rounded up.

PNECs for sediments and secondary poisoning

Since toluene does not preferentially partition into sediment and does not bioaccumulate to any significant extent, there is no justification for deriving PNECs based on the risks of secondary poisoning to mammals and birds.

Summary of proposed PNECs

Receiving medium/exposure scenario	**Proposed PNEC (µg l^{-1})**	**Existing EQS (µg l^{-1})**
Freshwater/long-term	74	50
Freshwater/short-term	380	500
Saltwater/long-term	74	40
Saltwater/short-term	370	400

Analysis

The lowest proposed PNEC derived for toluene is 74 µg 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 in the ng l^{-1} range, which suggests that they would be adequate for assessing compliance with the proposed PNECs for water.

Implementation issues

The proposed short-term PNECs are recommended for adoption as EQSs. However, existing long-term EQSs are lower (more stringent) than those proposed in this report and thus, under the 'no deterioration' principle, should be retained.

Contents

1. Introduction

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

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

An EU Risk Assessment Report (RAR) has been published for toluene [1] 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 toluene.

1.1 Properties and fate in water

Toluene is widely used in manufacturing and process industries. It has low solubility in water and volatilisation is expected to be an important fate process. While it is readily biodegradable at high concentrations in water, toluene exhibits a reduced degradation rate at lower concentrations.

[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
Toluene	108-88-3

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 [4], and existing EQSs obtained from the literature [13].

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

Table 2.2 Proposed overall PNECs as basis for quality standard setting

PNEC	TDG deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater short-term	380 µg l^{-1} (see Section 3.1.1)	-	500 µg l^{-1} (MAC)
Freshwater long-term	74 µg l^{-1} (see Section 3.1.1)	Insufficient data	50 µg l^{-1} (AA)
Saltwater short-term	370 µg l^{-1} (see Section 3.1.2)	-	400 µg l^{-1} (MAC)
Saltwater long-term	74 µg l^{-1} (see Section 3.1.2)	Insufficient data	40 µg l^{-1} (AA)
Freshwater sediment short-term	no PNEC derived (trigger criteria not met)	-	-
Freshwater sediment long-term	no PNEC derived (trigger criteria not met)	Insufficient data	-
Saltwater sediment short-term	no PNEC derived (trigger criteria not met)	-	-
Saltwater sediment long-term	no PNEC derived (trigger criteria not met)	Insufficient data	-

PNEC	TDG deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater secondary poisoning	no PNEC derived (trigger criteria not met)	-	-
Saltwater secondary poisoning	no PNEC derived (trigger criteria 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
F; R11 - Repr.Cat.3; R63 - Xn; R48/20-65 - Xi; R38 - R67	[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 toluene

Property	Value	Reference
Molecular formula	C_7H_8	
Vapour pressure	3,000 Pa at 20°C 3,800 Pa at 25°C 28.7 mmHg, ~3,826 Pa 22 mmHg at 20°C	[1] [1] [1] [9]
Henry's Law constant	6.64 x 10^{-3} atm-m^3/mol at 25°C	[7]
Solubility in water	515 mg l^{-1} at 20°C 534.8 mg l^{-1} at 25°C	[1, 9] [1]
Dissociation constant	-	
Molecular weight	92.14	[8]

2.5 Environmental fate and partitioning

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

Table 2.5 Environmental fate and partitioning of toluene

Property	Value	Reference
Abiotic fate	Volatilisation from water surfaces is an important fate process.	[7, 13]
	Volatilisation half-life of toluene in a water tank at ~5 m water depth ranged from 1.5–16 days.	[10]
	Volatilisation half-life of toluene in a water tank 25-cm deep ranged from 13–17 hours.	[11]
	Volatilisation is dependent on temperature, surface area, wind speed, dissolved organic carbon concentration, salinity and water depth.	[1]
Hydrolytic stability	Toluene is not expected to hydrolyse under normal environmental conditions due to the lack of hydrolysable functional groups.	[1, 7]
Photostability	The direct photolytic degradation of toluene is estimated to be negligible.	[1, 13]
Distribution in water/sediment systems	In water, toluene is not expected to adsorb to suspended solids and sediment to an appreciable degree.	[7]
Degradation in soil	The evaporation half-life of toluene added to sandy soil was found experimentally to be 4.9 hours.	[12]
	Based on the above study and the physico-chemical properties of toluene, evaporation is expected to constitute an important removal mechanism for toluene in the top centimetres of soil.	[1, 7]
	Adsorption coefficients (Kd) of toluene in soils range from 0.11–4.95 l/kg (with 0.2 and 3.7% organic content respectively).	[19]
Biodegradation	Several species of microorganisms that are able to degrade toluene have been isolated and the degradative pathway is well established. Toluene is readily biodegradable by microorganisms at high concentrations and biodegradation is expected to occur rapidly in water. At lower concentrations, however, it appears that toluene may persist in natural waters, with a reduced degradation rate.	[1, 7, 13] [1]
	Under anaerobic conditions, toluene is quickly degraded by adapted microorganisms when present in a high concentration. However, no information is available on the anaerobic degradability of toluene at environmentally realistic conditions and with non-adapted microorganisms.	[1]

Property	Value	Reference
Partition coefficient (log Kow) Koc Sediment–water Suspended matter–water	2.65 2.69 at 20°C 2.73 <3 – –	[1] [13] [7] [8]
Bioaccumulation BCF <u>Fish</u> *Anguilla japonica* (eel) *Leuciscus idus melanotus* (ide) *Clupea harengus* (herring) <u>Algae</u> *Chlorella fusca* <u>Other</u> *Mytilus edulis* (mussel) *Tapes semidecursata* (clam)	The BCF value of 90 observed in the fresh water fish *Leuciscus idus melanotus* was the largest value found in fish studies and was used as a worst case in the EU RAR. The log Kow for toluene of <3, combined with a low bioaccumulation potential in fish and molluscs and a rapid elimination rate (half-life <2 days in fish indicates that toluene is unlikely to bioconcentrate in the aquatic food chain). 13 90 8 380 4.2 1.7	[1] [14, 16] [14] [15] [16] [14] [17] [18]

BCF = bioconcentration factor

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 freshwater and saltwater data from existing EQS documents [13] and the EU Risk Assessment Report on toluene [1] were collated.

Further data published after derivation of the current UK EQS were then retrieved from:

- the US Environmental Protection Agency (US EPA) ECOTOX database;[3]
- Hazardous Substances Data Bank (HSDB®) database of the US National Library of Medicine;[4]
- World Health Organization (WHO) *Environmental Health Criteria 52: Toluene* [3].

[3] http://www.epa.gov/ecotox/
[4] http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

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

Toxicity data and other information on the inherent proprieties of toluene, taken from the EU RAR [1] were not subject to an additional quality assessment beyond that carried out in the RAR. Data contained in the RAR have already been subjected to quality assessment by the authors of the risk assessment 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).

Toxicity tests used in the context of the EU RAR were considered representative and valid on the basis of the following criteria stated in the report [1]:

> '*The included tests are considered representative and valid. When evaluating the validity of ecotoxicity tests results, it was considered whether standardised test methods have been followed, but also whether the effect concentrations were measured or nominal, from flow-through, semi-static or static tests, from experiment with nominal concentrations only, but performed in closed systems or open systems and whether or not solvents were used. When evaluating the test data validity information about the physico-chemical and environmental fate related properties of the substance were also considered. Generally, the results from static tests have been excluded due to the chemical nature of the substance (toluene is volatile) unless effect data were based on measured data or closed systems had been used.*'

2.6.1 Toxicity to freshwater organisms

Short-term toxicity data are available for eight different taxonomic groups of freshwater organisms, i.e. algae, crustaceans, fish, molluscs, insects, rotifers, amphibians and protozoans. Long-term data are available only for algae, crustaceans and fish.

Available freshwater data (cumulative distribution functions) for toluene are presented in Figures 2.1 and 2.2. These figures include all data regardless of quality and provide an overview of the spread of the available data. They should not be used as formal species sensitivity distributions and have not been used in this report to set the toluene PNECs. The lowest available freshwater data for toluene are presented in Tables 2.6 and 2.7.

The search for toluene toxicity data did not yield any field or simulated ecosystem studies for effects on aquatic communities.

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

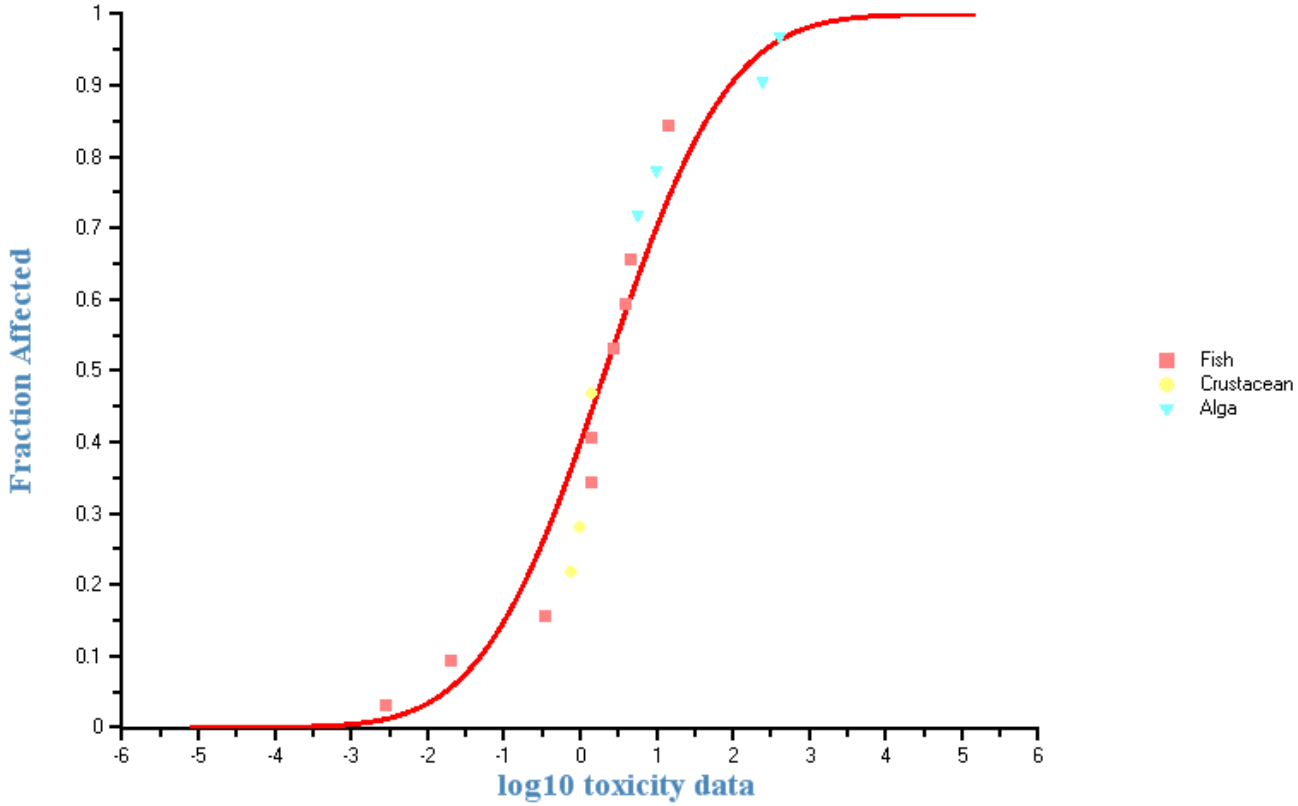

Figure 2.2 Cumulative distribution function of freshwater short-term data (mg l^{-1}) for toluene

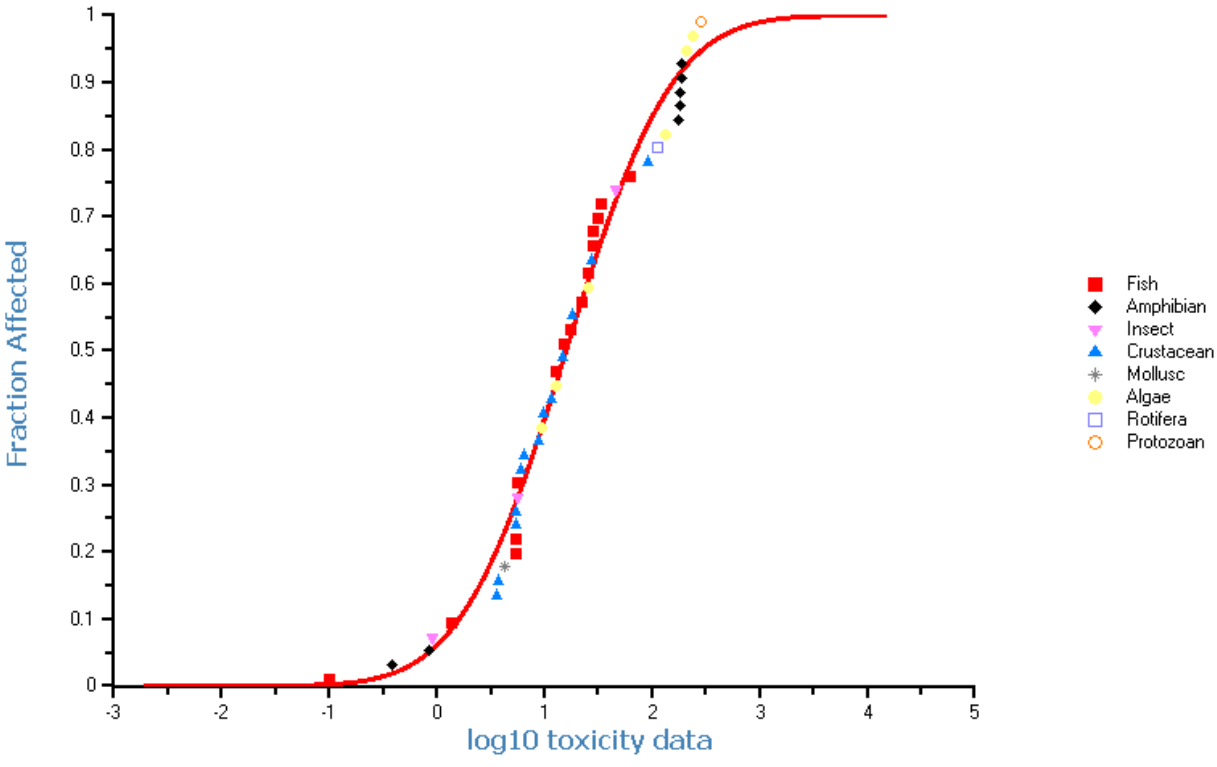

Table 2.6 Lowest available long-term aquatic toxicity data for freshwater organisms exposed to toluene

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Chlamydomonas reinhardtii	Green alga	ALG	EC10	Photosynthesis	2 hours	13	s	n	-	3	[24]
Selenastrum capricornutum	Green alga	ALG	NOEC		72 hours	12.5	CS	n	-	RAR	[36]
			NOEC		96 hours	10.0	CS	n	-	RAR	[47]
			Inhibition	GRO	8 days	**5.5**	s	m	21% inhibition of growth at 5.5 µg l^{-1}	2	[33]
			EC50	GRO	8 days	9.4	s	m	-	2	[33]
Ceriodaphnia dubia	Water flea	CRU	NOEC	REP	7 days	**0.74**	ss (CS)	m	MATC = 1.4 mg l^{-1} pH 7.6; hardness 68.3 mg l^{-1} $CaCO_3$	RAR (1)	[41]
			LOEC	REP		2.76					
			IC50	REP		3.23					
			LC50	MOR		3.41					
Daphnia magna	Water flea	CRU	NOEC	ITX	21 days	1	ss (CS)	m	NOEC based on measured concentration = 1 mg l^{-1}, based on nominal concentration = 2 mg l^{-1}	RAR	[51]
			EC50	DEV/REP	16 days	1.43	s	m	pH 8.2; 22°C; hardness 210 mg l^{-1} $CaCO_3$	2	[34]
Oncorhynchus kisutch	Coho salmon	FIS	NOEC	GRO	40 days	**1.4**	f	m	MATC = 2 mg l^{-1} Exposure of fry 7.6–10°C	RAR (1)	[39]
			LOEC	GRO	40 days	2.8					
Oncorhynchus mykiss	Rainbow trout	FIS	NOEC		27 days	**1.4**	f	m	MATC = 2.5 mg l^{-1} ELS (a second test under the same conditions resulted in a NOEC of 4.7 mg l^{-1} and a LOEC of 10.8 µg l^{-1}	RAR	[50]
			LOEC		27 days	4.4					

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Refer-ence
Pimephales promelas	Fathead minnow	FIS	NOEC		32 days	4	f	n	MATC = 4.9 mg l^{-1} embryo-larvae pH 7.6; 25°C; hardness 45 mg l^{-1} $CaCO_3$	RAR (3)	[26]
			LOEC	MOR	32 days	6	f	n			

[1] Critical data are highlighted in bold.
[2] Exposure: f = flow-through; s = static; ss = semi-static; CS = closed system.
[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 quality assessed in the EU RAR on toluene [1] and rated valid.
ALG = algae; CRU = crustaceans; FIS = fish
DEV = development; GRO = growth; ITX = intoxication; MOR = mortality; REP = reproduction
ELS = early life stage
LOEC = lowest observed effect concentration
MATC = maximum allowable toxicant concentration
NOEC = no observed effect concentration
ECx = concentration effective against X% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested
IC50 = concentration at which the population effect of the organisms tested is inhibited by 50%

Table 2.7 Lowest available short-term aquatic toxicity data for freshwater organisms exposed to toluene

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})[1]	Expo-sure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Chlamydomonas angulosa	Green alga	ALG	EC50	-	72–96 hours	134.0	CS	n	-	RAR	[35]
Chlorella vulgaris	Green alga	ALG	EC50	-	72–96 hours	207.0	CS	n	-	RAR	[35]
Selenastrum capricornutum	Green alga	ALG	EC50	GRO	8 days	**9.4**	s	m	-	2	[33]
Scenedesmus quadricauda	Alga	ALG	EC50	GRO	96 hours	25.8	s	m	-	1	[27]
Xenopus sp.	Clawed toad	AMP	LC50	MOR	96 hours	179, 181, 186, 191, 193	-	-	GM 186 mg l^{-1}	-	[37]
Ceriodaphnia dubia	Water flea	CRU	LC50	MOR	48 hours	**3.78**	ss (CS)	m	-	RAR	[41]
Ceriodaphnia dubia	Water flea	CRU	LC50	MOR	24 hours	9.0	s (CS)	m	-	2	[42]
Daphnia magna	Water flea 4–6 days old	CRU	LC50	MOR	48 hours	11.5	s (CS)	n	GM 13.1 mg l^{-1}	RAR	[23]
Daphnia magna	Water flea	CRU	LC50	MOR	48 hours	14.9	s	m		RAR	[20]
Daphnia spinulata	Water flea	CRU	EC50	NR	48 hours	5.53	s	m	pH 7.9; 20°C; hardness 95.8 mg l^{-1} $CaCO_3$	1	[27]
Hyalella curvispina	Amphipod	CRU	EC50	NR	96 hours	5.53	s	m	-	1	[27]
Bryconamericus iheringii	Characidae fish	FIS	LC50	MOR	96 hours	16.59	s	m	-	1	[27]
Lepomis macrochirus	Bluegill	FIS	LC50	MOR	96 hours	13	-	-	-	RAR	[25]
Oncorhynchus kisutch	Coho salmon	FIS	LC50	MOR	96 hours	**5.5**	-	-	7.6–10°C	1	[39]
Oncorhynchus mykiss	Rainbow trout	FIS	LC50	MOR	96 hours	5.76	ss (CS)	m	pH 7.58; 14°C, hardness 165.3 mg l^{-1} $CaCO_3$; oxygen 9.8 mg l^{-1}	1	[28]
Poecilia reticolata	Guppy	FIS	LC50	MOR	96 hours	28.2	ss (CS)	m	-	1	[28]

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})[1]	Expo-sure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Chironomus thummi	Midge	INS	LC50	MOR	48 hours	47	-	-	-	-	[43]
Chironomus thummi	Midge	INS	NOEC	MOR	48 hours	5.6	-	-	-	-	[43]
Chironomus riparius	Midge	INS	NOEC	BEH	96 hours	**0.92**	-	-	-	2	[48]
Corbicula fluminea	Clam	MOL	Increase in enzyme activity	PHY	5 days	4.3	-	-	26% increase in peroxidation	-	[54]
Tetrahymena pyriformis	Ciliate	PRO	IC50	GRO	2 days	289	-	-	-	-	[44]
Brachionus calyciflorus	Rotifer	ROT	LC50	MOR	24 hours	113.3	-	-	-	-	[30]

[1] Critical data are highlighted in bold. If more than one test per species with the same endpoint and test duration was available, geometric means (GMs) of these results were calculated. The GMs are presented in the 'Comments' column. Test results used to calculate GMs are underlined in the 'Conc.' column.
[2] Exposure: s = static; ss = semi-static; CS = closed system.
[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 quality assessed in the EU RAR on toluene [1] and rated valid.
ALG = algae; AMP = amphibians; CRU = crustaceans; FIS = fish; INS = insects; MOL = molluscs; PRO = protozoans; ROT = rotifers
BEH = behaviour; GRO = growth; MOR = mortality; PHY = physiology
NOEC = no observed effect concentration
EC50 = concentration effective against 50% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested
IC50 = concentration at which the population effect of the organisms tested is inhibited by 50%
NR = not reported

2.6.2 Toxicity to saltwater organisms

Single species toxicity data for saltwater organisms are available only for four different taxonomic groups, i.e. algae, crustaceans, fish and rotifers (acute only).

Available saltwater data for toluene are presented as cumulative distribution functions in Figures 2.3 and 2.4. These figures include all data regardless of quality and provide an overview of the spread of the available data. They should not be used as formal species sensitivity distributions and have not been used in this report to set the toluene PNECs. The lowest available saltwater data for toluene are presented in Tables 2.8 and 2.9.

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

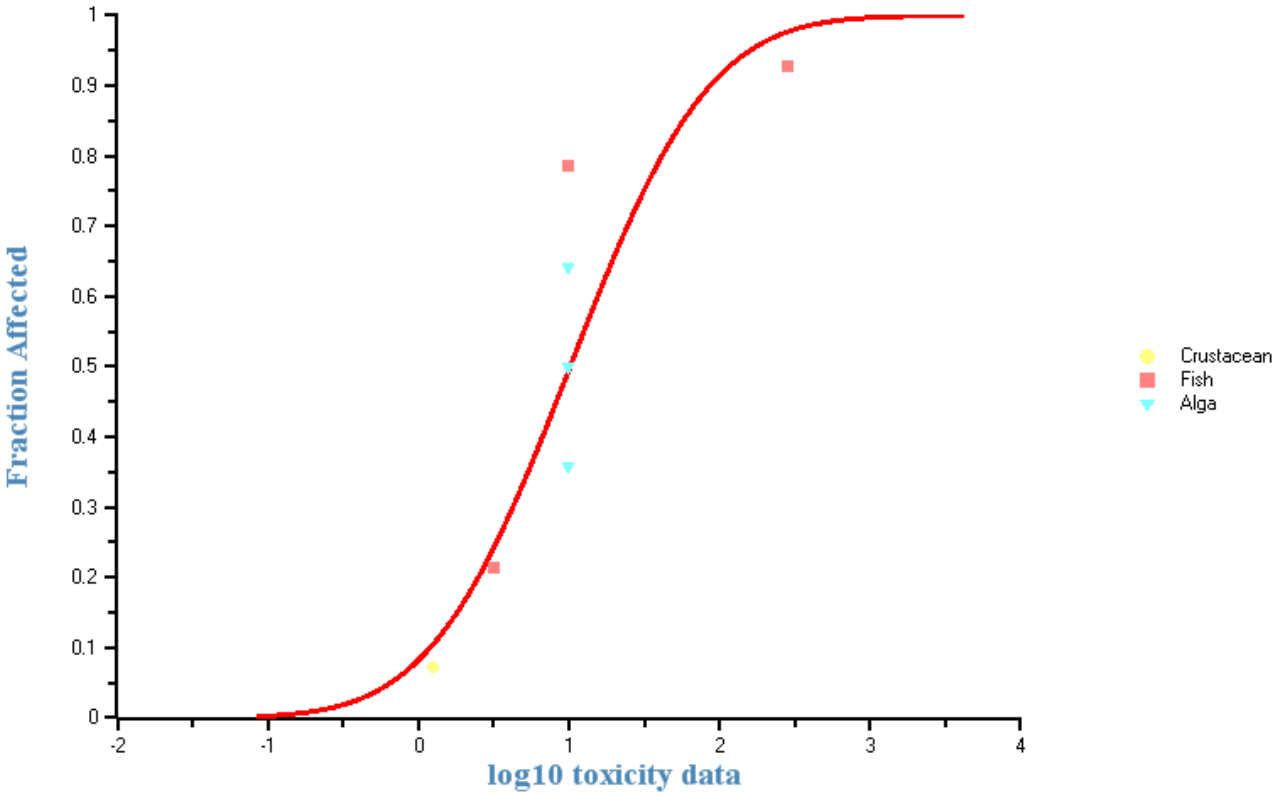

Figure 2.4 Cumulative distribution function of saltwater short-term data (mg l^{-1}) for toluene

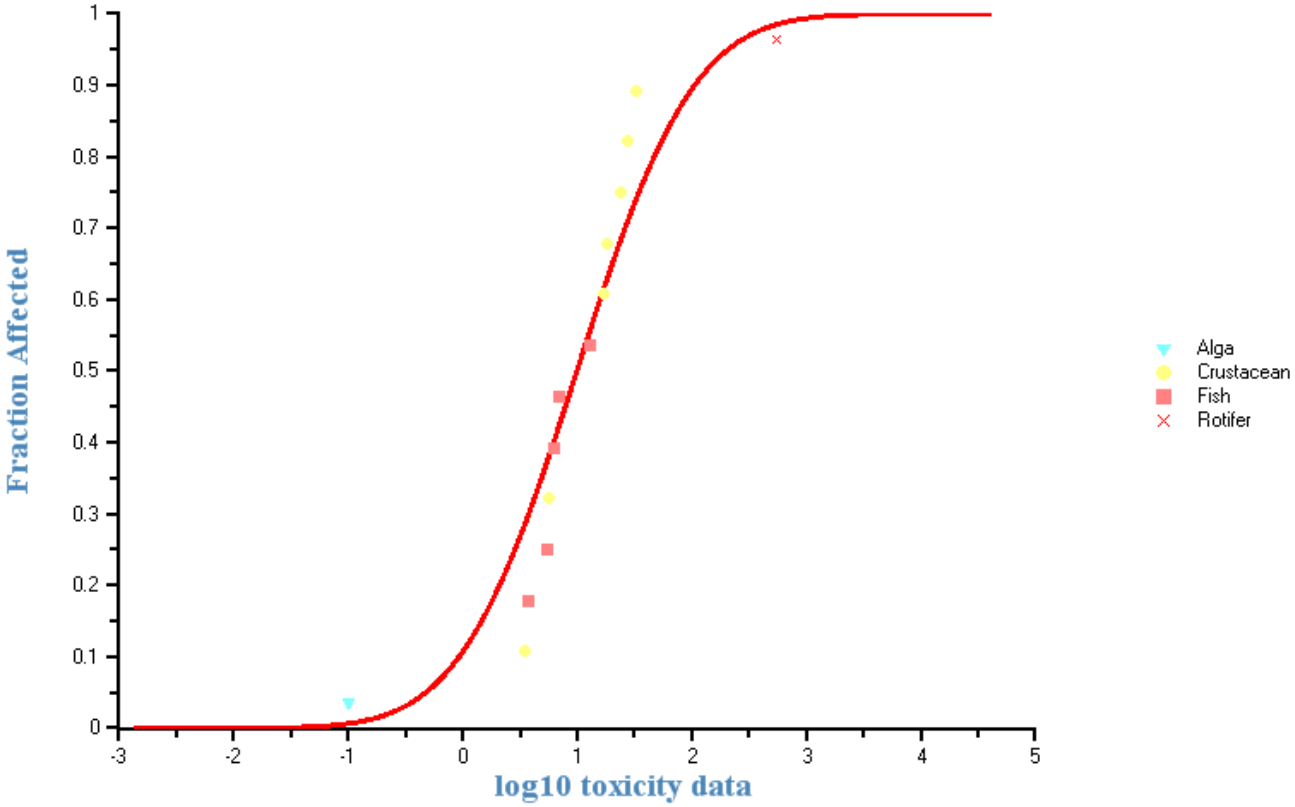

Table 2.8 Lowest available long-term aquatic toxicity data for saltwater organisms exposed to toluene

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})[1]	Exposure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Skeletonema costatum	Alga	ALG	NOEC		72 hours	**10**	s (CS)	n	-	RAR	[31]
Cirolana borealis	Isopod	CRU	NOEC ET50	Inactivity/ MOR	96 hours 400 hours	**1.25** 5.7	ss (OS)	n	-	3	[21]
Cyprinodon variegatus	Sheepshead minnow	FIS	Chronic effects	NR	NR	5	-	-	Chronic value	-	[47]
Cyprinodon variegatus	Sheepshead minnow	FIS	NOEC LOEC MATC	NR	28 days	**3.2** 7.7 4.96	f	-	ELS, egg-juvenile exposure 28 days	RAR	[49]

[1] Critical data are highlighted in bold.
[2] Exposure: f = flow-through; s = static; ss = semi-static; CS = closed system; OS = open system.
[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 quality assessed in the EU RAR on toluene [1] and rated valid.
ALG = algae; CRU = crustaceans; FIS = fish
MOR = mortality
ELS = early life stage
LOEC = lowest observed effect concentration
MATC = maximum allowable toxicant concentration
NOEC = no observed effect concentration
ET50 = exposure time required for a defined effect to be observed among 50% of a population when that population is treated with a known amount or concentration of a toxicant
NR = not reported

Table 2.9 Lowest available short-term aquatic toxicity data for saltwater organisms exposed to toluene

Scientific name	Common name	Taxonomic group	Endpoint	Effect	Test duration	Conc. (mg l^{-1})[1]	Expo-sure[2]	Toxicant analysis[3]	Comments	Reliability index[4]	Reference
Amphidinium carterae	Alga	ALG	NR	GRO	2–3 days	**0.1**	s (CS)	-	30% decrease	3	[29]
Palaemonetes pugio	Grass shrimp (mature)	CRU	LC50	-	24 hours	17.2	s	m	according to APHA 1965 guidelines	RAR	[11]
Nitocra spinipes	Copepod	CRU	LC50	-	24 hours	24.2	s	m	according to APHA 1965 guidelines	RAR	[11]
Chaetogammarus marinus	Scud (marine)	CRU	LC50	-	48 hours	18	s	-	initial concentration 96% of nominal	RAR	[20]
Crangon franciscorum	Bay shrimp	CRU	LC50	MOR	96 hours	**3.7**	s	m	-	2	[22]
Cyprinodon variegatus	Sheepshead minnow	FIS	LC50	MOR	96 hours	13	-	-	ELS	-	[45]
Morone saxatilis	Striped bass	FIS	LC50	MOR	24 hours	6.3	s	m	25 ppt salinity	RAR	[22]
Morone saxatilis	Striped bass	FIS	LC50	MOR	96 hours	6.3	s	m	25 ppt salinity	RAR	[22]
Oncorhynchus gorbuscha	Pink salmon	FIS	LC50	MOR	96 hours	6.4	s	m	GM 7 (6.4–8.1 mg l^{-1}) 4–12°C; salinity 28 ppt	RAR	[38]
Oncorhynchus gorbuscha	Pink salmon	FIS	Breathing rate	PHY	15 hours	**3.8**	f	m	increasing the breath rate	2	[46]
Oncorhynchus gorbuscha	Pink salmon	FIS	TLm	MOR	24 hours	5.4	f	m	-	RAR	[46]
Brachionus plicatilis	Rotifer	ROT	LC50	MOR	24 hours	552.6	-	-	-	-	[30]

[1] Critical data are highlighted in bold. If more than one test per species with the same endpoint and test duration was available, geometric means (GMs) of these results were calculated. The GMs are presented in the 'Comments' column. Test results used to calculate GMs are underlined in the 'Conc.' column.
[2] Exposure: f = flow-through; s = static; CS = closed system.
[3] Toxicant analysis: m = measured.
[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 quality assessed in the EU RAR on toluene [1] and rated valid.
ALG = algae; CRU = crustaceans; FIS = fish; ROT = rotifers
GRO = growth; MOR = mortality; PHY = physiology
ELS = early life stage
LC50 = concentration lethal to 50% of the organisms tested
TLm = median threshold limit
NR = not reported
ppt = parts per trillion

2.6.3 Toxicity to sediment-dwelling organisms

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

2.6.4 Endocrine-disrupting effects

Low-level toluene exposure (80 ppm) may have an impact on the levels of the hormones follicle-stimulating hormone (FSH), luteinising hormone (LH) and testosterone in humans by affecting the hypothalamus-pituitary axis. Possible explanations include a link to toluene-induced changes in neurotransmitter levels or to dopamine-like activity of toluene or its metabolites [1].

In the EU RAR [1] the relevance of the observations is interpreted as follows:

> ‘ *… the effects cannot be regarded as directly adverse, since the hormone levels were within reference limits, and the effects seemed to be reversible. Nonetheless, the fact that the levels were within normal reference limits does not imply that the effect on hormone levels is non-existing. The differences may seem small, but especially for hormones small changes may be of importance and it is considered as remarkable that two human studies show similar effects on hormone levels (i.e. lower FSH, LH and testosterone). These findings may indicate a possible interference with endocrine mechanisms by toluene. However, limited data from an earlier Danish study investigating substantially higher toluene exposure levels do not show similar effects and, therefore clear conclusions regarding the effects of toluene on hormone levels cannot be drawn.*’

No other information was found indicating that toluene may interfere with or disrupt the endocrine system of terrestrial or aquatic organisms.

2.6.5 Mode of action of toluene and occurrence of relevant metabolites in the aquatic environment

Aquatic organisms are exposed to toluene via respiration, resulting in changes in gill permeability and internal carbon dioxide poisoning. The acute effects of toluene are considered to be through a narcosis with minimal specific toxicity [1].

The most important atmospheric removal process for toluene is by reaction with hydroxyl radicals. The hydroxyl radical reaction leads to the initial formation of benzyl and hydroxycyclohexadienyl radicals or alkyl-substituted homologues. The reactions result in the formation of *o*-, *m*- and *p*-cresol, *o*-, *m*- and *p*-nitrotoluene, benzylnitrate, glyoxal, etc. The introduction of two oxygen atoms finally leads to the splitting of the aromatic ring. Photochemical oxidative degradation/transformation is considered to be an important degradation pathway in air; the half-life of toluene in air is estimated to be approximately 2 days according to the realistic worst-case concept [1].

Toluene is generally considered as readily biodegradable in water, sediment and soil. At lower concentrations, however, toluene may persist in natural waters, with a reduced degradation rate [1].

In animals and man, toluene is mainly converted into benzyl alcohol and excreted as hippurate. The toxicokinetics of toluene in humans have been extensively studied [1, 6].

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 reported by Black *et al.* [53], who observed an LC50 of 20 µg l^{-1} and a 27-day LC10 of 2.9 µg l^{-1} in an early-life-stage test with rainbow trout. In this study, the eggs were exposed in a flow-through system to the test substance within 30 minutes of fertilisation. The concentration was measured daily. Later work by WRc [50], which attempted to reproduce these results, reported NOEC values in two independent studies of 1.4 and 4.7 mg l^{-1}, respectively.

Experts have examined both studies without discovering reasons for the large discrepancy between them and both studies are regarded as valid. Even though an evaluation of the original study by Black *et al.* did not reveal any obvious invalidating factors, it does not appear appropriate to include this study in the consideration of the long-term NOEC for fish. This is because four other independently conducted long-term valid fish studies (including one with the same fish species) have reported NOECs within the same range as each other, but several orders of magnitude higher than the NOEC reported by Black *et al.* [53].

As the effect values found by Black *et al.* [53] for several organic substances are usually very low compared with effect values found by other authors, a careful examination of the entire information set provided by Black and co-workers was conducted. However, no plausible reason for the large discrepancies could be found. Nevertheless, it was decided by the EU Member State Experts of the Technical Meeting on Existing Substances not to use these data for the derivation of a $PNEC_{aqua}$.

This report follows the decision by the Experts of the Technical Meeting on Existing Substances not to use the data by Black *et al.* in the EU RAR [1] for PNEC and EQS derivation. This is because neither new toxicity data that could support their findings nor new information on the validity of their data has become available. Furthermore, in the context of the derivation of quality standards for the priority substances of Annex X of the Water Framework Directive, the decision of the Technical Meeting Experts has also been adopted (after the subject was discussed again by an expert group from Member States, industry and ‘green’ NGOs).

The data and outcomes of the toluene 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 annual average concentration
The available data suggest that the sensitivities of the most susceptible representatives of fish, crustaceans and algae are similar (see Figure 2.1). This observation agrees with the generally accepted opinion that toluene acts by non-specific narcosis.

There are a number of long-term (lt) algal data available for toluene. The lowest available value is an 8-day growth inhibition value of 5.5 mg l^{-1} in the alga *Selenastrum capricornutum* [33]. In addition, the same study reported an 8-day EC50 of 9.4 mg l^{-1}. These values come from a well-documented study with measured effect concentrations and are suitable for inclusion in the PNEC derivation. In addition, these values are supported by a good quality NOEC of 10 mg l^{-1} in the same species after a 96-hour exposure period [47]. This study was reported by the toluene RAR to be of good quality.

The lowest valid long-term NOEC for a crustacean was found in a 7-day reproduction study with the water flea *Ceriodaphnia dubia* (NOEC of 0.74 mg l^{-1}) [41]. This study is based on measured concentrations and was reported by the toluene RAR to be of high quality. This value is supported by a 21-day NOEC (intoxication) [51] and a 16-day EC50 (reproduction) [34] of 1 and 1.43 mg l^{-1}, respectively, in the water flea *Daphnia magna*. Both studies were based on measured concentrations and are suitable for inclusion in the PNEC derivation.

In addition to the available crustacean data, there are several good quality NOECs available for fish. In 40-day exposures with *Oncorhynchus kisutch*, a NOEC of 1.4 mg l^{-1} was reported in a flow-through study based on measured toluene concentrations [39]. In addition, a 27-day NOEC of 1.4 mg l^{-1} was reported in rainbow trout (*Oncorhynchus mykiss*) also exposed under flow-through conditions [50]. Both studies are suitable for PNEC derivation.

As good quality long-term data are available for algae, crustaceans and fish, the $PNEC_{freshwater\_lt}$ can be derived in accordance with the TGD [4] on the basis of the NOEC of 0.74 mg l^{-1} for effects on the reproduction of *Ceriodaphnia dubia* and the standard assessment factor (AF) of 10. The proposed study was also used in the EU RAR [1] to derive the $PNEC_{aqua}$:

$PNEC_{freshwater\_lt}$ = 740 µg l^{-1}/AF (10) = 74 µg l^{-1} toluene

PNEC accounting for transient concentration peaks
Short-term (st) toxicity data are available for eight different taxonomic groups, i.e. algae, crustaceans, fish, molluscs, insects, rotifers, amphibians and protozoans (Table 2.7). Crustaceans and fish appear to be the most sensitive species with regard to short-term acute effects, as was the case for long-term toxicity.

The lowest available algal value is an 8-day EC50 (growth) of 9.4 mg l^{-1} in the alga *Selenastrum capricornutum* [33]. This is a well-documented study with measured effect concentrations and is suitable for inclusion in the PNEC derivation. This value is

supported by a high quality 96-hour EC50 (growth) in *Scenedesmus quadricauda* of 25.8 mg l^{-1} [27]. This value was again based on measured concentrations and is suitable for PNEC derivation.

The lowest test result considered valid is a 48-hour LC50 of 3.78 mg l^{-1} found for *Ceriodaphnia dubia* [41]. This value was based on measured concentrations and was regarded by the toluene RAR as being of suitable quality for PNEC derivation.

A lower value (96-hour NOEC) of 0.92 mg l^{-1} was reported for changes in the activity pattern of midge larvae (*Chironomus riparius*) [48]. However, the ecological relevance of the observed toxic effects (i.e. gradual changes in ventilation activity and in other movements, including feeding activity and phases of inactivity) are unclear.

Fish appear to have similar sensitivity to the effects of toluene as crustaceans. After a 96-hour exposure period, LC50 values of 5.5 and 5.76 have been reported in coho salmon [39] and rainbow trout [28] respectively. The studies were carried out under renewal or flow-through conditions and effects were based on measured concentrations. Consequently, both are suitable for PNEC derivation.

Non-salmonid fish appear to be less sensitive than salmonids to toluene, with 96-hour LC50 values of 13 and 28 mg l^{-1} for the bluegill (*Lepomis macrochirus*) [25] and guppy (*Poecilia reticolata*) [28], respectively.

The lowest short-term value for toluene was a 96-hour NOEC of 0.92 for behaviour in midge larvae (*Chironomus riparius*) [48]. However, the ecological relevance of the observed toxic effects is questionable. Therefore, it is suggested that the lowest standard endpoint is used as the basis for the derivation of the $PNEC_{freshwater\_st}$.

The PNEC for effects following short-term exposure to toluene is thus calculated on the basis of the *Ceriodaphnia dubia* 48-hour LC50 of 3.78 mg l^{-1} and the guidance given in the TGD on effects assessment for intermittent releases (Section 3.3.2 of Part II of the TGD [4]). As crustaceans are the most sensitive species with regard to both short-term and long-term exposure and because toluene acts non-specifically by narcosis, a reduced assessment factor of 10 (instead of 100) is used in order to extrapolate from the 50 per cent acute effect level to the short-term no-effect level.

$PNEC_{freshwater\_st}$ = 3780 µg l^{-1}/AF (10) = 380 µg l^{-1} toluene

3.1.2 PNECs for saltwaters

The effects dataset for marine species is very small, comprising short- and long-term data for only algae, crustaceans and fish. In addition, a 24-hour LC50 for a marine rotifer species is available (see Tables 2.8 and 2.9).

The few available valid toxicity data for marine taxa do not differ significantly from the range of values obtained for their freshwater relatives (see Tables 2.6–2.9). However, the marine database is too small to draw firm conclusions on possible differences. In line with advice in the TGD [4], freshwater data should be used alongside saltwater data because obvious differences in the sensitivity of freshwater or saltwater species of the same taxonomic group are not apparent.

PNEC accounting for annual average concentration
The available saltwater effects dataset is considered too small to be the basis for derivation of an annual average PNEC for the marine pelagic community. Moreover, as the differences in sensitivity of freshwater and saltwater species belonging to the same taxonomic groups appear to be small, freshwater and saltwater data should be combined to derive PNECs for saltwater (see above).

The lowest available long-term algal study is a 72-hour NOEC of 10 mg l^{-1} in *Skeletonema costatum* [31]. There was no chemical analysis in this study, but it was performed in a closed system and the toluene RAR regarded it as valid for PNEC derivation.

Only one long-term invertebrate study could be located; this was on the effects of toluene on the isopod *Cirolana borealis* [21]. A 96-hour NOEC (mortality) of 1.25 mg l^{-1} was reported in a semi-static system. However, this study appears not to be reliable since the toxicant concentration was not analysed, although an open semi-static system (involving the replacement of test solutions every second day), which did not prevent the evaporative losses of toluene, was used to expose the animals. Consequently, this value was not deemed suitable for PNEC derivation.

A small number of long-term marine fish data were available. The lowest reliable study was an early life stage test with the sheepshead minnow (*Cyprinodon variegatus*) [49]. A 28-day NOEC of 3.2 mg l^{-1} (corresponding LOEC of 7.7 mg l^{-1}) was reported for this study. The test was run under flow-through conditions and the toluene RAR regarded it as of good quality.

The lowest NOEC available in the combined freshwater and saltwater dataset is the same as used for the derivation of the freshwater annual average PNEC (7-day NOEC of 0.74 mg l^{-1} for reproduction of the crustacean species *Ceriodaphnia dubia* [41] (see Section 3.1.1).

The NOEC of 0.74 mg l^{-1} would normally be divided by an assessment factor of 100 according to the TGD provisions for marine effects assessment (applicable when three long-term tests on freshwater or saltwater species representing 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, echinoderms) are available;
- the studies indicate that these species do not belong to the most sensitive group;
- it can 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 species belonging to the following groups: molluscs, rotifers and protozoa. The tests indicate that these organisms do not belong to the most sensitive groups (see Tables 2.6–2.9). Given the non-specific (narcotic) mode of action of toluene, it seems improbable that long-term tests with representatives of these additional taxonomic groups would result in lower chronic toxicity data than obtained for crustaceans. It therefore seems justified to use only a reduced assessment factor of 10 to obtain the $PNEC_{saltwater\_lt}$ on the basis of the NOEC of 0.74 mg l^{-1} found for the crustacean species *Ceriodaphnia dubia*:

$PNEC_{saltwater\_lt}$ = $PNEC_{freshwater\_lt}$ = 740 µg l^{-1}/AF (10) = 74 µg l^{-1} toluene

PNEC accounting for transient concentration peaks
A slightly larger database is available for short-term saltwater exposures with toluene with data available for algae, crustaceans, fish and rotifers.

Only limited saltwater algal data were available. A 30 per cent reduction in population growth of *Amphidinium carterae* after a 2–3-day exposure to 0.1 mg l^{-1} toluene was reported [29]. However, this study is not considered reliable as measurements of toluene concentrations in the closed vessels are incomplete and, where conducted, show large and inconsistent deviations from the target (i.e. nominal) concentrations. Moreover, a 30 per cent decrease in cell density and chlorophyll was reported at a nominal concentration as low as 0.1 mg l^{-1}. However, this percentage of decrease remained nearly constant at toluene concentrations over a range of more than two orders of magnitude (up to 50 mg l^{-1}). This lack of a clear dose–response relationship and the obvious difficulties with dosing and monitoring of toxicant concentrations renders this result unreliable.

A number of values are available for saltwater crustaceans with effect concentrations ranging from 3–24 mg l^{-1}. The lowest reliable value is a 96-hour LC50 of 3.7 mg l^{-1} in the bay shrimp (*Crangon franciscorum*) [22]. This value was generated in an open system, but was based on measured concentrations. In addition, data are available for the marine rotifer *Brachionus plicatilis*. However, this species appears to be less sensitive than other invertebrates with a 24-hour LC50 of 552 mg l^{-1} [30].

Marine fish appear particularly sensitive to the short-term effects of toluene. LC50 values for salmonid and non-salmonid fish range from 3–13 mg l^{-1}. The lowest reliable effect concentration is in pink salmon (*Oncorhynchus gorbuscha*) with a significant effect on breathing rate after a 15-hour hour exposure to 3.8 mg l^{-1} toluene [46]. This was a valid study with data generated in a flow-through test with measured toluene concentrations. In terms of standard effects measures, 96-hour LC50 values of 6.3 and 6.4 mg l^{-1} have also been reported in striped bass and pink salmon exposed to toluene [22, 38].

The lowest valid acute effects values reported for marine and freshwater species are nearly identical and in both environments the most sensitive organism is a crustacean species (LC50 of 3.78 mg l^{-1} for the freshwater crustacean *Ceriodaphnia dubia* and LC50 of 3.70 mg l^{-1} for the marine crustacean *Crangon franciscorum*; see Tables 2.7 and 2.9). The lowest value is, therefore, used for the derivation of the short-term PNEC for saltwater.

The TGD [4] does not provide specific guidance for assessment of acute effects of intermittent releases to marine water bodies. Therefore, the PNEC has been calculated on the basis of the general guidance given in the TGD on effects assessment for intermittent releases (Section 3.3.2 of Part II of the TGD [4]). As crustaceans are the most sensitive species with regard to both short-term and long-term exposure and because toluene acts non-specifically by narcosis only, a reduced assessment factor of 10 (instead of 100) is used in order to extrapolate from the 50 per cent acute effect level to the short-term no-effect level.

$PNEC_{saltwater\_st}$ = 3700 µg l^{-1}/AF (10) = 370 µg l^{-1} toluene

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

The minimum number of long-term toxicity studies defined by the TGD (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 1992 report [13], the most sensitive freshwater species to toluene was coho salmon (*Oncorhynchus kisutch*). The lowest reported 96-hour LC50 of 5.5 mg l^{-1} for this species was used to derive the long-term freshwater standard by applying an assessment factor of 100 to give a rounded EQS of 50 µg l^{-1} expressed as an annual average.

The same datum was used to derive the freshwater short-term value by applying an assessment factor of 10 to give a rounded EQS of 500 µg l^{-1} expressed as a maximum allowable concentration.

The long-term standard for the protection of saltwater life was based on a 96-hour LC50 of 3.7 mg l^{-1} for the bay shrimp *Crangon franciscorum*. An assessment factor of 100 was applied to this value to give a rounded EQS of 40 µg l^{-1} expressed as an annual average.

The same datum was used to derive the saltwater short-term value by applying an assessment factor of 10 to give a rounded EQS of 400 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 Kow $\geq$3) (Table 2.5) is not met in the case of toluene.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

Toluene toxicity is most prominent in the central nervous system after acute and chronic exposure. Reproductive toxicity has been observed in rats treated with toluene [6]. In vertebrates, toluene has low acute toxicity via inhalation and the oral route. In rats, an LC50 of 28.1 mg l^{-1} (4 hours) and an oral LD50 of 5.58 g/kg have been reported [1].

In the rat, an oral no observed adverse effect level (NOAEL) for general systemic toxicity of 625 mg/kg per day for repeated oral exposure was identified in a 90-day study [1]. At higher levels (1,250 mg/kg and above) neuronal necrosis and organ weight increases were found. In a similar 90-day mouse study, non-specific effects (liver enlargement and

one death) were found at 1,250 mg/kg. Also in the rat, a no observed adverse effect concentration (NOAEC) for general systemic toxicity of 625 ppm (2,344 mg/m^3) for repeated exposure via inhalation was identified in a 15-week study. At the higher exposure level [1,250 ppm (4,688 mg/m^3)] a decrease in leucocyte count in females and relative organ weight increases were observed [1].

The International Agency for Research on Cancer (IARC) [6] considers that there is:

- inadequate evidence in humans for the carcinogenicity of toluene;
- evidence suggesting lack of carcinogenicity of toluene in experimental animals.

Toluene is considered to be non-genotoxic, and was not carcinogenic to rats or mice in inhalation studies [1].

Table 3.1 summarises the no-effect values for toluene used by international bodies to set human health standards.

Table 3.1 Mammalian toxicity data relevant for the assessment of non-compartment specific secondary poisoning

Endpoint	Value	Species	Duration	Effect	Reference
LOAEL	312 mg/kg bw/day	Mice, gavage study	13 weeks	Marginal hepatoxic effects	[3]
NOAEL	223 mg/kg bw/day	Rat, gavage	13 weeks	Changes in liver and kidney weights	[52]
LOAEL	88 ppm (119 mg/m³)	Man, inhalation	5.7 years	Neurobehavioural changes	[52]
NOAEL	625 mg/kg bw/day	Rat, oral	90 days	Neuronal necrosis and organ weight increases	[1]
NOAEC	625 ppm (2,344 mg/m³)	Rat, inhalation	15 weeks	Decreased leucocytes, organ weight increases	[1]
NOAEC	300 ppm (1,125 mg/m³)	Rat, inhalation	2 years	Not stated, but assumed to be similar to above	[1]

LOAEL = lowest observed adverse effect level

3.5.2 PNECs for secondary poisoning of predators

The derivation of specific PNECs for the protection of predators against secondary poisoning by toluene is unnecessary because the trigger values, i.e. bioconcentration factor (BCF) ≥100 or biomagnification factor (BMF) >1, are not met.

In addition, the substance is completely metabolised or excreted if taken up orally by vertebrates. The combination of a rapid elimination rate (half-life <2 days in fish [14, 16]), together with the low bioaccumulation potential in fish and molluscs, indicates that toluene is unlikely to bioconcentrate in the aquatic food chain [1].

4. Analysis and monitoring

Methods are available for determining toluene concentrations in a variety of environmental matrices including water. Validated methods, approved by agencies and organisations such as the US EPA, the American Society for Testing and Materials (ASTM), the American Public Health Association (APHA) and the National Institute of Occupational Safety and Health (NIOSH) in the USA, are available for air, water and solid waste matrices [56, 57].

Gas chromatography (GC) is the most widely used analytical technique for quantifying concentrations of toluene in environmental matrices. Various detection devices used for GC include:

- flame ionisation detection (FID)
- mass spectrometry (MS)
- photoionisation detection (PID).

Sample preconcentration prior to GC analysis is generally required due to the complexity of the sample matrix and the usually low concentrations of volatile organic compounds (VOCs) in environmental media. Methods include:

- gas purge-and-trap
- headspace gas analysis
- extraction with organic solvent.

Purge-and-trap is the most widely used method for the isolation and concentration of VOCs in environmental samples [32]. It offers advantages over other techniques in that it allows easy isolation and concentration of target compounds, thereby improving overall limits of detection (LODs) and recovery of the sample.

Sampling techniques for air include collection in sample loops, on adsorbents, in canisters and by cryogenic trapping. The analysis is normally performed by GC-FID, GC-PID or GC-MS. Detection limits depend on the amount of air sampled, but values in the ng l^{-1} range have been reported [55].

Due to its volatility, toluene is lost relatively easily from biological samples such as plant and animal tissue and body fluids. To prevent analyte loss, samples should be collected and stored with care (e.g. at low temperatures in sealed containers).

Headspace techniques are usually used to separate toluene from biological fluids. This involves equilibrium of volatile analytes such as toluene between a liquid and solid sample phase and the gaseous phase. The gaseous phase is then analysed by GC. There are two main types of headspace methodology:

- static (equilibrium) headspace
- dynamic headspace – usually called the ‘purge and trap’ method [5].

The static headspace technique is relatively simple, but may be less sensitive than the purge-and-trap method. The purge-and-trap method, while providing increased sensitivity, requires more complex instrumentation [5].

Packed columns and capillary columns are used for chromatographic separation, followed by identification and quantitation using various detectors; FID, PID and MS are most often used.

Solvent extraction permits concentration, thereby increasing sensitivity, but the solvent can interfere with analysis. Direct aqueous injection is a very rapid method, but sensitivity is low and matrix effects can be a serious problem. Limits of detection for purge-and-trap methods are typically ≥50 ng l^{-1} [40].

The lowest proposed PNEC derived for toluene is 74 µg 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 in the ng l^{-1} range, which suggests that current analytical methodologies are more than adequate to analyse toluene for compliance with the derived PNECs for water.

5. Conclusions

5.1 Availability of data

Acute toxicity data are available for eight different freshwater taxonomic groups (algae, crustaceans, fish, amphibians, molluscs, insects, rotifers and protozoans). The freshwater chronic dataset is less extensive, providing coverage of only the first three of these taxa.

The saltwater toxicity data are very limited and available only for four taxonomic groups (algae, crustaceans, fish and rotifers). Chronic toxicity marine studies are again restricted to algae, crustaceans, and fish. There are no field or mesocosm data available for toluene.

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

Fish, crustaceans and algae appear to be of similar sensitivity. The lowest valid long-term datum reported was a NOEC of 0.74 mg l^{-1} for the water flea *Ceriodaphnia dubia* from a 7-day reproduction study. This value was supported by similar concentrations obtained from studies of *Daphnia magna*. Because good quality chronic datasets are available for algae, crustaceans and fish, an assessment factor of 10 is recommended, resulting in a $PNEC_{freshwater\_lt}$ of 74 µg l^{-1}.

This PNEC is 1.5 times higher than the existing EQS of 50 µg 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 coho salmon, *Oncorhynchus kisutch* (5.5 mg l^{-1}).

5.2.2 Short-term PNEC for freshwaters

Data for the acute exposure of eight taxonomic groups to toluene showed crustaceans and fish to be the most sensitive species. This is similar to the cases observed for chronic toxicity.

The lowest effect concentration was a 48-hour LC50 of 3.78 mg l^{-1} for *Ceriodaphnia dubia*. As crustaceans are the most sensitive species with respect to long- and short-term exposure, and because toluene acts non-specifically by narcosis, a reduced assessment factor (from 100 to 10) is recommended to extrapolate from the 50 per cent acute effect level to a short-term no-effect level. This results in $PNEC_{freshwater\_st}$ of 380 µg l^{-1}.

The proposed PNEC is 1.5 times lower than the existing EQS of 500 µg l^{-1}. This is a consequence of new more sensitive 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 coho salmon, *Oncorhynchus kisutch* (5.5 mg l^{-1}).

5.2.3 Long-term PNEC for saltwaters

The available data for marine species are too limited to be used as the basis for PNEC derivation. Because there are no apparent differences in the sensitivity of freshwater and saltwater species belonging to the same taxonomic groups, the freshwater and saltwater data are combined for PNEC derivation.

The lowest NOEC available in the combined freshwater and saltwater dataset was the same as that used for the derivation of the freshwater long-term PNEC (7-day NOEC of 0.74 mg l^{-1} for a reproduction study of *Ceriodaphnia dubia*).

According to Annex V of the Water Framework Directive, the NOEC of 0.74 mg l^{-1} would normally be divided by an assessment factor of 100. However, in the combined datasets, additional short-term tests are available for molluscs, rotifers and protozoa. These organisms do not belong to the most sensitive groups, though given the non-specific mode of action of toluene, it seems unlikely that long-term tests with representatives of these additional taxonomic groups would result in lower chronic toxicity than that obtained for crustaceans. Consequently, a reduced assessment factor of 10 applied to the *Ceriodaphnia dubia* NOEC of 0.74 mg l^{-1} is recommended, resulting in the same PNEC as that for freshwater, i.e. $PNEC_{saltwater\_lt}$ = $PNEC_{freshwater\_lt}$ of 74 µg l^{-1}.

The proposed PNEC is approximately two times higher than the existing tentative EQS of 40 µg l^{-1}. This reflects new data that have become available since the original EQS was derived and the use of the combined freshwater and saltwater dataset. The existing EQS was based on an assessment factor of 100 applied to an acute LC50 for bay shrimp, *Crangon franciscorum* (3.7 mg l^{-1}).

5.2.4 Short-term PNEC for saltwaters

A slightly larger dataset is available for short-term saltwater exposures with toluene. Crustaceans are the most sensitive taxonomic group for both marine and freshwater species with the lowest valid acute effects being nearly identical (LC50 of 3.78 mg l^{-1} for the freshwater crustacean *Ceriodaphnia dubia* and LC50 of 3.70 mg l^{-1} for the marine crustacean *Crangon franciscorum*). As a result, the saltwater data is used for the derivation of the short-term PNEC.

As crustaceans are the most sensitive species with respect to long- and short-term exposure, and because toluene acts non-specifically by narcosis, a reduced assessment factor (from 100 to 10) is recommended to extrapolate from the 50 per cent acute effect level to the short-term no-effect level. This results in a $PNEC_{saltwater\_st}$ of 370 µg l^{-1}.

The proposed PNEC is slightly lower than the existing EQS of 400 µg l^{-1}. It has the same basis, but differs slightly because it has been rounded up.

5.2.6 PNECs for sediments and secondary poisoning

Since toluene does not preferentially partition into sediment and does not bioaccumulate to any significant extent, there is no justification for deriving PNECs based on the risks of secondary poisoning to mammals and birds.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1})	Existing EQS (µg l^{-1})
Freshwater/long-term	74	50
Freshwater/short-term	380	500
Saltwater/long-term	74	40
Saltwater/short-term	370	400

5.3 Analysis

The lowest proposed PNEC derived for toluene is 74 µg 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 in the ng l^{-1} range, which suggests that they would be adequate for assessing compliance with the proposed PNECs for water.

5.4 Implementation issues

The proposed short-term PNECs are recommended for adoption as EQSs. However, existing long-term EQSs are lower (more stringent) than those proposed in this report and thus, under the 'no deterioration' principle, should be retained.

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List of abbreviations

AA	annual average
AF	assessment factor
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstracts Service
EC50	concentration effective against 50% of the organisms tested
ECx	concentration effective against X% of the organisms tested
EHC	Environmental Health Criteria
ELS	early life stage
EQS	Environmental Quality Standard
ET50	exposure time required for a defined effect to be observed among 50% of a population when that population is treated with a known amount or concentration of a toxicant
FID	flame ionisation detection
GC	gas chromatography
GLP	Good Laboratory Practice (OECD)
HSDB	Hazardous Substances Data Bank
IC50	concentration at which the population effect of the organisms tested is inhibited by 50%
IUPAC	International Union of Pure and Applied Chemistry
LC50	concentration lethal to 50% of the organisms tested
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
lt	long term
MAC	maximum allowable concentration
MATC	maximum allowable toxicant concentration
MS	mass spectrometry
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PID	photoionisation detection
PNEC	predicted no-effect concentration

ppm	parts per million
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
TLm	median threshold limit
UKTAG	UK Technical Advisory Group
US EPA	US Environmental Protection Agency
VOC	volatile organic compound
WFD	Water Framework Directive
WHO	World Health Organization

ANNEX 1 Data quality assessment sheets

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

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

Table A1 Klimisch Criteria*

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

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

Reference number	21

Information on the test species	
Test species used	*Criolana borealis*
Source of the test organisms	Caught in Skogsvaag (60°16'N, 05°06'E) in 90 m depth
Holding conditions prior to test	Running sea water (8–10°C, salinity 33.5–34.5 ppt)
Life stage of the test species used	individuals of similar size (100–200 mg ash free dry weight)

Information on the test design	
Methodology used	Not stated
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Sea water
Test concentrations used	0, 0.0125, 1.25, 5.7, 12.5, 25 and 125 ppm
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)	Semi-static (open) – replacement of test media each second day
Measurement of exposure concentrations	Nominal
Measurement of water quality parameters	Temperature, salinity
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	NOEC, ET50
Study conducted to GLP	Not stated

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

Reference number	**22**

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

Information on the test design	
Methodology used	Not stated
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static (open)
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Temperature 16°C, salinity 25 ppt
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	LC50
Study conducted to GLP	Not stated

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

Reference number	24

Information on the test species	
Test species used	*Chlamydomonas reinhardtii*
Source of the test organisms	Strain 11-32A SAG (+)
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Toluene formulation
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	2E +6 cells per ml
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static (2 hours duration)
Measurement of exposure concentrations	Unmeasured
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	EC10
Study conducted to GLP	Not stated

Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference number	**26**

Information on the test species	
Test species used	*Pimephales promelas*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	30 days

Information on the test design	
Methodology used	Not stated
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	No measurement
Measurement of water quality parameters	pH 7.6, temperature 25°C, hardness 45 mg l^{-1} $CaCO_3$
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	LOEC
Study conducted to GLP	Not stated

Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference number	27

Information on the test species	
Test species used	*Daphnia spinulata*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	<24 hours

Information on the test design	
Methodology used	US EPA 1982*
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Artificial pond water
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Temperature 20 (19–21) °C, pH 7.8 (7.6–8.0), hardness 95.8 (89.8–101.8) mg l^{-1} $CaCO_3$, alkalinity 189.3 (174.8–203.8) mg l^{-1} $CaCO_3$
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	EC50 – results expressed in mM
Study conducted to GLP	Yes

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

* US Environmental Protection Agency, 1982 *Environmental effects test guidelines*. EPA 560/6-82-002. Washington, DC: Office of Pesticide and Toxic Substances.

Reference number	28

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

Information on the test design	
Methodology used	OECD 1981*
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Triplicate
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static (closed)
Measurement of exposure concentrations	Nominal (checked beginning and end)
Measurement of water quality parameters	Oxygen, hardness, pH, conductivity and temperature.
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Results presented in log 1/LC50 (mg l^{-1}) Converted into LC50 mg l^{-1}. Confirmed conversion with author.
Study conducted to GLP	Yes

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

Reference number	**29**

Information on the test species	
Test species used	*Amphidinium carterae* and *Dunaliella tertiolecta*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Sea water
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static (closed)
Measurement of exposure concentrations	Yes, but not all. Where measured, large deviations from target (i.e. nominal) concentrations
Measurement of water quality parameters	Temperature 18°C
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	Cell growth/inhibition/stimulation/NOEC
Study conducted to GLP	Not stated

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

Reference number	33

Information on the test species	
Test species used	*Selenastrum capricornutum*
Source of the test organisms	Not stated
Holding conditions prior to test	Bristol medium
Life stage of the test species used	Exponential growth phase

Information on the test design	
Methodology used	Not stated
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Bristol medium
Test concentrations used	Five concentrations
Number of replicates per concentration	Four replica experiments per concentration
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static (8 days)
Measurement of exposure concentrations	Measured (CG)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	21.5% inhibition of growth at 5.5 mg l^{-1}
Study conducted to GLP	Not stated

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

Reference number	34

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	<2 days old

Information on the test design	
Methodology used	Not stated
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Temperature 22°C, pH 8.2, hardness 210 mg l^{-1} $CaCO_3$
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	EC50/LC50
Study conducted to GLP	Not stated

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

Reference number	38

Information on the test species	
Test species used	*Oncorhynchus gorbuscha*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Fry

Information on the test design	
Methodology used	Not stated
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static (open)
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Temperature 4°C, salinity 26–28 ppt
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	LC50
Study conducted to GLP	Not stated

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

Reference number	39

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

Information on the test design	
Methodology used	Not stated
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow through
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	pH (7.6–10)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Endpoint comment	LC50 and NOEC
Study conducted to GLP	Yes

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

Reference number	**41**

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

Information on the test design	
Methodology used	US EPA 1994*
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Artificial moderately hard water
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static (closed) Fed
Measurement of exposure concentrations	Measured beginning and end
Measurement of water quality parameters	Hardness 68.3 (49.5–87.1) mg l^{-1} $CaCO_3$ pH 7.6 (7.41–7.79)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	NOEL
Study conducted to GLP	Yes

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

Reference number	**42**

Information on the test species	
Test species used	*Pimephales promelas*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Larvae

Information on the test design	
Methodology used	Not stated
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	pH 7.65, temperature 25°C, hardness 45.5 mg l^{-1} $CaCO_3$
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	LOEC
Study conducted to GLP	Not stated

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

Reference number	46

Information on the test species	
Test species used	*Oncorhynchus gorbuscha*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Fry

Information on the test design	
Methodology used	Not stated
Form of the test substance	Toluene
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Measured
Measurement of water quality parameters	Temperature 12°C, salinity 26–28 ppt
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Endpoint comment	TLm Breathing rate
Study conducted to GLP	Not stated

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

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