Proposed Environmental Quality Standards for Ethylbenzene in Water

R&D Technical Report P2-115/TR4

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This report reviews the available data on the use, fate/behaviour and aquatic toxicity of ethylbenzene. Environmental Quality Standards have been proposed for the protection of aquatic life which will assist Agency staff in assessing the potential effects of this substance on water quality.

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R&D TECHNICAL REPORT P2-115/TR4

FOREWORD

This report, which proposes Environmental Quality Standards (EQSs) for the protection of fresh and saltwater life and water intended for human consumption from exposure to ethylbenzene, is one of a series produced under the Environment Agency/SNIFFER co-funded Phase V EQS contract.

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EXECUTIVE SUMMARY

This report, prepared for the Environment Agency and the Scottish Environment Protection Agency, reviews and critically assesses information available on the environmental fate and toxicity of ethylbenzene. The information is used to propose standards for the protection of aquatic life and a reference level for the protection of water abstracted to potable supply.

Ethylbenzene is an aromatic hydrocarbon mainly used in the production of polystyrene. It is also a component of petroleum, and the derived product is a mixture with benzene, toluene and xylenes, commonly known as BTEX. As a component of 'mixed xylenes' it is widely used as an organic solvent, for example in the rubber and chemical industries and as a diluent in paints and lacquers and as a motor fuel additive.

Volatilisation is likely to be the major process contributing to losses of ethylbenzene from water. In this medium it has a half life ranging from a few hours to several weeks. Aerobic biodegradation of ethylbenzene is rapid after a period of acclimatisation and half lives of between 2-20 days for this process have been reported. Other fate processes, such as photolysis, oxidation, and hydrolysis are unlikely to be significant. Few studies have been conducted on the fate and behaviour of ethylbenzene in soil. However, its moderate adsorption to soil indicates that some leaching may occur into groundwater.

The available aquatic toxicity data for ethylbenzene are limited mainly to short-term single species laboratory tests, and in the majority of cases, static laboratory tests. Few studies are available on the effect of long term exposure to aquatic organisms. No multispecies (e.g. mesocosms) or field studies have been reported in the open literature. Interpretation of toxicity data is dependent on appropriate preparation of stock and test solutions and the extent to which test concentrations have been maintained, particularly due to losses from volatilisation.

In studies where appropriate measures have been taken in the preparation of test solutions and maintenance of test concentrations, ethylbenzene is of moderate toxicity to aquatic organisms with lowest reliable effect concentrations of *ca.* 2 mg l^{-1}. Algae, *Daphnia* and fish are well-represented in the available acute toxicity data but few data exist for other aquatic taxa. Data obtained from experiments with marine species indicates a similar sensitivity to freshwater species. Ethylbenzene may exhibit moderate bioaccumulation in aquatic organisms, based on its log BCF and K_{ow} values of approximately 2 and 3, respectively.

Proposed standards are shown in Table S1.

A method for the determination of ethylbenzene in potable waters has been produced by the Standing Committee of Analysts (SCA). The method involves solvent extraction of the sample with pentane and separation and detection by gas chromatography with a flame ionization detector. The detection limit is 0.941 μg l^{-1}. This method would be adequate for the analysis of the proposed standards.

Table S1 Proposed standards for ethylbenzene (µg l^{-1})

Use	AA	MAC	Notes
Protection of freshwater life	20	200	
Protection of saltwater life	20	200	
Abstraction to potable supply	20		1

Notes to Table S1:

AA Annual Average
MAC Maximum Allowable Concentration
1 Based on taste and odour

KEY WORDS

Environmental Quality Standards, EQS, ethylbenzene, aquatic toxicity, bioaccumulation, freshwater, saltwater, mammalian toxicity, potable supply.

1. INTRODUCTION

Ethylbenzene is an aromatic hydrocarbon mainly used in the production of polystyrene. It is also a component of petroleum, and the derived product is a mixture with benzene, toluene and xylenes, commonly known as BTEX. As a component of 'mixed xylenes' it is widely used as an organic solvent, for example in the rubber and chemical industries and as a diluent in paints and lacquers and as a motor fuel additive.

This report reviews and critically assesses the information available on the inputs and concentrations of ethylbenzene in the environment (Section 2), the analytical methods available for its detection (Section 3), the fate and behaviour of ethylbenzene in the environment (Section 4 and Appendix A) and the aquatic and mammalian toxicity of ethylbenzene (Section 5 and Appendices B to D). This information is used, where possible, to derive Environmental Quality Standards (EQSs) for the protection of fresh and saltwater life and to recommend reference levels for the abstraction of water to potable supply.

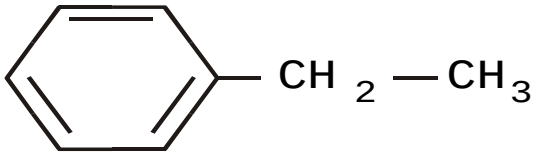
2. ETHYLBENZENE IN THE ENVIRONMENT

2.1 Physico-chemical properties

Ethylbenzene is an aromatic hydrocarbon. It is a component of the liquid petroleum product containing benzene, toluene, ethylbenzene and xylenes known as BTEX. The following sections of the report refer to ethylbenzene itself rather than the BTEX mixture, unless otherwise stated. A summary of the physico-chemical properties of ethylbenzene is provided in Table 2.1

Ethylbenzene is a colourless liquid which is immiscible with water and volatilizes readily due to its low solubility and relatively high vapour pressure at ambient temperatures. This is reflected in the high Henry's law constant (Table 2.1).

Table 2.1 Chemical and physical properties of ethylbenzene

IUPAC CHEMICAL NAMES	Ethylbenzene
SYNONYMS	Phenylethane, EB, Ethylbenzol
CAS NUMBER	100-41-4
MOLECULAR FORMULA	C_8H_{10}
MOLECULAR STRUCTURE	
MOLECULAR WEIGHT (g)	106.16
COMPOSITION	C 90.5% H 9.5%
APPEARANCE	Colourless liquid[3]
MELTING POINT (^oC)	-95.01[3]
BOILING POINT (^oC)	136.25[3]
FLASH POINT (^oC)	18[1]
VAPOUR PRESSURE (mm Hg)	9.53[3]
WATER SOLUBILITY (mg l^{-1}) @ 20^oC	152[2]
HENRY'S LAW CONSTANT (atm m^3 mol^{-1})	8.44 x 10^{-3}[3]
log K_{ow}	3.13[2], 3.15[3]
log K_{oc}	1.98-3.04[1]
Half life (days)	Several days to 2 weeks[3]

References:

1. Merck (1989)
2.WHO (1996)
3.Howard (1989)

2.2 Manufacture

Ethylbenzene is produced from petroleum as a mixture with benzene, toluene and xylenes (BTEX). It is not economical to use fractionation and ultrafractionation to extract the ethylbenzene from the mixture. Around 90% of ethylbenzene used in the chemicals industry is manufactured via alkylation of benzene using a soluble aluminium chloride catalyst. Ethyl chloride or hydrogen chloride can be used as a catalyst promoter. In 1983, the production of ethylbenzene in Western Europe was around 3 million tonnes (ECETOC 1986) with a similar amount produced in the USA (WHO 1996). Plant capacity in the UK was reported as around 310 000 tonnes in 1986, with the annual production figures predicted to remain at the 1986 level of 295 000 tonnes (DoE 1992).

2.3 Uses

The majority of ethylbenzene is manufactured and used on site to produce the styrene monomer for polystyrene. Ethylbenzene as a component of “mixed xylenes” is used as a motor fuel additive, component of solvents, as a diluent in paints and lacquers and as a solvent in the rubber and chemical manufacturing industries (WHO 1996). Ethylbenzene is also a constituent of asphalt and commercial naphtha (Verschueren 1996).

2.4 Entry into the aquatic environment

Ethylbenzene can enter the aquatic environment from industrial sites as a result of surface run-off, although this will be a minor route due to its low solubility and high vapour pressure. Point sources include effluent discharges or spills from manufacturing plants and petroleum refining. As ethylbenzene comprises around 4.6% of gasoline, diffuse sources will include the hydrocarbon in run-off from roads and in seepage from boats etc. Crude oil is also an important source of ethylbenzene (Benville and Korn, 1977).

2.5 Concentrations in the aquatic environment

Ethylbenzene has a low solubility in water and a high volatility. It is therefore likely to partition into the vapour phase and concentrations in the aquatic environment will be low. Water samples collected from UK monitoring stations (estuaries and offshore sites) in 1992 did not contain ethylbenzene above the detection limit (10 ng l^{-1}) except, on one occasion, in a sample taken from the Tees mid-estuary (Dawes and Waldcock 1994).

3. ANALYSIS

3.1 Analytical requirements for EQS monitoring

The adequate monitoring of EQSs requires a suitably accurate analytical method. The accepted approach for the derivation of the accuracy requirements of an analytical system (when monitoring to a particular water quality standard) is described in WRc Report NS30 (Cheeseman *et al* 1989).

For an EQS of X units, the error on a single analytical result should not be larger than X/10 concentration units or 20% of the concentration in the sample, whichever is the greater. Following the convention of dividing the tolerable error equally between random and systematic sources, this implies:

- a maximum tolerable standard deviation of X/40 concentration units or 5% of the concentration in the sample, whichever is the greater; and
- a maximum tolerable bias of X/20 concentration units or 10% of the concentration in the sample, whichever is the greater.

It is recommended that the target limit of detection should be set at X/10 concentration units.

For example, for a proposed EQS of 1 mg l^{-1}:

- the limit of detection should be 0.1 mg l^{-1} or less;
- the total error should not exceed 0.1 mg l^{-1} or 20% of the determinand concentration (whichever is the greater);
- the systematic error or bias should not exceed 0.05 mg l^{-1} or 10% of the determinand concentration (whichever is the greater); and
- the total standard deviation of individual results should not exceed 0.025 mg l^{-1} or 5% of the determinand concentration (whichever is the greater).

3.2 Analytical Techniques

A method for the determination of ethylbenzene in potable waters has been produced by the Standing Committee of Analysts (SCA) in the Blue Book "Determination of very low concentrations of hydrocarbons and halogenated hydrocarbons in water" (HMSO 1987).

The method involves solvent extraction of the sample with pentane; removal of non-hydrocarbons with florisil; concentration; and separation and detection by gas chromatography (GC) with a flame ionization detector (FID). The recovery is around 100%. The calibration is linear over the range of application (0–20 μg l^{-1}), with a detection limit (LOD) of 0.941 μg l^{-1}. The reported total standard deviation is 0.04 μg l^{-1} for the low

concentration standard (1 μg l^{-1}) and 2.60 μg l^{-1} for the higher concentration standard (20 μgl^{-1}).

A number of other methods have been reported in the open literature for the analysis of ethylbenzene, however few are applicable for the determination of low concentrations in environmental samples. One exception is a new methodology for the analysis of groundwater samples using compound-specific isotopic analysis (Dempster *et al* 1997). Pentane extraction was followed by compositional analysis using GC-FID and isotopic analysis using GC/combustion/isotope ratio mass spectrometry (GC/C/IRMS). Extraction efficiencies of 80-85% were obtained by this method and analysis was conducted in the range 0.1-100 mg l^{-1}.

An automated headspace analysis method has been developed by Otson and Kumarathasan (1995) for analysis of blood and water samples. An automated head space sampler was connected to a GC with FID detection. A minimum detection limit of around 1 μg l^{-1} was achieved.

Dawes and Waldcock (1994) analysed estuarine and marine samples for 13 volatile organic compounds including ethylbenzene using an automatic purge and cryotrapping method. In this method, separation was achieved by GC and analysis by quadrupole MS (electron impact ionization). The internal standard was deuterated toluene. The linear range for ethylbenzene was unknown, but the reported limit of detection was 10 ng l^{-1}.

Leonard *et al* (1998) reported a high speed gas extraction-gas chromatography method for ethylbenzene. The hydrocarbon was extracted from samples (1-2 ml) using elevated temperatures (95 °C) and a reflux condenser. Extract was injected on a 250 nm DB-1 column (8 m x 0.25 mm i.d.) with hydrogen as the carrier gas (200 cm/s) and with an oven temperature of 65 °C. Detection was with FID. The calibration graphs were linear from 100-10 000 μg l^{-1}, with an LOD of 2.3 μg l^{-1}. The RSD was 8.9% (n=4) and recovery was approximately 90%.

4. FATE AND BEHAVIOUR IN THE ENVIRONMENT

Ethylbenzene has a low solubility in water and a relatively high vapour pressure. It therefore volatilises readily from water with a half life ranging from a few hours to several weeks. Ethylbenzene does not absorb light above 290 nm and is therefore unlikely to be directly photolysed in water. Hydrolysis is not expected to be a significant loss process. Oxidation by hydroxyl radicals in air is rapid and it is possible that ethylbenzene is removed from aquatic systems by similar processes (WHO 1996).

The log K_{ow} and K_{oc} for ethylbenzene have been reported as 3.13-3.15 and 1.98-3.04, respectively (Merck 1989, WHO 1996, and Howard 1989). These data suggest that ethylbenzene will have a moderate to low tendency to adsorb to soils, sediments and suspended matter although, in practice, such residues are likely to be low due to the predominating effect of losses by volatilisation.

Aerobic biodegradation of ethylbenzene is rapid after a period of acclimatisation. In seawater, half lives of 2.1 and 20 days were observed in summer and spring, respectively, with appreciable lag times occurring in spring (Wakeham *et al* 1983). Laboratory bioreactor studies have shown biodegradation up to 98% within 37 days (van der Hoek *et al.* 1989). Anaerobic biodegradation is slower than aerobic degradation and a 30 day lag period has been observed in an aquifer microcosm under denitrifying conditions (Hutchins *et al.* 1991). Under methanogenic conditions, no degradation was observed after 20 weeks, however after 40 and 120 weeks ethylbenzene was degraded to 26% and <1% respectively (Wilson *et al.* 1986).

Few studies have been conducted on the fate and behaviour of ethylbenzene in soil. However, the data available suggest that some leaching may occur into groundwater. This has been illustrated by a study set up following a pipeline break in the USA (Cozzarelli *et al.* 1990). In soil, hydrolysis and photolysis are unlikely to be important fate processes and biodegradation is probably an important loss process for ethylbenzene following a period of acclimatisation. *Pseudomonas* has been shown to degrade ethylbenzene, with the formation of hydroxy intermediates (Gibson *et al*. 1973).

5. SUMMARY OF AQUATIC TOXICITY AND BIOACCUMULATION

5.1 Introduction

This section summarises the available data on the aquatic toxicity of ethylbenzene, highlighting those values of most significance to the derivation of EQS values (Table 5.1). A more detailed consideration of the data is made in Appendices B, C and D.

The available aquatic toxicity dataset for ethylbenzene is limited to short-term single species laboratory tests, and in the majority of cases, static laboratory tests. Although data for only 16 freshwater species have been located (and 13 saltwater species) numerous acute studies have been carried out with several of them (notably freshwater algae, *Daphnia* and fish). By contrast, few studies are available on the effect of long term exposure to aquatic organisms. No multispecies (e.g. mesocosms) or field studies have been reported in the open literature. No data could be located for freshwater macrophytes, annelids, molluscs, insects or amphibians, or for marine bacteria, annelids and echinoderms. Additionally, the dataset for ethylbenzene was insufficient to enable any assessment of the influence of water quality parameters (e.g. hardness, pH) or environmental conditions (e.g. temperature) on observed toxicity.

The most important consideration in the evaluation of toxicity data for ethylbenzene concerns the extent to which reported effect concentrations reflect actual exposure concentrations. Inaccurate preparation of stock and test solutions is likely unless dilutions are prepared from a saturated stock solution from which undissolved test substance has been removed. A number of reports describe effects at concentrations in excess of the water solubility of ethylbenzene and these must be regarded as suspect. Furthermore, losses due to volatilisation may lead to a marked under-estimation of test concentrations and hence, to estimated effect concentrations. Whilst a significant number of studies (particularly with *Daphnia* and to a lesser extent with unicellular algae) have been carried out using closed test systems and with analytical confirmation of test solutions, many others have not. Only where testing has been performed using closed test vessels, and/or test solutions have been confirmed by analysis, have test data been considered suitable for the derivation of standards. Table 5.1 summarises those studies where this requirement has been met. These also tend to be the lowest effect concentrations, probably because of more accurate generation and maintenance of test concentrations with the result that the risks of under-estimating toxicity have been reduced.

The toxic effects in aquatic organisms of ethylbenzene result from nonpolar narcosis (Niederlehner *et al.*, 1998). Compared to other related aromatic hydrocarbons, ethylbenzene is more toxic to aquatic organisms than benzene or toluene and exhibits similar acute toxicity to xylenes, isopropyl benzene and *n*-propyl benzene (Benville and Korn, 1977; Galassi *et al.*, 1988).

5.2 Freshwater toxicity

Freshwater bacteria do not appear to be very sensitive to ethylbenzene with 24 hour IC50 values of around 159 mg l^{-1} (corrected for pKa and pressure) for *Nitrobacter* (Tang *et al.*

1992) and 96 mg l^{-1} for *Nitrosomonas* (Blum and Speece (1991). The lowest data are for *Pseudomonas putida* with a Toxicity Threshold value of 12 mg l^{-1} (Bringmann and Kuhn 1980). No data are available for saltwater species.

The data relating to the effect of ethylbenzene on freshwater algae covers an order of magnitude. The lowest reliable data are from two closed static tests using the green alga (*Selenastrum capricornutum*). EC50 (growth) values of 4.6 and 5.4 mg l^{-1} were reported by Galassi *et al.* 1988 and Masten *et al.* 1994, respectively, following 72 hours exposure. After 96 h, the EC50 to this species had declined to 3.6 mgl^{-1} (Masten *et al.*, 1994). The tests were conducted under standard OECD and EPA guidelines respectively, modified for the testing of volatile substances and were supported by analysis of test solutions.

The majority of EC50 data for freshwater invertebrates relate to *Daphnia magna* and range from around 2 mg l^{-1} to 200 mg l^{-1}. The higher values are mainly reported in older, less reliable studies where exposure conditions are either not well described or where losses due to volatilisation have not been adequately accounted for. The lowest *Daphnia* EC50 values (*ca.* 2 mgl^{-1}) are the lowest of all the acute data reported for ethylbenzene and were achieved in static test regimes but using closed vessels and with analytical verification of test concentrations. Probably the most reliable is that reported by Galassi *et al*. (1988) where tests were initiated using saturated solutions of ethylbenzene, closed test vessels were employed in which any air space above the test solutions was eliminated, and test solutions were subjected to analytical confirmation. This study gave rise to a 24 h EC50 of 2.2 mgl^{-1}.

Only one chronic study (on the effect of ethylbenzene on reproduction in the freshwater crustacean, *Ceriodaphnia dubia*) has been located. A 7-day NOEL of 9 mg l^{-1} and LOEL of 16 mg l^{-1} for reproductive effects was reported by Niederlehner *et al*. (1998) in a study carried out according to methods described in the USEPA's Whole Effluent Testing Programme, with modifications to minimise the volatilisation of test chemical. Compared with a corresponding acute (48h) LC_{50} of 30 mgl^{-1} in the same study, only a small acute:chronic ratio is indicated.

Fish toxicity data reported in the open literature indicate LC_{50} values ranging between 1 mg l^{-1} and 285 mgl^{-1} and relate to short term (acute) exposure only. No data could be located on the long term effects of ethylbenzene to freshwater fish. The lowest acute LC_{50} value to fish was cited in Vittozi and De Angelis (1991) but it has not been possible to examine or assess the quality of the data. However, Galassi *et al.* (1988) determined a 96 h LC50 of 4.2 mg l^{-1} for rainbow trout under static conditions using closed bottles and with renewal of test solution at 48 hours. The test was carried out according to OECD guideline 203 (modified for testing volatile compounds) and test concentrations were analysed by HPLC.

5.3 Saltwater toxicity

Freshwater algal toxicity data are comparable with those reported for the marine alga *Skeletonema costatum* (EC50s (growth inhibition) of 4.9-8 mg l^{-1}) (Masten *et al.* 1994). The lowest reliable effect concentration is a 96h EC_{50} of 7.7 mgl^{-1} conducted under EPA standard guidelines, adapted for the volatile test compound.

The dataset for marine invertebrates relates mainly to crustaceans and EC50 values are in the same order of magnitude as those seen with the algae. The lowest reliable effects concentrations arise from a study using the mysid shrimp (*Mysidopsis bahia*) and reported an

acute (96h) NOEC and LOEC (for survival) of 1.0 and 2.7 mg l^{-1}, respectively (Masten *et al.* 1994). Although a lower value has been reported (a 96h LC_{50} to the bay shrimp, *Crago franciscorum,* of 0.49 µl l^{-1}), this experiment was performed in open vessels and it is clear that most of the ethylbenzene introduced into the test vessels (>99%) had volatilised after 48h. It is noteworthy that the LC_{50} declined from 24h to 96h by a factor of 4 (from 2.2 µl $l^{-1}$to 0.49 µl l^{-1}) even though most of the ethylbenzene had volatilised from test vessels at concentrations bracketing the 24h LC50 (>99% loss at 1.0 µl $l^{-1}$and 67% at 4.9 µl l^{-1}). It is not clear from the paper describing this study the basis for the reported LC50 values. Furthermore, the absence of density data for ethylbenzene prevents conversion of toxicity data reported in terms of µl l^{-1} to µg l^{-1}. Consequently, this study is considered unsuitable for the derivation of standards.

Tokuda (1984) investigated the behaviour in light of the motile spores of the seaweed, *Ulva pertusa*. Although phototaxis was impaired at concentrations as low as 0.19 mg l^{-1}, the extent of inhibition is not defined. Coupled with uncertainties about the ecological significance of the endpoint used, it is felt these data are not suitable for the derivation of standards.

Marine data for molluscs are restricted to a 96 hour LC50 of 1030 mg l^{-1} developing embryo-larvae of the Pacific oyster *Crassostrea gigas.* (LeGore cited in ECDIN 1991).

Effects concentrations for marine fish species are broadly similar to freshwater toxicity values. The lowest reliable effects concentration is a 96 h LC50 of 5.1 mg l^{-1}, estimated from a study conducted according to ASTM/EPA standard guidelines for the Atlantic silverside (*Menidia menidia*). A NOEC and LOEC (survival) of 3.3 and 5.9 mg l^{-1} were also reported for this study (Masten *et al.* 1994).

5.4 Bioaccumulation

With a log K_{ow} value of approximately 3, a moderate tendency to bioaccumulate is indicated, and this is borne out in experimental studies using fish and algae (Herman *et al.*, 1991; EUCLID, 1996; Mancha *et al.*, 1997). Herman *et al.* (1991) noted that there was a greater bioaccumulation potential for ethylbenzene than there was for benzene (BCF 1.63) and toluene (BCF 1.99) and values were comparable to the xylenes (2.34 - 2.41). This ranking is the same as that emerging from studies into the acute toxicities of these substances to aquatic life (Benville and Korn, 1977; Galassi *et al.*, 1988).

Further details of bioaccumulation studies are to be found in Appendix B2.

Table 5.1 Lowest reliable acute and chronic toxicity data for ethylbenzene to freshwater and saltwater organisms

Species	Exposure time	Concn ($mg\ l^{-1}$)	Effect	Ref
Freshwater				
Selenastrum capricornutum (Green alga)	72 h	4.6	EC50 (growth inhibition)	1
Selenastrum capricornutum (Green alga)	72 h 96h	5.4 3.6	EC50 (growth inhibition) EC50 (growth inhibition)	2
Daphnia magna (Water flea)	24 h	2.2	EC50 (immobilisation)	1
Daphnia magna (Water flea)	48 h	2.93-2.97	EC50 (immobilisation)	5
Daphnia magna (Water flea)	24 h 48h	2.27-2.89 1.81-2.41	EC50 (immobilisation) EC50 (immobilisation)	6
Daphnia magna (Water flea)	48 h	2.93-2.97	EC50 (immobilisation)	5
Ceriodaphnia dubia (Water flea)	7 d 7 d	9 16	NOEL (reproduction) LOEL	4
Oncorhynchus mykiss (Rainbow trout)	96 h	4.2	LC50	1
Saltwater				
Skeletonema costatum (Diatom)	96 h	7.7	EC50 (growth)	2
Artemia salina (Brine shrimp)	48 h	6.5	EC50 (immobilisation)	5
Mysidopsis bahia (Mysid shrimp)	96 h	2.6 2.7 1.0	LC50 LOEC (survival) NOEC (survival)	2
Menidia menidia (Atlantic silverside)	96 h	5.1 3.3	LC50 NOEC (survival)	2

References:

1. Galassi *et al.* (1988)
2. Masten *et al.* (1994)
3. Tosato *et al.* (1993)
4. Niederlehner *et al.* (1998)
5. Maclean and Doe (1989)
6. Vigano (1993)

6. DERIVATION OF EQSs

6.1 Standards in other countries

The Water Supply (Water Quality) Regulations (HMSO 1989) implementing the EC Drinking Water Directive, do not stipulate a standard specifically for ethylbenzene.

However, Canada has adopted an interim standard for ethylbenzene of 90 μg l^{-1} expressed as a Maximum Allowable Concentration (MAC). The standard was derived by applying a safety factor of 20 to the 48 h EC50 (immobilisation) value of 1800 μg l^{-1} for *Daphnia magna* (water flea), identified as the most sensitive organism tested (Vigano, 1993). The safety factor was reported to take account of the non-persistent nature of ethylbenzene and extrapolation of acute data to a NOAEL in the field (CCME 1999).

An interim standard for the protection of saltwater organisms of 25 μg l^{-1} has been adopted by Canada and is expressed as a MAC. It was derived by applying a safety factor of 20 to the 24 h LC50 value for the bay shrimp (*Crago franciscorum*) (Benville and Korn 1977). Again, the safety factor was intended to take account of the non-persistent nature of ethylbenzene and extrapolation of acute data to a NOAEL in the field (CCME 1999).

A number of Environmental Quality Objectives have been derived in the Netherlands (RIVM 1999) and these are summarised in Table 6.1.

Table 6. 1 Maximum permissible concentrations (MPCs) and negligible concentrations (NCs) for different environmental compartments

	Surface Water (μg l^{-1})	Soil (mg kg^{-1})	Sediment (mg kg^{-1})	Air (μg/m^3)
MPC	370	3.1	3.1	39
NC	3.7	0.031	0.031	0.39

6.2 Protection of freshwater life

Data on the toxicity of ethylbenzene to freshwater and saltwater life are limited to 16 freshwater species. Nevertheless, numerous studies have been performed with some of the species (Table B1), and a high proportion have been carried out according to standard test guidelines (e.g. OECD, EPA), often with modifications to reduce losses due to volatilisation (e.g. use of sealed test vessels) and with analytical verification of stock and/or test concentrations.

Ethylbenzene is of moderate toxicity to freshwater organisms, with no taxon exhibiting particularly high sensitivity. Effects concentrations for the most reliable studies (Table 5.1) lie between 2 and 4 mg l^{-1} for algae, crustaceans and fish following acute exposure. The only chronic study is one describing the effects of ethylbenzene on reproduction of *Ceriodaphnia*

dubia (Neiderlehner *et al.*, 1998). This study led to a 7 d LOEC and NOEC of 16 and 9 mg l^{-1}, respectively.

The lowest reliable effects concentration is a 24 hour EC50 (immobilisation) of 2.2 mg l^{-1} for *Daphnia magna* (Galassi *et al*., 1988) which was generated from a study where appropriate measures were taken to control loses of ethylbenzene due to volatilisation and where test concentrations were verified by analysis. To protect freshwater life from short term exposure to ethylbenzene, an EQS of 200 $\mu g l^{-1}$ is proposed. This is derived by applying a safety factor of approximately 10 to the 24 hour EC50 (immobilisation) of 2.2 mg l^{-1} for *Daphnia magna.* This EQS should be expressed as a Maximum Allowable Concentration (MAC). To protect freshwater life from long term exposure to ethylbenzene, an Annual Average (AA) of 20 $\mu g\ l\text{-}^{1}$ is proposed, derived by applying a safety factor of 100 to the same effect concentration. Although chronic toxicity data are not plentiful, this AA should provide adequate protection to aquatic life in the event of prolonged exposure to ethylbenzene by virtue of (a) the substantial safety factor involved, (b) the standard being substantially higher than the reported *Ceriodaphnia* chronic NOEC and (c) also higher than chronic effects seen in studies with related aromatic hydrocarbons such as xylenes and benzene (Hedgecott and Lewis, 1997a; 1997b).

6.4 Protection of saltwater life

The toxicity dataset for saltwater organisms is represented by a similar number of species (13) as the freshwater dataset but these species have been subject to much less intensive investigation. The data presented in Table B1 indicate that saltwater organisms have a similar sensitivity to ethylbenzene as freshwater organisms. The lowest reliable effects concentration is a 96 h LC_{50} of 2.6 mgl^{-1} and corresponding LOEC of 2.7 mgl^{-1} to the mysid shrimp (*Mysidopsis bahia*) (Masten *et al.*, 1994). Applying a safety factor of approximately 10 to this value, and bearing in mind the similar sensitivities of freshwater and saltwater species, a standard of 200 μgl^{-1} results. As with the proposed standards for freshwater life, an Annual Average of 20 μgl^{-1} is also proposed to protect against the effects of possible long-term or continuous exposure.

In proposing these saltwater standards, it should be noted that the data used by Canada's CCME (24 h LC50 value for the bay shrimp (*Crago franciscorum*) (Benville and Korn 1977)) to derive its standard have been excluded from this EQS derivation for the reasons explained in Section 5.3.

6.6 Abstraction of water to potable supply

This section outlines the derivation of reference levels for abstraction of water to potable supply. Reference levels may be based on either mammalian toxicology (to determine a level for the protection of human health) or on the taste and odour properties of a substance. A summary of the mammalian toxicity data is given in Appendix D.

The mammalian toxicity of ethylbenzene was reviewed by the World Health Organization (WHO) in its 1993 revision of the Drinking Water Guidelines (WHO 1993, WHO 1996) and also under the International Programme on Chemical Safety (IPCS 1996).

In 1993, WHO recommended a tolerable daily intake (TDI) of 97.1 μg kg^{-1} body weight day^{-1} (WHO 1993). This was based on applying an uncertainty factor of 1000 to a NOAEL of 97.1 mg kg^{-1} body weight day^{-1} established in a limited 6-month gavage study in rats. Assuming a 60 kg adult drinking 2 litres of water per day and 10% allocation of the TDI to drinking water, a health-based guideline value of 300 μg l^{-1} was proposed.

However, low odour and taste threshold concentrations of between around 2-550 and 30-780 μg l^{-1}, respectively, have been reported for ethylbenzene (WHO 1996). The taste and odour threshold concentration is most likely to be between 20 to 200 μg l^{-1}, although the lowest reported odour threshold is around 2 μg l^{-1}.

A reference level of 20 μg l^{-1} is proposed for ethylbenzene based on taste and odour considerations.

7. CONCLUSIONS

1. Ethylbenzene is an aromatic hydrocarbon mainly used in the production of polystyrene. It also occurs as a component of crude oil and petroleum, and the derived product is a mixture with benzene, toluene and xylenes, commonly known as BTEX. As a component of 'mixed xylenes', it is widely used as an organic solvent, for example in the rubber and chemical industries and as a diluent in paints and lacquers and as a motor fuel additive.

2. Ethylbenzene is a colourless liquid which floats on water and volatilizes readily due to its low solubility, and relatively high vapour pressure. Its volatilisation half life ranges from a few hours to several weeks. Photolysis, oxidation, hydrolysis and sorption are unlikely to be significant loss processes from water.

3. Ethylbenzene is biodegraded relatively rapidly under aerobic conditions following a period of acclimatisation. In seawater, half lives of 2.1 and 20 days were observed in summer and spring respectively. However, anaerobic biodegradation is slower.

4. Few studies have been conducted on the fate and behaviour of ethylbenzene in soil. However, its moderate adsorption to soil indicates that volatilisation is likely and some leaching into groundwater is possible.

5. The available toxicity dataset for ethylbenzene is limited mainly to short-term single species laboratory tests, in the majority of cases using static test regimes. Few studies are available on the effect of long term exposure to aquatic organisms. No multispecies (e.g. mesocosms) or field studies have been reported in the open literature.

6. In studies where appropriate measures have been taken in the preparation of test solutions and maintenance of test concentrations, ethylbenzene is of moderate toxicity to aquatic organisms with lowest reliable effect concentrations of *ca.* 2 mg l^{-1}. Algae, *Daphnia* and fish are well-represented in the available acute toxicity data but few data exist for other aquatic taxa.

7. Ethylbenzene may exhibit moderate bioaccumulation in aquatic organisms, based on its log BCF and K_{ow} values of approximately 2 and 3, respectively.

8. A Maximum Acceptable Concentration of 200 μg l^{-1} and an Annual Average of 20 μg l^{-1} are proposed for the protection of freshwater and saltwater life.

9. For the abstraction of water to potable supply, a reference level of 20 μg l^{-1} is proposed for ethylbenzene based on aesthetic considerations.

10. Current analytical methods are adequate for monitoring the proposed guidelines and reference level.

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APPENDIX A FATE AND BEHAVIOUR IN THE ENVIRONMENT

A1 FATE AND BEHAVIOUR IN SOIL

A1.1 Abiotic processes

Ethylbenzene exhibits a moderate adsorption to soil (Howard 1989) and volatilisation will predominate. Hydrolysis and photolysis are unlikely to be significant fate processes in soil.

A1.3 Biodegradation

Little information is known regarding the biodegradation of ethylbenzene in soil. However, biodegradation studies in water indicate rapid aerobic biodegradation after an initial lag period, and this is likely to be the case with unacclimated soil. Microbial oxidative degradation has been shown to proceed via hydroxylation of the benzene ring to give 2,3-dihydroxy-1-ethylbenzene (Gibson *et al.* 1973) and similarly a 2,3-hydroxy intermediate has been postulated in the degradation of ethylbenzene by *Pseudomonas* cultures followed by a meta-cleavage of the benzene ring (Smith and Ratledge 1989).

A1.4 Sorption

The moderate adsorption to soil indicates that leaching is likely to occur (Howard 1989). This has been illustrated in a study following pollution from a pipeline break, where ethylbenzene was observed to leach into a shallow aquifer (Cozzarelli *et al* 1990).

A2 FATE AND BEHAVIOUR IN WATER

Ethylbenzene has a low solubility in water and a relatively high vapour pressure. The calculated Henry's Law Constant is 8.44 x 10^{-3} atm m^3 mol^{-1}, which suggests that volatilisation will be an important fate process in the aquatic environment. Half-lives ranging from a few hours to several weeks have been reported (Howard 1989).

The rate of volatilisation was illustrated in a laboratory study using an upflow aerated column (UAC) designed for the removal of ethylbenzene from contaminated groundwater and soil. Approximately 8% of ethylbenzene was collected in the vapour phase (van der Hoek *et al* 1989). Other studies concerning the volatilisation of ethylbenzene include a study on its removal from an acclimated activated sludge reactor. Around 22% was volatilised (six day sludge residence time) compared to 78% biodegradation. A mesocosm experiment to determine the fate and persistence of VOCs in seawater indicated that volatilisation was an important fate process, particularly in winter with a degradation half life of 13 days (Wakeham *et al* 1983).

The moderate K_{ow} of ethylbenzene indicates that some adsorption to sediments and suspended solids will occur. However, losses due to volatilisation are expected to be more important under most circumstances.

A2.1 Abiotic processes

Abiotic degradation is unlikely to be a significant loss process in the aquatic environment. Ethylbenzene does not absorb light above 290 nm and is therefore unlikely to be directly photolysed in water. Additionally, hydrolysis is not expected to be a significant loss process. Oxidation by hydroxyl radicals in air is rapid and it is possible that ethylbenzene could be removed from aquatic systems by similar processes (WHO 1996). However, this is unlikely to be a significant removal mechanism from water.

A2.2 Biodegradation

Biodegradation has been shown to be an important degradation pathway in aquatic systems, with half lives in spring and summer of 2.1 and 20 days in seawater, respectively (Wakeham *et al.* 1983). A lag period was observed indicating that acclimation was taking place, and was faster at warmer temperatures. Ethylbenzene was degraded to concentrations under 5 $\mu g\ l^{-1}$ within seven days under aerobic conditions in a mesocosm prepared from aquifer material (Hutchins 1991). In an aquifer field test, BTEX compounds underwent rapid biodegradation and were almost completely attenuated during a 16 month observation period (Schirmer and Barker 1998). In upflow aerated column (UAC) and rotating disc biological contactor (RBC) experiments, 96% of ethylbenzene in contaminated groundwater and soil was removed in 146 days (RBC) whereas 98% of ethylbenzene was removed after 37 days and 100% after 92 days in the UAC (van der Hoek *et al.* 1989).

A2.2.1 Anaerobic biodegradation

The bacterial metabolism of ethylbenzene has been recently reviewed (Heider *et al.* 1998). Ethylbenzene is oxidised at the methylene carbon to 1-phenylethanol and subsequently to acetophenone. After which it is carboxylated to 3-oxophenylpropionate and converted to benzoyl-CoA and acetyl-CoA.

In an aquifer microcosm study under methanogenic conditions, a long lag time was observed before the onset of biodegradation. Ethylbenzene was not degraded during the first 20 weeks, however after 40 weeks the concentration of ethylbenzene was reduced to 26% of the original concentrations. After 120 weeks, the concentration was less than 1% of the original (Wilson *et al.* 1986). A pipeline break in Minnesota, USA, resulted in contamination of a shallow aquifer. Degradation of ethylbenzene and other monoaromatic hydrocarbons occurred in the anoxic groundwater with the formation of organic acids (Cozzarelli *et al.* 1990).

A microcosm study using uncontaminated aquifer materials under denitrifying conditions indicated a 30 day lag period before biodegradation. Biodegradation was then rapid with high nitrate concentrations (75 mg l^{-1}) and significant even with a low concentration of nitrate (30 mg l^{-1}). With contaminated aquifer materials, the lag time was longer and biodegradation was much slower with insignificant biodegradation after six months (Hutchins *et al.* 1991). It has also been shown that in the absence of oxygen, nitrate or nitrous oxide was required for degradation of ethylbenzene (Hutchins 1991).

A3 BEHAVIOUR IN SEWAGE TREATMENT PROCESSES

A number of studies indicate that ethylbenzene is readily degraded after a period of acclimatisation. Weber (1987) reported that 78% (out of 82% total degradation) of ethylbenzene was biodegraded in a bench scale mixed batch reactor with a six day sludge residence time at a concentration of 0.1 mg l^{-1}. The innoculum used was adapted activated sludge and analysis of test concentrations was by gas chromatography. IUCLID (1996) reported 78% degradation of 0.029 mg l^{-1} ethylbenzene in a wastewater plant of an organic chemical manufacturing site using a combined powdered carbon-biological process. Test concentrations were analysed. The Japanese Chemicals Testing and Inspection Institute reported 81-100% degradation (of the ThOD) of 100 mg l^{-1} ethylbenzene after 14 days in the MITI test (IUCLID 1996).

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APPENDIX B FRESHWATER TOXICITY AND BIOACCUMULATION

B1 INTRODUCTION

Flow through tests are often preferred for rapidly degraded chemicals. Hydrolysis, photolysis, and biodegradation (for unacclimatised organisms) would not be significant over the duration of acute tests with ethylbenzene and, therefore, static tests would be adequate for assessing short term exposure to ethylbenzene. However, ethylbenzene is a volatile liquid hydrocarbon with a vapour pressures of 9.53 mmHg and a Henry's Law constant of 8.44×10^{-3} atm m^3 mol^{-1}. It will have a tendency to volatise from water and therefore, it is important for toxicity studies to be carried out in sealed test units to minimise its loss, and to allow an accurate assessment of toxicity.

Chronic studies would need semi-static or flowthrough test designs to maintain the desired concentration of the substance, ideally supported by the analysis of actual exposure concentrations to ensure that losses due to volatilisation are accounted for, and that overall toxicity is not underestimated.

The data relating to the freshwater toxicity of ethylbenzene are given in Table B1. Many of these studies have been performed in the context of deriving QSARs for nonpolar narcotic chemicals, of which ethylbenzene is an example (Niederlehner *et al.*, 1998).

B1.2 FRESHWATER TOXICITY

The available toxicity dataset for ethylbenzene (see Table B1) is limited mainly to short-term single species laboratory tests, and in the majority of cases, static laboratory tests. Only one chronic study for C*eriodaphnia dubhia* reproduction has been located in the open literature. No multispecies (e.g. mesocosms) or field studies could be located in the open literature. The dataset reported in subsections B1.2.1 to B1.2.4 covers three bacteria species, one protozoan species, three algal species, two crustacean species, and seven fish species.

No data are available at the present time for macrophytes, annelids, molluscs, insects or amphibians.

B1.2.1 Bacteria

Bacteria do not appear to be very sensitive to ethylbenzene with 24 hour IC50 values of around 159 mg l^{-1} (corrected for pKa and pressure) for *Nitrobacter* (Tang *et al.* 1992) and 96 mg l^{-1} for *Nitrosomonas* (Blum and Speece 1991). In the latter test, the inhibition of ammonia consumption was used as the criterion for toxic inhibition and nitrite was checked to ensure that only toxicity to *Nitrosomonas* and not to *Nitrobacter* was controlling the rate of metabolic activity. The Tang *et al.* study used a similar test method to ensure that the results were comparable, albeit for a different species. The lowest data are for *Pseudomonas putida* with a Toxicity Threshold value of 12 mg l^{-1} (Bringmann and Kuhn 1980). This test measured the concentration of bacterial suspension using a turbidity meter to assess the inhibition of growth. However, few details relating to test conditions were reported.

B1.2.2 Algae

The data relating to the effect of ethylbenzene on freshwater algae are variable, with EC50 values ranging from 3.6 to 51 mg l^{-1}. The lowest reliable data are for two closed static tests with the green algae (*Selenastrum capricornutum*). EC50 values (72 hour) of 4.6 and 5.4 mg l^{-1} were reported by Galassi *et al.* (1988) and Masten *et al.* (1994), respectively. The growth inhibition test reported by Galassi *et al.* was conducted under OECD standard guidelines, modified as appropriate in order to prevent hydrocarbon loss by volatilization. The concentrations of the substrates in the test solutions were also periodically measured by HPLC. Masten *et al.* followed an EPA test procedure modified by the use of sealed, filled vials as test vessels in place of partially filled Erlenmeyer flasks.

Other algal studies have shown that 13, 38, 46 and 48% inhibition of oxygen uptake by *Chlorella vulgaris* occurred at 13, 33, 65 and 130 mg l^{-1} respectively (Potera cited in ECDIN 1991).

B1.2.3 Invertebrates

Data relating to the toxicity of ethylbenzene to invertebrates are limited to crustaceans and mainly for the crustacean, *Daphnia magna*. Toxicity values for this species generally range from around 2 mg l^{-1} to 200 mg l^{-1} (Table B1). The higher values are mainly reported in older, less reliable studies where exposure conditions are either not well described or where losses due to volatilisation have not been adequately accounted for. The lowest *Daphnia* EC50 values (ca. 2 mgl^{-1}) were achieved in static test regimes but using closed vessels and with analytical verification of test concentrations. Probably the most reliable is that reported by Galassi *et al.* (1988) where tests were initiated using saturated solutions of ethylbenzene, closed test vessels were employed in which any air space above the test solutions was eliminated, and test solutions were subjected to analytical confirmation. This study gave rise to a 24 h EC50 of 2.2 mgl^{-1}.

One study has been conducted on *Ceridaphnia dubia* where a 48 h LC50 of 30 mg l^{-1} (Niederlehner *et al.* 1998) resulted in an experiment using sealed test vessels and with analytical verification of test solutions. This suggests that *Daphnia* may be less sensitive to ethylbenzene than *Ceriodaphnia*.

Only one study appears to be available on the effect of ethylbenzene on reproduction in crustacea. A 7-day NOEL of 9 mg l^{-1} and LOEL of 16 mg l^{-1} for reproductive effects was reported by Niederlehner *et al.* (1998). This study was carried out according to procedures described in the USEPA's Whole Effluent Testing Programme, with modifications to minimise volatilisation of test chemicals.

B1.2.4 Fish

Unlike the studies using daphnids, few of the toxicity studies with fish indicate measures to control losses of ethylbenzene due to volatilisation, possibly because of the practical difficulties in conducting fish studies in sealed vessels and the consequent depletion of dissolved oxygen levels.

Fish toxicity data reported in the open literature range from around 1 mg l^{-1} to 285 mg l^{-1} and relates to short term exposure only. No data could be located which describe possible effects of long term exposure of freshwater fish to ethylbenzene.

The lowest effect concentrations obtained with fish (1.15, 1.51 and 1.69 mg l^{-1} for 96 h LC50 with rainbow trout, bluegill sunfish and fathead minnows, respectively) were cited in Vittozi and De Angelis (1991). The original study reports are not available and, therefore, it is not possible to assess the quality of the data giving rise to these results.

Arguably the most reliable fish toxicity data arise from a study performed by Galassi *et al.* (1988). They determined a 96 h LC50 of 4.2 mg l^{-1} for rainbow trout under static conditions using closed bottles and with renewal of test solution at 48 hours. The test was carried out according to OECD guideline 203, modified as appropriate for the testing volatile compounds, and concentrations were analysed by HPLC.

Table B1 Toxicity of ethylbenzene to freshwater life

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3 l^{-1}$)	pH	Exposure time	Concn (mg l^{-1})	Effect	Ref
BACTERIA										
Nitrobacter	nd	S,cs	N	25	nd	8.5-9.1	24 h	259 (observed) 159 (H)	IC50	1
Nitrosomonas	nd	S,cs	N	25	nd	6.5-8	24 h	96 (H)	IC50	2
Methanogens	nd	S,cs	N	35	nd	7	48 h	160 (H)	IC50	2
Aerobic heterotrophs	nd	S,cs	N	35	nd	7	15 h	130 (H)	IC50	2
Pseudomonas putida	nd	S,cs	N	25	nd	nd	nd	12	TT (Toxicity threshold)	3
Pseudomonas putida	nd	S	nd	nd	nd	nd	nd	>100	Oxygen consumption inhibition test	4
PROTOZOA										
Entosiphon sulcatum Protozoa	nd	S,cs	N	25	nd	6.9	72 h	140	TT (Toxicity threshold)	3
ALGAE										
Scendesmus quadricauda	nd	S,cs	N	25	nd	nd	7 d	>160	TT (Toxicity threshold)	3
Selenastrum capricornutum	nd	S,cs	Y	nd	nd	nd	72 h	4.6	EC50 (growth)	5
Selenastrum capricornutum	nd	S,cs	Y	nd	nd	nd	8 d	4.8	EC50 (growth)	6

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3 l^{-1}$)	pH	Exposure time	Concn (mg l^{-1})	Effect	Ref
Selenastrum capricornutum	nd	S,cs*	Y	20±1	nd	7.5	24 h 48 h 72 h 96 h	13.4 (0.02-938) 7.2 (3.4-15.1) 5.4 (2.6-11.3) 3.6 (1.7-7.6)	EC50 (growth)	7
Chlamydomonas angulosa	nd	S	nd	nd	nd	nd	nd	51	EC50 photosynthetic inhibition	8
Chlorella vulgaris	nd	S	nd	nd	nd	nd	3h	63	Inhibition of photosynthesis	8
Chlorella vulgaris	nd	nd	nd	20	nd	nd	nd	13	17% inhibition of O_2 consumption	22
	nd	nd	nd	20	nd	nd	nd	130	48% inhibition of O_2 consumption	
	nd	nd	nd	30	nd	nd	nd	130	59% inhibition of O_2 consumption	
ARTHROPODS - CRUSTACEANS										
Daphnia magna Water flea	nd	S,cs*	Y	nd	nd	nd	24 h	2.2	IC50	5
Daphnia magna Water flea	neonates <24 hr	S,cs	Y	20	nd	7.8	48 h	2.9 (2.5-4.4) 18.4 (11.5-25.4) 13.9 (10.6-17.2)	EC50 (immobilisation) EC50 (immobilisation) EC50 (immobilisation)	9
Daphnia magna Water flea	4-6 d	S,cs*	N	23±2	nd	nd	48 h	2.1 (20mmol/m3)	LC50	10

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3 l^{-1}$)	pH	Exposure time	Concn (mg l^{-1})	Effect	Ref
Daphnia magna Water flea	neonates	S,cs*	Y	20±1	nd	nd	24 h 48 h	2.27-2.89 1.81-2.41	EC50 (immobilisation)	11
Daphnia magna Water flea	nd	S	N	nd	nd	nd	48 h	75	LC50	12
Daphnia magna Water flea	<24 hr	S,cs	N	22±1	72	6.7-8.1	24 h 48 h NOEC	77 (57-100) 75 (50-120) 6.8	LC50	13
Daphnia magna Water flea	nd	S	nd	nd	nd	nd	24 h	2.08 (1.55-2.63)	EC50 (immobilisation)	14
Daphnia magna Water flea	<24	S, os	nd	20	16°	8.0 ±2	24 h	184 (173-196) 137 200	EC50 (immobilsation) EC0 EC100	15
Daphnia magna Water flea	<24 h	S,os	nd	20-22	16°	7.6-7.7	24 h	190 120 200	EC50 EC0 EC100	16

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3 l^{-1}$)	pH	Exposure time	Concn (mg l^{-1})	Effect	Ref
Ceridaphnia dubia Water flea	nd	S,cs*	Y	nd	nd	nd	48 h 7 d 7 d 7 d 31 (25-35) µM	30 (23-39) 34 (29-40) 9 16 	LC50 LC50 NOEL (reproduction) LOEL (reproduction) IC50 (reproduction)	17
FISH										
Pimephales promelas Fathead minnow	nd	nd	nd	nd	nd	nd	96 h	1.69	LC50	18
Pimephales promelas Fathead minnow	nd	F	nd	nd	nd	nd	96 h	12	LC50	2
Pimephales promelas Fathead minnow	nd	F	nd	26	45.6	7.4	96 h	12.1	LC50	12
Pimephales promelas Fathead minnow	3.8-6.4 cm 1-2 g	S	N	25	20 360	7.5 8.2	24 h 48 h 96 h 24 h 48 h 96 h	48.5 48.5 48.5 (38.9-62.83) 42.3 42.3 42.3 (33.52-53.47)	LC50	19

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3 l^{-1}$)	pH	Exposure time	Concn (mg l^{-1})	Effect	Ref
Lepomis macrochirus Bluegill sunfish	0.2 g	nd	nd	17	nd	nd	96 h	1.94	LC50	18
Lepomis macrochirus Bluegill sunfish	nd	nd	nd	nd	nd	nd	96 h	1.51	LC50	18
Lepomis macrochirus Bluegill sunfish	young 0.32-1.2 g	S, os	N	20-24	28-44	6.7-7.4	24 h 96 h	169 150 (130-200)	LC50 LC50	20
Lepomis macrochirus Bluegill sunfish	nd	S	nd	17	40-50	7.2-7.5	96 h	88 (62-122)	LC50	12
Lepomis macrochirus Bluegill sunfish	3.8-6.4 cm 1-2 g	S,os	N	25	20	7.5	24 h 48 h 96 h	35.1 (26.74-43.67) 32 (32 -32) 32 (32-32)	Tlm	19
Lepomis macrochirus Bluegill sunfish	0.2	S	nd	nd	44	7.4	24 h 96 h	100 (70-149) 84 (57-124)	LC50	21
Lepomis macrochirus Bluegill sunfish	0.2	S	nd	nd	44	7.4	24 h 96 h	160 (113-226) 140 (93-211)	LC50	21

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3 l^{-1}$)	pH	Exposure time	Concn (mg l^{-1})	Effect	Ref
Lepomis macrochirus Bluegill sunfish	0.2 g	S	nd	nd	44	6.5	24 h 96 h	135 (66-276) 56 (40-78)	LC50	21
Lepomis macrochirus Bluegill sunfish	0.2 g	S	nd	nd	44	7.5	24 h 96 h	220 (129-376) 86 (55-134)	LC50	21
Lepomis macrochirus Bluegill sunfish	0.2 g	S	nd	nd	44	8.5	24 h 96 h	285 (189-431) 285 (189-431)	LC50	21
Lepomis macrochirus Bluegill sunfish	0.5 g	S	nd	nd	12	8.0	24 h 96 h	198 (142-277) 135 (113-162)	LC50	21
Lepomis macrochirus Bluegill sunfish	0.5 g	S	nd	nd	44	8.0	24 h 96 h	134 (112-170) 134 (122-170)	LC50	21
Lepomis macrochirus Bluegill sunfish	0.5 g	S	nd	nd	162	8.0	24 h 96 h	80 (64-101) 80 (64-101)	LC50	21
Lepomis macrochirus Bluegill sunfish	0.5 g	S	nd	nd	300	8.0	24 h 96 h	135 (113-162) 135 (113-162)	LC50	21
Poecilia reticulata Guppy	nd	S,cs	Y	21±1	nd	nd	96 h	9.6	LC50	5

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3 l^{-1}$)	pH	Exposure time	Concn (mg l^{-1})	Effect	Ref
Poecilia reticulata Guppy	nd	nd	nd	nd	nd	nd	96 h	1.99	LC50	18
Poecilia reticulata Guppy	1.9-2.5cm 0.1-0.2 g	S,os	N	25	20	7.5	24 h 48h 96 h	97.1 97.1 97.1 (81.5-114.8)	TLm	19
Ictalurus punctatus Channel catfish	nd	S	nd	22	40-50	7.2-7.5	96 h	210 (134-330)	LC50	21
Carassius auratus Goldfish	3.8-6.4 cm 1-2 g	S,os	nd	25	20	7.5	24 h 48 h 96 h	94.4 94.4 94.4 (79.62-110.8)	Tlm	19
Leuciscus idus melanotus Golden Orfe	nd	S,os	nd	20	nd	7-8	48 h	26 44 70	LC0 LC50 LC100	12
FISH (salmonid)										
Oncorhynchus mykiss Rainbow Trout	nd	S,cs	Y	12±1	nd	nd	96 h	4.2	LC50	5
Trout	2.4 g	nd	nd	12	nd	nd	96 h	1.15	LC50	18
Oncorhynchus mykiss Rainbow Trout	nd	S	nd	12	40-50	7.2-7.5	96 h	14	LC50	12

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg $CaCO_3 l^{-1}$)	pH	Exposure time	Concn (mg l^{-1})	Effect	Ref
Oncorhynchus mykiss Rainbow Trout	2.4 g	S	nd	12	44	7.4	24 h 96 h	14 (11-18) 14 (11-18)	LC50	21

Notes:

(H) Corrected for H
F Flow through
S Static
os Open system
cs Closed system
cs* Closed system with headspace eliminated
nd no data
h hours
d days

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15. Bringmann and Kuhn (1982)
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17. Niederlehner *et al.* (1998)
18. Cited in Vittozzi and De Angelis (1991)
19. Pickering and Henderson (1966)
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21. Mayer and Ellersieck (1986)
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B2. BIOACCUMULATION

The log K_{ow} values for ethylbenzene have been reported to be 3.13 and 3.15 by WHO (1996) and Howard (1989), respectively. These data suggest that that ethylbenzene may exhibit moderate bioaccumulation in freshwater organisms although competing losses due to volatilisation are likely to reduce exposure concentrations in the field and to reduce the extent of bioaccumulation.

Few studies are available that have examined the bioaccumulation of ethylbenzene in freshwater organisms. Herman *et al.* (1991) determined a log bioconcentration factor of 2.31 for *Selenastrum capricornutum*. Bioaccumulation was calculated using the loss-from-headspace technique. The authors noted that there was a greater bioaccumulation potential for ethylbenzene than there was for benzene (BCF 1.63) and toluene (BCF 1.99) and values were comparable to the xylenes (2.34 - 2.41). This ranking is the same as that emerging from studies into the acute toxicities of these substances to aquatic life (Benville and Korn, 1977; Galassi *et al.*, 1988). Additionally, Herman *et al*. (1991) observed that there was a good correlation between bioconcentration and log Kow for ethylbenzene.

Mancha *et al.* (1997) determined experimentally a log K_{ow} of 3.25 for ethylbenzene using the OECD method 117, based on the retention time in an HPLC system. They calculated a log BCF value of 2 for fish using the Connel and Hawker equation and Veith equation (Mancha *et al.* (1997).

IUCLID (1996) reported a log BCF of 1.9 for the goldfish *Carrasius auratus*. No further details were provided.

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APPENDIX C SALTWATER TOXICITY AND BIOACCUMULATION

C1. SALTWATER TOXICITY

The available toxicity dataset for ethylbenzene (Table C1) is limited mainly to short-term single species laboratory tests, and in the majority of cases, static laboratory tests. No data are available on the effect of chronic exposure of ethylbenzene on marine organisms. No multispecies (e.g. mesocosms) or field studies have been reported in the open literature. The dataset reported in subsections C1.1 to C1.4 covers one algal/diatom species, one species of macroalga, six crustacean species, one mollusc species and four fish species.

C1.1 Algae

Toxicity data for ethylbenzene to marine algae are restricted to a single study using the marine diatom, *Skeletonema costatum* and the macroalga, *Ulva pertusa*.

EC50 (growth inhibition) values of 8, 7.5, 4.9 and 7.7 mg l^{-1} to *Skeletonema costatum* were reported by Masten *et al.* (1994) following 24, 48, 72 and 96 hours, respectively. The test was carried out according to EPA standard guidelines, adapted for testing of volatile test compound by the use of sealed test vessels. Ethylbenzene concentrations in test solutions were also measured by gas chromatography. Although Masten *et al*. took careful precautions to control losses of ethylbenzene during exposure, they noted a 'salting out' effect which is likely to have impaired interpretation of the results, in particular the concentrations responsible for observed effects.

Tokuda (1984) investigated the behaviour in light of the motile spores of the seaweed, *Ulva pertusa*. He found that inhibition of sporal phototaxis was complete at 1.9 mg l^{-1} ethylbenzene, with some inhibition (not defined) at 0.62 and 0.19 mg l^{-1}. The test used headspace gas chromatography to analyse concentrations at the start of the test and sporal inhibition was assessed visually following illumination of the solution for 30-60 min. The test solutions were kept in beakers sealed with aluminium foil and polychlorinated vinylidene film.

C1.2 Invertebrates

The data relating to the effect of ethylbenzene on marine invetebrates are limited to crustaceans and molluscs.

Effects concentrations for crustaceans range from 0.49-87.6 mg l^{-1}. A 48 h EC50 (immobilisation) of 6.5 mg l^{-1} was reported for *Artemia salina* by Maclean and Doe (1989) with confirmation of test concentrations by fluorescence spectroscopy. The results from this study compare well with the 48 h and 72 h LC50 of >5.2 and 4.0 mg l^{-1}, respectively, reported by Masten *et al.* (1994) for the mysid shrimp (*Mysidopsis bahia*). The latter study also reported a NOEC and LOEC of 1.0 and 2.7 mg l^{-1} respectively. Masten *et al*. were again careful to control losses of ethylbenzene during exposure and to confirm test concentrations by analysis. Their results are also consistent with reported values for 24 h LC50s of 14.4 -17.3 for *Palaemonetes pugio*, 15.4 mg l^{-1} for *Artemia* and 16 mg l^{-1} for *Nitocra bahia*. (Potera cited in ECDIN 1991 and Abernethy *et al.* 1986).

The lowest reported LC50 values are for the bay shrimp *Crago franciscorum*. The 24h and 96 h LC50 values were reported as 2.2 and 0.49 mg l^{-1}, respectively (Benville and Korn 1977) but this experiment was performed in open vessels and it is clear that most of the ethylbenzene introduced into the test vessels (>99%) had volatilised after 48 h. Even after 24 h, most of the ethylbenzene had volatilised from test vessels at concentrations bracketing the 24 h LC50 (>99% loss at 1.0 µl l^{-1} and 67% at 4.9 µl l^{-1}). It is not clear from the paper describing this study the basis for the reported LC50 values. Furthermore, the absence of density data for ethylbenzene prevents conversion of toxicity data reported in terms of µl l^{-1} to µg l^{-1}.

The only study that has examined the toxicity of ethylbenzene to molluscs was performed on the larvae of Pacific oyster *Crassostrea gigas* in a static test, without analysis of test concentrations. A 96 hour LC50 of 1030 mg l^{-1} was reported (LeGore cited in ECDIN 1991).

C1.4 Fish

Few data have been reported on the effect of ethylbenzene on marine fish species. The lowest reliable 96 h LC50 of 5.1 mg l^{-1} was reported from a study conducted under ASTM/EPA standard guidelines for atlantic silversides (*Menidia menidia*). A NOEC and LOEC (survival) of 3.3 and 5.9 mg l^{-1} was reported for this study (Masten *et al.* 1994). The data reported by Masten *et al.* are similar to data reported by Benville and Korn (1977), who reported a 96 h LC50 of 4.3 mg l^{-1} for *Morone saxatilis.* However, few experimental details are available for this latter study and again, apparently high losses of ethylbenzene during exposure make interpretation difficult.

C2. SALTWATER BIOACCUMULATION

There are no data in the open literature on the bioaccumulation of ethylbenzene to marine organisms.

Table C1 Toxicity of ethylbenzene to marine life

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (%)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Ref
ALGAE / DIATOM											
Skeletonema costatum Diatom	nd	S,cs	Y	20±1		nd	8	24 h 48 h 72 h 96 h	8.0 (6.2-10.4) 7.5 (5.0-11.2) 4.9 (2.4-9.8) 7.7 (5.9-10.0)	EC50 (growth) EC50 EC50 EC50	1
Ulva pertusa Seaweed	zoo-spores	S-cs	nd	20	seawater	nd	nd	0.5 -1 h	 1.9 0.62 0.19	Inhibition of phototaxis: 100% some some; ca 60% spores with abnormal movement, 40% with extremely abnormal movement	4

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (%)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Ref
ARTHROPODS (CRUSTACEANS)											
Artemia Brine shrimp	nd	S,cs*	N	20±1	30	nd	nd	24 h	15.4 (145 mmol/m3)	LC50	5
Artemia Brine shrimp	nd	S, cs	Y	23-24	30	nd	8.1-8.2	48 h	6.5 - 13.3 8.8 - 13.3	EC50 (immobil-isation) LC50	6
Artemia salina Brine shrimp	nd	S,cs	Y	23-24	30	nd	8.1	48 h	9.2	EC50	6
Crago franciscorum Bay shrimp	nd	S,os	Y	16	25	nd	nd	24 h 96 h	2.2 0.49	LC50 LC50	7
Mysidopsis bahia Mysid shrimp	<24 h	F,cs	Y	25±1		nd	nd	48 h 72 h 96 h	> 5.2 4.0 (2.7-5.2) 2.6 (2.0-3.3) 1.0 2.7	LC50 LC50 LC50 NOEC (survival) LOEC (survival)	1
Mysidopsis bahia Mysid shrimp	nd	S	N	nd	nd	nd	nd	96 h	87.6	LC50	8
Nitocra bahia Copepod	nd	S,os	nd	10-20	15-20	nd	nd	24 h	16	LC50	3
Palaemonetes pugio Shrimp	nd	S,cs	nd	10-20	15-20	nd	nd	24 h	14.4-17.3	LC50	3

Species	Life stage	Test type	Analysis	Temp (°C)	Salinity (%)	Hardness (mg $CaCO_3$ l^{-1})	pH	Exposure time	Concn (mg l^{-1})	Effect	Ref
Cancer magister Dana Dungeness crab	larvae first stage zoeae	F	Y	13		nd	nd	48 h 96 h	40 13	LC50 LC50	9
MOLLUSCS											
Crassostrea gigas Pacific oyster	larvae	S	N	17-20	25.3-30.8	nd	nd	96 h	1030	LC50	10
FISH											
Cyprinodon variegatus Sheepshead minnow	8-15 mm 14-28 d	S,os	Y	25-31		nd	nd	24 h 48 h 72 h 96 h 96 h	300 (250-340) 360 (310-440) 320 (270-380) 280 (260-290) 88	LC50 LC50 LC50 LC50 NOEC	11
Menidia menidia Atlantic silversides	9-13 mm	F,cs	Y	22±1		63	8	96 h >96 h	5.1 (4.4-5.7) 3.3 5.9	LC50 NOEC LOEC	1
Oncorhynchus kisutch Coho salmon	young 5-40 g	S,csa	N	8	30	nd	8.1	96 h 96h	10 <50	LOEC LC50	12
Morone saxatilis Striped Bass	nd	S	Y	16	25	nd	nd	24 h 96 h	4.3 (3.9-4.7) 4.3 (3.9-4.7)	LC50	7

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APPENDIX D MAMMALIAN TOXICOLOGY

The mammalian toxicity of ethylbenzene was extensively reviewed by the World Health Organization (WHO) in its 1993 revision of the Drinking Water Guidelines (WHO 1993) and more recently, under the International Programme on Chemical Safety (IPCS 1996).

Ethylbenzene is readily absorbed by ingestion or through the skin and is rapidly excreted. In man, it is readily converted into soluble metabolites (mainly mandelic acid and phenylglyoxalic acid) which are excreted rapidly in the urine (WHO 1996).

Most toxicity data relate to the inhalation route of exposure and, at high doses, it can cause irritation of the eyes, nose and throat and CNS depression (Ware 1988, Fawell and Hunt 1988). Based on laboratory animal studies, ethylbenzene is of very low acute oral toxicity (rat oral LD_{50} range from 3.5 - 4.7 g kg^{-1}) (WHO 1996). Short-term oral studies have been carried out in laboratory animals which indicate that it is of low chronic oral toxicity, with target organs being the liver and kidney. In a six-month gavage study in female rats, no adverse effects were seen at a dose of 136 mg kg^{-1} (administered five days/week), whereas higher doses (408 or 608 mg kg^{-1}) had slight effects on the liver and kidney (WHO 1996, IPCS 1996).

Ethylbenzene is considered not to be mutagenic in a variety of tests in bacteria, yeast, insects, mammalian cells and intact mammals (WHO 1996, IPCS 1996) although there are currently inadequate data to assess its carcinogenicity (WHO 1996, IPCS 1996). It can cross the placenta but it is not fetotoxic in rodents (Clayton and Clayton 1994) and, although poorly studied, is unlikely to be teratogenic based on inhalation studies in rodents (Ware 1988).

In 1993, WHO recommended an tolerable daily intake (TDI) of 97.1 μg kg^{-1} body weight day^{-1} (WHO 1993, 1996) This was based on applying an uncertainty factor of 1000 to a NOAEL of 136 mg kg^{-1} body weight day^{-1} which was based on liver and kidney toxicity observed in a limited six-month study in rats (administration five days/week; equivalent dose 97.1 mg kg^{-1}). An uncertainty factor of 1000 was applied to allow for intra- and interspecies variation and 10 for the limited database and short duration of the study. Assuming a 60 kg adult drinking two litres of water per day and 10% allocation of the TDI to drinking water, a health-based guideline value of 300 μg l^{-1} was proposed.

However, WHO noted that this value exceeded reported odour thresholds in drinking water. Odour threshold concentrations reported in the literature range from 1.6, 3.2, 10, 29, 100, 140, 150, 200 and 550 μg l^{-1} (Ware 1988, Fawell and Hunt 1988, Van Gemert and Nettenbriejer 1977, Alexander *et al.* 1982, Young *et al.* 1996). The lowest odour concentrations were reported at 60 ^{o}C whereas the threshold value of 150 μg l^{-1} was reported at 40 ^{o}C (Alexander *et al.* 1982, Young *et al.* 1996). Taste threshold concentrations of 29, 64, 72, 80, 200, 390 and 780 μg l^{-1} have been reported (Ware 1988, Alexander *et al.* 1982, Young *et al.* 1996, WHO 1996). The taste concentrations of 64 and 80 μg l^{-1} were reported at 40 ^{o}C whereas the value of 390 μg l^{-1} was reported at 25 ^{o}C (Alexander *et al.* 1982, Young *et al.* 1996).

The taste and odour threshold concentration for ethylbenzene is most likely to be between 20 to 200 μg l^{-1}.

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APPENDIX E SPECIES CITED

Scientific name	Common name	Organism	Water
Artemia salina	Brine shrimp	Crustacean	SW
Cancer magister	Dungeness crab	Crustacean	SW
Carassius auratus	Goldfish	Fish	FW
Ceridaphnia dubia	Water flea	Crustacean	FW
Chlamydomonas angulosa	Green alga	Algae	FW
Chlorella vulgaris	Green alga	Algae	SW
Crago franciscorum	Bay shrimp	Crustacean	SW
Crassostrea gigas	Pacific oyster	Mollusc	SW
Cyprinodon variegatus	Sheepshead minnow	Fish	SW
Daphnia magna	Water flea	Crustacean	FW
Entosiphon sulcatum	Protozoa	Protozoa	FW
Ictalurus punctatus	Channel catfish	Fish	FW
Lepomis macrochirus	Bluegill sunfish	Fish	FW
Leuciscus idus melanotus	Golden orfe	Fish	FW
Menidia menidia	Atlantic silversides	Fish	SW
Morone saxatilis	Striped bass	Fish	SW
Mysidopsis bahia	Mysid shrimp	Crustacean	SW
Nitocra bahia	Copepod	Crustacean	SW
Nitrobacter	Bacteria	Bacteria	FW
Nitrosomonas	Bacteria	Bacteria	FW
Oncorhynchus kisutch	Coho salmon	Fish	SW
Oncorhynchus mykiss	Rainbow trout	Fish	FW
Palaemonetes pugio	Brown shrimp	Crustacean	SW
Pimephales promelas	Fathead minnow	Fish	FW
Poecilia reticulata	Guppy	Fish	FW
Pseudomonas putida	Bacteria	Bacteria	FW
Scendesmus quadricauda	Green alga	Algae	FW
Selenastrum capricornutum	Green alga	Algae	FW
Skeletonema costatum	Diatom	Diatom	SW
Ulva pertusa	Seaweed	Algae	SW

APPENDIX F ABBREVIATIONS USED

BCF = bioconcentration factor

EC50 = concentration required to elicit specified effect in 50% of exposed population

EQS = environmental quality standard

LC50 = concentration required to cause mortality in 50% of exposed population

LC100 = concentration required to cause mortality in 100% of exposed population

LOAEL = lowest observed adverse effect level

LOEC = lowest observed effect concentration

ND = no data

NOAEL = no observed adverse effect level

NOEC = no observed effect concentration

NOEL = no observed effect level

MATC = maximum acceptable toxicant concentration

S = static test

ADI = admissible daily intake