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Proposed Environmental Quality Standards for Styrene in Water

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PROPOSED ENVIRONMENTAL QUALITY STANDARDS FOR STYRENE IN WATER

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Statement of Use
This report reviews the available data on the environmental fate, behaviour and toxicity of Styrene both in the freshwater and marine environments. Environmental Quality Standards (EQSs) for the protection of aquatic life and for the abstraction of water to potable supply has been proposed. These will assist NRA staff in assessing the effects of Styrene on water quality and the setting of appropriate standards.

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EXECUTIVE SUMMARY

This report prepared for the National Rivers Authority reviews the properties and uses of styrene, its fate, behaviour and reported concentrations in the environment, and critically assesses the available data on its toxicity and bioaccumulation. All the available data have been examined and used, where possible, to derive Environmental Quality Standards (EQSs) for the protection of fresh and saltwater life as well as for the abstraction of water to potable supply. The proposed EQSs are presented in Table S1.

Table S1 Proposed EQSs and guideline values for styrene (expressed as $\mu g\ l^{-1}$)[1]

Use	AA	MAC	Notes
Protection of freshwater life	50	500	T
Protection of saltwater life	50	500	G
Abstraction to potable supply	-	20	

Notes: AA = Annual average
MAC = Maximum allowable concentration
T = Tentative value
G = Guideline values
1 = Lowest Limit of Detection reported is 0.008 $\mu g\ l^{-1}$

Styrene has a wide range of commercial uses, in particular it is used as a chemical intermediate in the production of polymers, copolymers and reinforced plastics, and is present in petroleum products and some adhesives. The major use of styrene is in the manufacture of polystyrene.

The major release of styrene to the aquatic environment is through industrial effluents (in particular from chemical and plastic manufacturing plants), either directly or indirectly via sewage treatment. It is not, however, very persistent in the aquatic environment (half-lives in rivers in the order of hours), with volatilisation being the major removal mechanism.

Styrene is of moderate to low acute toxicity to aquatic organisms, although the available toxicity data were generally found to be of poor quality. No reliable chronic toxicity data were available to assess the potential effects of continuous long-term exposure of aquatic organisms to styrene (e.g. from point source discharges).

An EQS of 50 $\mu g\ l^{-1}$, expressed as an annual average (AA) concentration, for the protection of freshwater life against long-term exposure to styrene is derived by applying a safety factor of approximately 100 to the lowest reliable 96-hour LC_{50} of 5.9 $mg\ l^{-1}$ reported for rainbow trout

(*Oncorhynchus mykiss*). In addition, an EQS of 500 $\mu g\ l^{-1}$, expressed as a maximum allowable concentration (MAC) is proposed to protect against short-term acute effects. The MAC is derived by applying a safety factor of approximately 10 to the 96-hour LC_{50} for *O. mykiss*.

These EQSs should be considered "tentative" because of the limited data available, in particular the lack of well conducted acute toxicity tests and chronic toxicity data.

Insufficient data are available to derive separate standards for the protection of saltwater life. Until further data become available, it is proposed that the EQSs derived for the protection of freshwater life should be adopted as guidelines for the protection of saltwater life. Comparison of these standards with toxicity values reported for saltwater organisms suggests that these EQSs will be adequately protective.

An EQS of 20 $\mu g\ l^{-1}$, based on the health-based guideline recommended by the World Health Organisation, expressed as a MAC is proposed for waters abstracted to potable supply. However, aesthetic (taste and odour) problems may arise in drinking water at concentrations below this EQS.

Some current analytical techniques appear adequate to monitor the proposed EQSs.

KEY WORDS

Environmental Quality Standards (EQSs), styrene, aquatic toxicity, freshwater, saltwater, mammalian toxicity.

1. INTRODUCTION

This report, prepared for the National Rivers Authority (NRA), reviews and critically assesses the information available on the inputs to and concentrations of styrene in the environment (Section 2), the analytical methods available for the analysis of styrene (Section 3), the fate and behaviour of styrene in the environment (Section 4 and Appendix A) and its aquatic and mammalian toxicity (Section 5 and Appendices B to D).

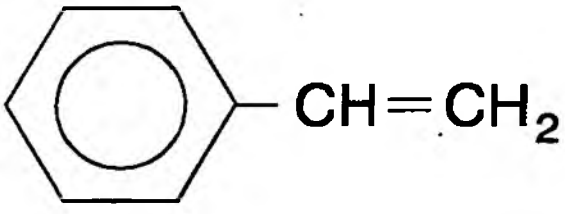
The information is used to derive Environmental Quality Standards (EQSs) for the protection of fresh and saltwater life and for the abstraction of water to potable supply (Section 6).

2. STYRENE IN THE ENVIRONMENT

2.1 Physico-chemical properties

A summary of the physico-chemical properties of styrene is provided in Table 2.1. Styrene is a colourless to yellow viscous liquid which is flammable and volatile. A density of 0.9060 (d_4^{20}) and lower and upper flammability limits of 1.1-6.1 % at 20 °C have been reported (WHO 1983, Clayton and Clayton 1994).

Table 2.1 Chemical and physical properties of styrene monomer

IUPAC CHEMICAL NAME	Styrene
SYNONYMS	Vinylbenzene[1], ethenylbenzene, cinnamene[2], cinnamol, phenylethylene, phenylethene, phenethylene, styrol, styrole, styrolene, vinylbenzol[3]
CAS NUMBER	100-42-5
MOLECULAR FORMULA	C_8H_8
MOLECULAR STRUCTURE	
MOLECULAR WEIGHT	104[1][2]
COMPOSITION	C 92.26%, H 7.74%[4]
DESCRIPTION	Colourless to yellowish, viscous, flammable liquid with a sweet floral odour at low levels but disagreeable odour at high levels[4][9][10]
MELTING POINT (°C)	-30.63 [1]
BOILING POINT (°C)	145.2[1][9]
DENSITY (d_4^{20} i.e. specific density at 20°C referred to water at 4°C)	0.9060 [9][10]

VAPOUR PRESSURE (mm Hg)	6.6 at 25 °C (extrapolated)[1] 5.0 at 20 °C[2] 9.5 at 30 °C[2] 10.0 at 35 °C[10]
VAPOUR DENSITY (unitless)	3.6[5] [9]
HENRY'S LAW CONSTANT (atm m^3 mol^{-1})	2.810×10^{-3} [1] 5.200×10^{-3} [1] 2.633×10^{-3} [6] 2.280×10^{-3} [8]
FLASH POINT (°C)	31 (closed cup)[4] 37 (open cup)[5] 32[9]
FLAMMABILITY LIMITS (%) (lower limit - upper limit)	1.1-6.1 at 20 °C[9] [10]
AUTO-IGNITION TEMPERATURE (°C)	490[10]
WATER SOLUBILITY (mg l^{-1})	310 at 25 °C (water)[1] 280 at 15 °C (water)[2] 300 at 20 °C water)[2] 400 at 40 °C (water)[2] "Floats on water"[5] Soluble in ethanol, diethylether and acetone. Very soluble in benzene and petroleum ether[10]
pKa	Not applicable
log K_{ow}[3]	2.59[11] 2.95[1] 3.16[7]
log K_{oc}[3]	2.43 (estimated [1])[1] 2.74 (estimated [2])[1]
HALF-LIVES	Air: 3.5 hours (with hydroxyl radicals), 9 hours (with ozone)[1]; Water: 3.4 hours[8]
STABILITY	Polymerized easily at room temperature in the presence of oxygen and oxidizes on exposure to light and air. Stored in inert atmosphere or with added inhibitors (e.g. 0.001% tertiary butylcatechol)[10]

Notes: [1] Estimated from water solubility of 160
[2] Estimated from log K_{ow} 2.95
[3] Low <2, High >3.5

References:

(1) Howard (1990)
(2) Verscheuren (1983)
(3) RTECS (1994)
(4) MERCK (1989)
(5) Environment Canada (1984)
(6) Yaws *et al.* (1991)
(7) RREL Treatability Database Version 5.0 (1994)
(8) Watts and Moore (1988)
(9) Clayton and Clayton (1994)
(10) WHO (1983)
(11) Ogata *et al.* (1984)

Styrene is moderately soluble in water (300 mg l^{-1} at 20 °C) but dissolves more freely in alcohols, ethers and organic solvents (e.g. acetone). Examination of the reported partition coefficients for styrene (log K_{ow} 2.59-3.16, log K_{oc} 2.43-2.74) suggests only a moderate tendency to adsorb to particulate matter (e.g. soil and sediment particles). In water this tendency is likely to be obviated by its high potential for volatilisation (vapour pressure 6.6 mm Hg at 25 °C, Henry's Law constant 2.28 x 10^{-3} - 5.20 x 10^{-3}). Howard (1990) indicates that styrene will react rapidly with hydroxyl radicals and ozone in the atmosphere, with reaction half-lives given as 3.5 and 9 hours, respectively. Styrene polymerises readily at room temperature in the presence of oxygen and oxidizes on exposure to light and air (WHO 1983).

2.2 Manufacture

The estimated annual production of styrene in the UK is 270 000 tonnes/year. The majority of this (approximately 220 000 tonnes/year) is manufactured by British Petroleum (BP) at their plant in Baglan Bay (Crookes and Howe 1992) and the remainder by Enichem Elastomers Ltd. at Hythe. The approximate annual production at Hythe is 50 000 tonnes (W.Craft, Pers. Comm. 1994). European Chemical News (1990, cited by Crookes and Howe 1992) made reference to plans by Huntsman Chemicals for the development of a styrene production plant in the UK. However, information provided at the time of writing indicates that Huntsman Chemicals do not currently manufacture styrene (D. Evans, Pers. Comm. 1994).

Styrene is principally produced from the catalytic (mixed metal oxide catalyst) dehydrogenation of ethylbenzene. The overall yield of this process is about 90-92% styrene with the remainder being comprised of various impurities (Crookes and Howe 1992). The co-production of styrene and propylene oxide from the oxidation and subsequent dehydration of ethylbenzene is also reported (WHO 1983). The estimated annual production of ethylbenzene, which is used mainly for the production of styrene, is 295 000 tonnes (Crookes and Howe 1992). Another possible pathway for the production of styrene is the demethylation of cumene (Clayton and Clayton 1994).

2.3 Uses

Styrene has a wide range of commercial uses, particularly in the plastic and chemical industries. It is used extensively in the production of polymers (e.g. polystyrene) copolymers (e.g. styrene-butadiene) and reinforced plastics. Styrene is also used in dental fillings and is present in a number of petroleum products and adhesives. It is reportedly found in motor vehicle exhaust fumes, tobacco smoke and combustion/pyrolysis products (WHO 1983). The World Health Organisation (WHO 1983) reported the following typical use patterns of styrene in the United States in 1980:

- polystyrene (45%)
- exports (17%)
- acrylonitrile-butadiene-styrene and styrene-acrylonitrile resin (10%)
- styrene-butadiene rubber (8%)
- styrene-butadiene latex (6%)

- unsaturated polyester (5%)
- miscellaneous (4%)

Clayton and Clayton (1994) have determined that styrene-based plastics rank fourth in the overall production of thermoplastics in the US. The major markets for styrene-based plastics are packaging and durables (e.g. disposable containers, components of domestic appliances).

2.4 Entry into the aquatic environment

Environmental contamination of styrene will arise entirely from anthropogenic sources. The only known natural occurrence of styrene is in the sap of the styracaceous tree (Clayton and Clayton 1994). Releases of styrene to the environment as a whole are varied and may arise from both point (e.g. industrial effluents) and diffuse (e.g. as a combustion product of petroleum compounds) sources. The possible routes of entry of styrene into the aquatic environment are discussed below.

The majority of styrene released to the environment will be to the atmosphere through the manufacture of styrene itself, from the vents of distillation columns and other processing equipment (WHO 1983), the incineration of styrene-based plastics and rubbers, motor vehicle exhaust emissions and the combustion of petroleum products (WHO 1983). However, styrene reacts rapidly with hydroxyl radicals and ozone in the atmosphere with a combined half-life of about 2.5 hours (Howard 1990). Atmospheric deposition to the aquatic environment is, therefore, not considered to be a major source of contamination.

Manufacturing processes can result in the release of styrene directly to surface waters or sewers via wastewater effluents. Accidental spillages and leakages from storage or transport containers, may be responsible for episodic releases of styrene-containing products to catchment areas. Surface run-off and storm water drainage may result in direct surface water contamination with styrene. Styrene which is stored for any period of time is likely to contain tertiary butylcatechol, which is added to inhibit atmospheric degradation. Tertiary butylcatechol may also therefore enter the environment along with styrene in certain pollution incidents, but probably only in trace amounts.

The potential for styrene to enter groundwater is, however, less certain. Burback and Perry (1993) described styrene as a major groundwater contaminant. The US National Academy of Sciences (cited by CCREM 1987) has suggested that some styrene may enter water and soil from discarded products in landfills and chemical waste dumps. However, as most of these products (including polystyrene, styrene-acrylonitrile and acrylonitrile-butadiene-styrene) are known to be quite resistant to natural degradation processes (Clayton and Clayton 1994), it is likely that only low levels of styrene will be released in this way, and over a relatively long period of time.

Octachlorostryrene may also be released to the aquatic environment and there is evidence to suggest that it may be accumulated by fish (approximately 2.0-400.0 ng g^{-1} wet weight). However, octachlorostyrene is not intentionally produced (i.e. for commercial use) but instead can be formed as a by-product of the electrolytic production of chlorine using graphite anodes and coal tar pitch, or by the electrolytic production of magnesium, or as a result of high

temperature carbon/chlorine reactions (US EPA 1992b). The sites in which it was detected during a fish residue survey conducted by the US Environmental Protection Agency (US EPA 1992b) were all located in areas where industrial organic chemicals are manufactured, although no information on the water concentrations of octachlorostyrene was provided. There is no evidence to suggest that chlorinated styrenes will result from the release of styrene monomer to surface waters or groundwaters. Infact, the reaction of styrene with chlorine in water is likely to result in oxidation rather than substitution. For this reason chlorinated styrenes have not been considered further in this report.

2.5 Recorded levels in the environment

At the time of writing, the only available data on concentrations of styrene in the UK analysed in the past year by the National Rivers Authority and Scottish River Purification Boards were from the Severn Trent NRA Region. Although styrene was monitored for 1994 in a number of watercourses and sediments, the only reported value was a concentration of 0.5 $\mu g\ l^{-1}$ in a lake (A Wallwork, Pers. Comm 1995). In addition a number of pollution incidents have occurred in the Thames Region of the NRA following the use of products containing styrene to lined pipes.

Law *et al.* (1991) monitored a number of surface waters and estuaries around the UK between June 1988 and December 1989. By far the highest concentration of styrene recorded was in the River Tees in the North East (1.7 $\mu g\ l^{-1}$, unfiltered samples). Concentrations below 0.001 $\mu g\ l^{-1}$ (unfiltered samples) were reported for samples taken from the River Mersey (three sampling stations), the River Thames (one sampling station) and Plymouth Sound (four sampling stations). Watts and Moore (1988) recorded a styrene concentration of 0.175 $\mu g\ l^{-1}$ in the effluent of a sewage treatment plant which received wastewater from local plastic manufacturers. Concentrations in the receiving river, the River Clywedog, varied from 0.0465 $\mu g\ l^{-1}$ 30 m downstream of the outfall to 0.0191 $\mu g\ l^{-1}$ 6500 m downstream of the outfall. Upstream of the effluent discharge a styrene concentration of 0.0079 $\mu g\ l^{-1}$ was recorded. In another survey of the same river the following year, Tynan *et al.* (1989) detected concentrations of 0.205, 0.122 and 0.058 $\mu g\ l^{-1}$ in the effluent and at sites 30 and 6400 m downstream, respectively. A styrene concentration of 0.127 $\mu g\ l^{-1}$ was found just upstream of the outfall which suggests that there were additional discharges of styrene to the river prior to the sewage treatment outfall.

Styrene is found in a variety of other industrial effluents released to surface waters. For example, in the United States styrene was found in effluents from petroleum refining (31 $\mu g\ l^{-1}$), chemical (30 $\mu g\ l^{-1}$), rubber (2.6-3.0 $\mu g\ l^{-1}$) and textile manufacturing plants (Shackelford and Keith 1976, cited by CCREM 1987). Watts and Moore (1988) recorded a styrene concentration of 0.175 $\mu g\ l^{-1}$ in the effluent of a sewage treatment plant which received wastewater from local plastic manufacturers.

Although Burback and Perry (1993) described styrene as a major groundwater contaminant in the United States, Kenrick *et* al. (1985) analysed samples of groundwater from a total of 32 public and private supply boreholes from the three major UK aquifers and detected styrene at only one site, at a concentration of 0.01 $\mu g\ l^{-1}$.

Fielding *et al.* (1981) analysed drinking waters derived from 14 different sites in the UK (groundwater and various surface water sources) using GC-MS (gas chromatography-mass spectrometry) for a range of organic micropollutants. Although actual concentrations are not reported, styrene was detected in treated drinking water from only four sites. The water at two of these sites originated from the same groundwater source while at the remaining sites water originated from an upland reservoir and a lowland reservoir.

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 1.0 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

No SCA "Blue Book" method for the analysis of styrene has been adopted but a number of methods have been produced by the US Environmental Protection Agency (EPA) for the analysis of styrene in finished drinking water, raw source water and drinking water at different treatment stage.

EPA Method 502.2 for the analysis of volatile organic compounds in water is based on purge and trap capillary column gas chromatography (GC) using photo ionisation detection (PID) with a range of application of up to 200 $\mu g\ l^{-1}$ for styrene.

A 5-ml sample is purged by bubbling an inert gas through it for 11 minutes at ambient temperature after which the purge and trap system is placed in the desorb mode. The trapped materials are introduced to the GC column by rapidly heating the trap to 180 °C while backflushing the trap with an inert gas at 15 ml min^{-1} for about four minutes. As soon as desorption starts the GC temperature program is started. Analytical method performance data were given for the following two columns:

Column 1

Wide bore capillary column, 60 m long x 0.75 mm id VOCOL (Supelco Inc.) with 1.5 µ film thickness. The flow rate of helium carrier gas is adjusted to about 6 ml min^{-1}. The column temperature is held at 10 °C for 8 min then programmed to 180 °C at 4 °C min^{-1} and held until all compounds have eluted. The PID base temperature is 250 °C and the reactor temperature 810 °C. The limit of detection is 0.01 $\mu g\ l^{-1}$ and at 10 $\mu g\ l^{-1}$ the mean recovery 104% with a relative standard deviation (RSD) of 1.3% (number of samples (n) = 7).

Column 2

Mega-bore capillary column, 105 m long x 0.53 mm id, RTX-502.2 (O.I Corporation/RESTEK Corporation) with 3.0 µ film thickness. The flow rate of helium carrier gas is adjusted to about 8 ml min^{-1}. The column temperature is held for 10 min at 35 °C then programmed to 200 °C at 4 °C min^{-1} and held until all compounds have eluted. The PID base temperature is 250 °C and the reactor temperature 950 °C. The limit of detection is 0.1 $\mu g\ l^{-1}$ and at 10 $\mu g\ l^{-1}$ the mean recovery 96 % with 1.9 % RSD (n = 7).

Method 503.1 for volatile aromatic and unsaturated organic compounds in water also uses purge and trap GC-PID but with a packed column. The column is 2.0 m x 0.085 in ID packed with 5% SP-1200 and 1.75 % Bentone 34 on Supelcoport (80/100 mesh). The flow rate of the helium carrier gas is 30 ml min^{-1} and the column temperature is held at 50 °C for 2 min and then programmed at 3 °C min^{-1} to 110 °C and then held until all peaks have eluted. The limit of detection is 0.008 $\mu g\ l^{-1}$ and the range of application up to 1500 $\mu g\ l^{-1}$. No accuracy or precision data are given.

Methods 524.1 & 524.2 for the measurement of purgeable organic compounds in water uses purge and trap GC-mass spectrometry (GC-MS) again with either packed (Method 524.1) or capillary columns (Method 524.2) giving detection limits of 0.2 $\mu g\ l^{-1}$ and 0.04 $\mu g\ l^{-1}$ respectively. Typical recoveries are 95-120% with RSDs of 3.1-7.2% and the range of application up to 200 $\mu g\ l^{-1}$. The GC-MS method can be used with a sample size of 25 ml although 5 ml is recommended if the required detection limits can be met.

There is also a method for styrene and other semi-volatile organic compounds in wastewater by isotope dilution GC-MS (EPA method 1625). A stable isotopically labelled analog of styrene is added to a 1 litre sample which serves to correct the variability of the technique. The sample is

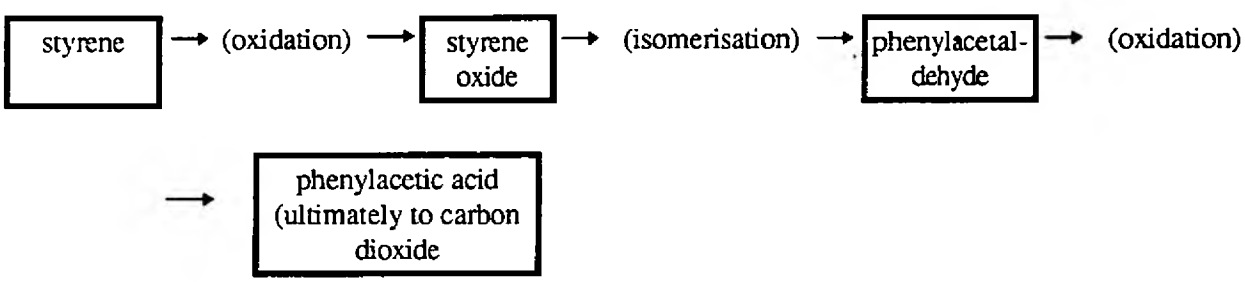
extracted with dichloromethane at pH 12-13 and then at pH < 2 using a continuous extraction technique. The extracts are then dried with sodium sulphate and concentrated to 1 ml before being analysed by GC-MS with on column injection. A 30 m x 0.25 mm id 5 % phenyl , 94% methyl, 1 % vinyl silicone column is used with a gas velocity of 30 cm sec^{-1} and a temperature programme of 5 min at 30 °C; 30-280 °C at 8 °C.min^{-1} and held until all compounds have eluted. The limit of detection is 10 µg l^{-1}.

4. FATE AND BEHAVIOUR AND IN THE ENVIRONMENT

Information on the likely fate and behaviour of styrene in the environment indicate that it may be subject to volatilisation, biodegradation and sorption to varying degrees. In the atmosphere styrene oxidises rapidly. However, chemical oxidation and other abiotic processes such as hydrolysis and photolysis are generally not expected to influence the concentrations of styrene in the terrestrial and aquatic environments. Details of the studies reviewed for this report are presented in Appendix A and summarised in Tables A1-3.

The majority of styrene released to the environment is likely to be to the atmosphere (see Section 2.4). Styrene reacts rapidly with hydroxyl radicals and ozone in the atmosphere with a combined half-life of about 2.5 hours (Howard 1990). Atmospheric deposition is therefore not considered to be a major source of contamination of the aquatic or terrestrial environments.

If released to the terrestrial environment, some styrene will initially be volatilised from the surface layers of soil. For example, 26% of [^{14}C]styrene was lost by volatilisation from a 1.5 cm deep soil column (Fu and Alexander 1992). Styrene may also be completely mineralised under aerobic conditions (half-lives range from 30 days to 8 weeks) and biotransformed under anaerobic conditions, depending on the concentrations applied and the type of soil. Hartmans *et al.* (1990) proposed the following metabolic pathway for styrene by aerobic soil microorganisms:



Information on the anaerobic degradation of styrene in soil indicates that non-methanogenic biotransformation of styrene can occur, although probably quite slowly. Grbic-Galic *et al.* (1990) reported that the anaerobic removal of styrene as a sole organic carbon and energy source, its degradation to a range of aromatic, alicyclic and aliphatic intermediates, and ultimately carbon dioxide and a small amount of reduced organic compounds, was observed with all the inocula tested (ferulate- and toluene- degrading inocula and styrene-enriched sludge consortia) except the benzene-degrading consortia. Using ferulate-degrading, toluene-degrading and styrene-enriched consortia, 98.1, 96.3 and 99% respectively of styrene was found to be transformed, (initial concentration 8.0 mmol carbon l^{-1}) after four months. A tentative transformation pathway for styrene was proposed by Grbic-Galic *et al.* (1990) and this is summarized in Figure A1.1 (Appendix A).

The log K_{oc} values of 2.43 and 2.74 reported for styrene indicate a moderate potential for sorption in soil. This has to some extent been confirmed in laboratory studies (e.g. Fu and Alexander 1992, Fu *et al.* 1994). The degree of sorption of styrene in soil will also depend on

the type of soil and concentration applied. Styrene sorption was found to be lowest in aquifer sand (0.4% organic matter) and highest in an organic soil (32.9% organic matter), with 57-72% and 84-93% (initial concentrations 1.0-100 mg l^{-1}) sorbed, respectively (Fu and Alexander 1992). The sorption of styrene in soil does not appear to significantly affect its degradation. Regardless of its sorption potential, some styrene may still be leached from soil under certain conditions (e.g. high rainfall). From an initial concentration of 1.0 mg kg^{-1}, 31% of styrene was leached after 14 leachings with distilled water (Fu *et al.* 1994). However, the likelihood of styrene entering groundwater via soil is relatively low (see Sections 2.4 and 2.5). The terrestrial environment is not likely to be a major route for the entry of styrene into surface waters either, unless the release to soil is very close to a water body or high levels of rainfall are experienced soon after release.

Styrene will enter the aquatic environment mainly in industrial effluents, either directly or indirectly via sewage treatment plants, or as a result of accidental spillage or leakage. Styrene can be effectively removed from effluents by activated sludge and sedimentation processes (see A3, Appendix A). For example, influent concentrations of up to 0.1 mg l^{-1} were 96.4% and 91.4% removed from domestic wastewaters by activated sludge and sedimentation, respectively (Canviro Consultants 1988, cited by RREL 1994). Used in combination, activated sludge and sedimentation were reported to remove >79% from an industrial effluent containing 1.0 mg styrene l^{-1}. Biological filters may be more sensitive to styrene. Jordan (1989, cited by RREL 1994) recorded 0% and 8% removals of styrene for influent concentrations of 0-0.1 and 0.1-1.0 mg l^{-1}, respectively, in highly polluted effluents (USEPA superfund effluents). This, however, could have been due to the presence of other pollutants which may have been toxic to microorganisms. Lapinskas (1993) reported that a submerged fixed-film reactor was successfully used to treat groundwater polluted by a variety of volatile organic compounds, but in particular ethylbenzene, styrene and xylene. The extent of styrene removal was not reported, although >98.8% of ethylbenzene (the compound from which styrene is usually manufactured) was consistently removed (influent concentrations ranged from 14 to 152 mg l^{-1} with an average residence time of 8.3 hours).

Tolerance thresholds for aerobic biological treatment processes are not available in the literature. Studies on the aerobic biodegradation of styrene in soil suggest that high concentrations (e.g. concentrations equal to or greater than 5.0 mg kg^{-1}) become increasingly less biodegradable. Fu and Alexander (1992), for example, found that only 17% of [^{14}C]styrene mineralised at the highest concentration tested (4000 mg kg^{-1}). This suggests that high concentrations of styrene may inhibit microbial activity. Burback *et al.* (1994), for example, estimated a toxic concentration of 4.0 mM (approximately 417 mg l^{-1}) for the soil mycobacterium *Mycobacterium vaccae* exposed to styrene for seven days in suspended media.

Information on the effects of styrene on anaerobic sludge digestion is very limited. Grbic-Galic *et al.* (1990), however, indicated that styrene may be biologically transformed (non-methanogenically) under strictly anaerobic conditions (see above).

Styrene is highly volatile in water, with half-lives generally of only a few hours (e.g. 3.5-14 hours) for rivers, depending on factors such as wind speed, river flow and water depth. Biodegradation and sorption are only likely to play minor roles in the removal of styrene from surface waters (Fu and Alexander 1992, Tynan *et al.* 1989, Watts and Moore 1988). As styrene is relatively insoluble in water, if it is released to water in very high concentrations, and

where the mixing capacity of the receiving water is low, an insoluble film may appear on the surface. Such an event may lead to reduced levels of dissolved oxygen in the underlying water with subsequent effects on aquatic organisms. This is not, however, likely to be a frequent occurrence since it will probably only occur as a result of accidental spillages and leakages.

5. SUMMARY OF AQUATIC TOXICITY AND BIOACCUMULATION

5.1 Freshwater

The available toxicity data for styrene (see Table B1, Appendix B) are limited to single-species laboratory tests, and in the majority of cases, to static laboratory tests with only nominal concentrations. Most of the reported studies have examined the effects of styrene to freshwater life after short-term exposure. Data for the most sensitive species of each taxonomic group tested are summarised in Table 5.1.

Styrene is highly volatile in aqueous media and exposure concentrations will therefore rapidly decrease during testing unless measures are taken to minimize the effects of volatilisation (e.g. covering test vessels, renewing test media). In the majority of studies reported no such measures appear to have been taken and very few have attempted to measure actual styrene concentrations during exposure. Consequently, reported LC_{50} or EC_{50} estimates may underestimate the toxicity of styrene, but the magnitude of any such underestimate is unknown. It is perhaps significant that the lowest reported LC_{50}/EC_{50} values are from studies in which flow-through conditions were employed (see Table 5.1). Styrene is also only moderately soluble in water (300 mg l^{-1} at 20 °C) but the majority of effects appear to have occurred within the solubility limit of styrene. A small number of toxicity studies did, however, use solvent carriers (e.g. acetone) as an aid to dissolution and dispersion.

Examination of the available toxicity data for freshwater organisms indicate that styrene is of moderate to low acute toxicity to those species tested, with the majority of effect concentrations above 20 mg l^{-1}.

The limited data for lower organisms such as bacteria, cyanobacteria, green algae and protozoans suggest that these species are not particularly sensitive to styrene (toxicity thresholds for cell inhibition multiplication vary from 67 and 72 mg l^{-1} for bacteria and cyanobacteria, and in excess of 100 mg l^{-1} for algae and protozoans), although more data are needed to confirm this. No data are available on the sensitivity of vascular aquatic plants.

The data for invertebrates are restricted to the Phyla Mollusca and Crustacea. The concentrations reported to have caused adverse effects on freshwater crustaceans after short-term exposure vary considerably, from 23 to 300 mg l^{-1} (both for the water flea *Daphnia magna*).

Freshwater fish generally appear to be the most sensitive organisms to styrene. The lowest acute toxicity value reported is for the fathead minnow (*Pimephales promelas*) in a well conducted flow-through study (96-hour LC_{50} 4.02 mg l^{-1} - measured concentration). *P. promelas* is a sub-tropical species. (A temperature of 21.3 °C was maintained throughout the test). Rainbow trout (*Oncorhynchus mykiss*) juveniles were found to be similarly sensitive to styrene in another flow-through study, with 12-, 24-, 48-, 96- and 168-hour LC_{50}s of 8.8, 6.5, 6.3, 5.9 and 4.9 reported, respectively. In this particular test nominal concentrations were

not confirmed with analysis. Styrene was, however, solubilised in high concentrations of acetone although acetone controls were not employed. The authors cited acute toxicity values which suggest that the concentrations of acetone used (maximum acetone concentration used was 2400 mg l^{-1} for a styrene concentration of 100 mg l^{-1}) were relatively non-toxic to the fish tested, but the inclusion of solvent controls is nevertheless important to account for the additional stress that may or may not be induced by the solvent.

Styrene mainly enters the aquatic environment through point source discharges (e.g. sewage and industrial effluents) (see Section 2.4). although styrene is not very persistent in water, organisms in the immediate vicinity of a discharge may be subjected to long-term continuous exposure to styrene, depending on their ability to avoid exposure (e.g. fish may respond by swimming to more tolerable conditions). An assessment of the chronic toxicity of styrene to aquatic organisms is therefore important for the calculation of Environmental Quality Standards (EQSs). However, there are no reliable chronic toxicity data reported in the literature. Due to its high volatility, styrene is difficult to test in the laboratory and this might explain the lack of chronic toxicity.

The low solubility of styrene in water may itself have potentially hazardous implications in the field. If released to freshwaters in high concentrations (e.g. in the event of an accidental spillage or container leakage), styrene may form an insoluble slick or film over the surface of the water, although this will depend largely on the mixing capacity of the receiving water. In shallow, slow-flowing waters this may significantly reduce the natural aeration of the water body from the atmosphere, thus causing an additional perturbation to the ecosystem. There is also some indication from one laboratory study (Erben and Pisl 1993) that styrene may form a film over the bodies of aquatic organisms. If this is the case, indirect effects may rapidly result (e.g. respiratory surfaces and sensory mechanisms may be hindered). However, considering the relatively small volumes of water used in laboratory toxicity tests it is unlikely that the smothering effects observed by Erben and Pisl (1993) will be repeated to any great extent in the field. Because of the high volatility of styrene this phenomenom may not exist for extended periods.

Since most of the studies reported do not meet the ideal criteria for testing sparingly soluble, volatile organic chemicals in the laboratory the overall quality of the freshwater toxicity dataset is questionable. It is conceivable that studies which have not measured or maintained exposure concentrations in some way (e.g. static tests) or which have not completely dissolved styrene in the test media, will have underestimated the final toxicity results.

Styrene mainly enters the aquatic environment through point source discharges (e.g. sewage and industrial effluents) (see 2.4). Although styrene is not very persistent in water, organisms in the immediate vicinity of a discharge may be subjected to long-term continuous exposure to styrene, depending on their ability to avoid exposure (e.g. fish may respond by swimming to more tolerable conditions). An assessment of the chronic toxicity of styrene to aquatic organisms is therefore important for the calculation of Environmental Quality Standards (EQSs). However, there are no reliable chronic toxicity data reported in the literature. Due to its high volatility, styrene is difficult to test in the laboratory and this might explain the lack of chronic toxicity.

No field or mesocosm tests appear to have been performed with styrene. Such tests would provide a useful link between laboratory and field responses to styrene.

There is no experimental evidence to suggest that styrene is accumulated by freshwater organisms even though the *n*-octanol-water partition coefficients (log K_{ow}) reported (e.g. 2.59-3.16) suggest that styrene has a moderate potential to bioaccumulate. However, this is not supported by the only experimental bioconcentration factor reported for styrene (13.5 for the goldfish *Carassius auratus*).

Table 5.1 Acute toxicity of styrene to freshwater organisms

Species	Test type	Analysis	Duration (hours)	Effect	Conc (mg l^{-1})	Ref
Bacteria						
Pseudomonas putida (bacterium)	S	N	16	toxicity threshold [1]	72	1
Cyano-bacteria						
Microcystis aeruginosa (blue-green alga)	S	N	ND	toxicity threshold [1]	67	2
Algae						
Scenedesmus quadricauda (green alga)	S	N	72	toxicity threshold [1]	>200	1
Protozoans						
Uronema parduczi Chatton-Lwoff (protozoan)	S	N	ND	toxicity threshold [1]	185	3
Molluscs						
Amphimelania holandri (freshwater snail)	S	N	48	LC_{50}	111.2	4
Amphimelania holandri (freshwater snail)	S	N	96	LC_{50}	96.9	4

Species	Test type	Analysis	Duration (hours)	Effect	Conc (mg l^{-1})	Ref
Crustaceans						
Daphnia magna (water flea)	S	N	24	LC_{50}	27	5
Daphnia magna (water flea)	S	N	48	LC_{50}	23	5
Daphnia magna (water flea)	S	N	48	NOEC	<6.8	5
Asellus aquaticus (water slater)	S	N	96	LC_{50}	52.9	4
Gammarus fossarum (freshwater shrimp)	S	N	96	LC_{50}	52.0	4
Fish (non-salmonid)						
Pimephales promelas (fathead minnow)	F	A	96	LC_{50}	4.02	6
Pimephales promelas (fathead minnow)	S	N	96	LC_{50}	32	7
Carassius auratus (goldfish)	S	A	24	LC_{50}	26	8
Lepomis macrochirus (bluegill sunfish)	S	N	96	LC_{50}	25.1	9
Fish (salmonid)						
Oncorhynchus mykiss (rainbow trout)	S	N	24	LC_{50}	2.5	10
Oncorhynchus mykiss (rainbow trout)	F	N	96	LC_{50}	5.9	11
Oncorhynchus mykiss (rainbow trout)	F	N	168	LC_{50}	4.9	11

Notes to Table 5.1

S: Static
A: Concentrations analysed
F: Flow-through
[1]Cell multiplication inhibition
N: Nominal concentrations

References

1. Bringmann and Kuhn (1980)
2. Bringmann and Kuhn (1978)
3. Bringmann *et al.* (1980)
4. Erben and Pisll (1993)
5. LeBlanc (1980)
6. Gieger *et al.* (1990)
7. Mattson *et al.* (1976)
8. Bridié *et al.* (1979)
9. Pickering and Henderson (1966)
10. Qureshi *et al.* (1982)
11. Abram and Collins (1981)

5.2 Saltwater

The data relating to the sensitivity of marine species are limited to three studies looking at short term exposure:

1. Price *et al.* (1974) reported a 24-hour median threshold limit (Tlm) of 68 mg l^{-1} for the brine shrimp (*Artemia salina*) under static conditions (no further study details were reported).

2. Qureshi *et al.*(1982) performed a MICROTOX test with styrene, using the luminescent bacteria *Photobacterium phosphoreum*, and determined a 5 minute EC_{50} of 5.4 mg l^{-1}.

3. Heitmuller *et al.* (1981) reported 24, 48, 72 and 96 hour LC_{50}s as 9.1 mg l^{-1} with a no observed effect concentration (NOEC) of 5.1 mg l^{-1}. The tests were carried out according to EPA guideline under static conditions and results reported as nominal values.

The only study to have investigated chronic exposure was carried out using the crustacean *Pontoporeia affinis* (a small amphipod found in Northern Europe but not Britain). However, the effects observed in this study appear to have been rather subjective. At concentrations from 2.3 to 23 mg l^{-1}, an immediate increase in swimming activity was observed, while at concentrations ranging from 35 to 46 mg l^{-1}, immediate cessation of swimming activity was observed, although the crustaceans recovered within a few days. In both instances the total duration of exposure was 40 days. Further details of the test design were not available. Thus as a consequence of the lack of information available on the test itself, this study cannot be considered in the derivation of EQSs.

From these data it is not possible to derive even a tentative EQS for the saltwater environment.

No information exists on the bioaccumulation potential of styrene in the saltwater environment, although it is not expected to differ significantly from the freshwater environment.

6. DERIVATION OF EQSS

6.1 Standards in other countries

Standards for the protection of aquatic life have not been established by the EC, the United States or Canada. However, the EC Freshwater Fish Directive (78/659/EEC) (CEC 1978) requires that "petroleum products" are not present in such quantities that they form a visible film on the surface of the water or form coatings on the beds of water-courses and lakes; impart a detectable "hydrocarbon" taste to fish; or produce harmful effects in fish.

Mandatory EC standards of 0.05, 0.2 and 1.0 mg l^{-1} have been adopted for dissolved or emulsified hydrocarbons (after extraction by petroleum ether) for A1, A2 and A3 treatments[1], respectively, for surface waters intended for potable supply (CEC 1975). However, specific standards for styrene have not been adopted.

Drinking water standards have not been set for styrene in the EC Drinking Water Directive. The World Health Organisation (WHO 1993) have recommended a health-based guideline value of 20 µg l^{-1} for styrene in drinking water. The United States Environmental Protection Agency has adopted a Maximum Contaminant Level (MCL) of 100 µg l^{-1} as a drinking water standard in the US (US EPA 1992a).

6.2 Protection of freshwater life

The available toxicity data indicate that styrene is of moderate to low acute toxicity to the freshwater species tested, with the majority of effect concentrations above 20 mg l^{-1}. However, no information is available on the effects of styrene on freshwater insects or macrophytes, nor are there any reliable chronic toxicity data or field data. A summary of the lowest toxicity data reported for each taxonomic group is provided in Table 5.1.

Styrene is highly volatile in aqueous media and therefore not very persistent. The majority of toxicity studies do not, however, appear to have taken account of this (e.g. by analysing exposure concentrations, by adopting flow-through or semi-static conditions; or by covering test vessels). The lowest toxicity values reported generally come from flow-through studies.

The lowest toxicity value reported is a 24-hour LC_{50} of 2.5 mg l^{-1} for the rainbow trout (*Oncorhynchus mykiss*) (Qureshi *et al.* 1982). This value is, however, thought to be unsuitable for the derivation of Environmental Quality Standards (EQSs), principally because it was based on nominal concentrations of styrene applied in an open, static test system. The most credible study reported was a flow-through test with the fathead minnow (*Pimephales promelas*). Based on measured concentrations of styrene, a 96-hour LC_{50} of 4.02 mg l^{-1} was determined

[1] A1 refers to simple physical treatment and disinfection; A2 normal full physical and chemical treatment with disinfection; and A3 intensive physical and chemical treatment with disinfection.

(Gieger *et al.* 1990). However, *P. promelas* is a warm water fish and therefore cannot be considered an ideal species on which to base EQSs. A 96-hour LC_{50} of 5.9 mg l^{-1} was calculated for rainbow trout (*Oncorhynchus mykiss*) in another flow-through test (Abram and Collins 1982). In this study, nominal concentrations of styrene were applied without confirmation by analysis, although a solvent carrier (acetone) was used to aid dissolution in the test media. However, because the authors claimed that the maximum concentration of acetone used (2400 mg l^{-1}) was well below the acute toxicity values reported for acetone with *O. mykiss* (96-hour LC_{50}s 8000-10 000 mg l^{-1}), control tests with equal concentrations of acetone were not employed. The use of solvents such as acetone may itself cause some stress to the test organisms, in addition to that caused by exposure to styrene. Because this source of additional stress was not accounted for, it is conceivable that the toxicity values reported for styrene in this study were overestimated. Using these values to derive EQSs could therefore be considered as somewhat precautionary.

An EQS of 50 µg l^{-1}, expressed as an annual average concentration is proposed. This is derived by applying a safety factor of approximately 100 to the 96-hour LC_{50} of 5.9 mg l^{-1} reported for *O. mykiss*. A maximum allowable concentration (MAC) of 500 µg l^{-1} is also recommended. The MAC is derived by applying a safety factor of approximately 10 to the 96-hour LC_{50} for *O. mykiss*. The analytical techniques available appear to be adequate for the monitoring of the proposed EQSs (see Section 3). However, these EQSs should be considered "tentative" because of the limited amount of credible toxicity data available. The EQSs will need to be reviewed when additional data become available. Due to the volatile nature of styrene in water, there is a particular requirement for well conducted studies (measured concentrations, flow-through or semi-static tests), and, to ensure the protection of organisms present in the vicinity of styrene outfalls (e.g. sewage or industrial effluents), there is a need for reliable chronic toxicity data. Data from field studies would also be useful to verify the EQSs proposed.

6.3 Protection of saltwater life

There are insufficient toxicity data available for the derivation of Environmental Quality Standards (EQSs) to protect saltwater life. Until further data become available, it is suggested that the EQSs derived for the protection of freshwater life be adopted as guidelines for the protection of saltwater life: an annual average concentration of 50 µg l^{-1} and a maximum allowable concentration (MAC) of 500 µg l^{-1}. Comparison of these standards with the two toxicity values reported for saltwater organisms (24-hour TLm of 68 mg l^{-1} for the brine shrimp *Artemia salina*, a 5-minute EC_{50} of 5.4 mg l^{-1} for the bacterium *Photobacterium phoshoreum* in the Microtox test) and a 96-hour LC_{50} of 9.1 mg l^{-1} for the sheepshead minnow (*Cypronodan variegatus*) suggests that they will be adequately protective. However, these standards should be reviewed as more data become available.

6.4 Abstraction of water for potable supply

An Environmental Quality Standard (EQS) of 20 µg l^{-1}, expressed as a MAC, is proposed for the protection of water abstracted to potable supply. This is based on the health-based guideline recommended by the World Health Organisation (WHO 1993) which was calculated

from a Tolerable Daily Intake (TDI) of 7.7 $\mu g\ kg^{-1}$ body weight, assuming a 60 kg adult drinks 2 litres of water per day, and allocating 10% of the TDI to drinking water.

Aesthetic problems may, however, occur at concentrations in drinking water below this EQS. WHO (1993) suggested that taste and odour problems may arise at concentrations between 2 and 200 $\mu g\ l^{-1}$. The taste threshold established for styrene by WRc (99% purity and dissolved in still, bottled water equilibrated at 25 °C) is about 94 $\mu g\ l^{-1}$. Further details are provided in Appendix D.

7. CONCLUSIONS

- Styrene has a wide range of commercial uses, particularly in the plastic and chemical industries. It is used extensively in the production of polymers, copolymers and reinforced plastics and is present in petroleum products and some adhesives. The major use of styrene is in the manufacture of polystyrene.

- The major release of styrene to the aquatic environment is through industrial effluents (in particular from chemical and plastic manufacturing plants), either directly or indirectly via sewage treatment.

- Styrene is effectively removed from effluents by activated sludge and sedimentation processes at low concentrations. For example, influent concentrations of up to 0.1 mg l^{-1} were 96.4 and 91.4% removed from domestic wastewater by activated sludge and sedimentation, respectively. High concentrations of styrene may become progressively less biodegradable (only 17% of [^{14}C]styrene was mineralised in soil at a concentration of 4000 mg kg^{-1}).

- Styrene is highly volatile in water, with half-lives generally only of a few hours. Half-lives reported for styrene in rivers vary from 3.5 to 14 hours, depending on wind speed and water depth. Biodegradation and sorption may play minor roles in the removal of styrene. Abiotic processes such as oxidation, hydrolysis and photolysis are not expected to influence styrene in the aquatic environment.

- Significant concentrations of styrene are not likely to enter groundwaters through the soil (e.g. as a result of accidental spillages and leakages) as it is prone to sorption as well as aerobic and anaerobic biological degradation. A wide range of half-lives for aerobic biodegradation are reported (5 days to 21.7 weeks), depending on the concentration and soil type. Monitoring data for UK groundwaters confirms that styrene is not a common contaminant.

- The majority of laboratory studies reported for styrene were unsuitable for the accurate estimation of its toxicity. Analysis of exposure concentrations was rarely carried out and few tests attempted to maintain constant exposure concentrations by using flow-through or semi-static test designs. No credible chronic toxicity studies were available to assess the possible effects of continuous long-term exposure to aquatic life (e.g. as a result of point source discharges).

- Examination of the available toxicity data for freshwater organisms indicate that styrene is of moderate to low acute toxicity to those species tested, with the majority of effect concentrations above 20 mg l^{-1}. No data were available for freshwater insects or macrophytes.

- The toxicity data for saltwater organisms are restricted to three studies looking at short term exposure and a chronic study using the crustacean *Pontoporeia* which indicate a similar order of toxicity as to freshwater life.

- The octanol-water partition coefficients reported (log K_{ow} 2.59-3.16) suggest that styrene may have a moderate potential for bioaccumulation, although this is not supported by the only experimental bioconcentration factor reported (13.5 for the goldfish, *Carassius auratus*). There is no information on the metabolism or depuration of styrene by fish.

- EQSs expressed as an annual average concentration of 50 $\mu g\ l^{-1}$ and a maximum allowable concentration of 500 $\mu g\ l^{-1}$ are tentatively proposed for the protection of freshwater and as guidelines values for the protection of saltwater life. These EQSs should be reviewed as more data become available. Due to the volatile nature of styrene, there is a particular requirement for well conducted toxicity studies (measured concentrations, flow-through or semi-static conditions), especially chronic toxicity studies.

- An EQS of 20 $\mu g\ l^{-1}$ expressed as a MAC is proposed for the protection of water abstracted to potable supply.

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APPENDIX A FATE AND BEHAVIOUR IN THE ENVIRONMENT

A1. FATE AND BEHAVIOUR IN SOIL

The major processes that affect the fate and behaviour of styrene in soil are biodegradation, sorption and volatilisation. Biodegradation and sorption are probably the most important mechanisms, with volatilisation accounting for losses from surface layers. Abiotic processes such as hydrolysis and photolysis are not expected to significantly influence the degradation of styrene in soil. Details of the studies reported on the fate and behaviour of styrene in soil are given below and summarized in Table A1.

Fu and Alexander (1992) carried out a series of experiments to study the possible significance of volatilisation, biodegradation and sorption of styrene in different environmental samples, including aquifer sand, Lima loam (pH 7.23, 7.5% organic matter), Kendaia loam (pH 7.46, 5.3% organic matter), silt loam (pH 4.87, 5.7% organic matter), organic soil (pH 7.50, 32.9% organic matter), primary sewage, lake water, and groundwater. Approximately 26% of styrene (initial concentration 1.0 mg kg^{-1}) was found to volatilise from a 1.5 cm depth of soil (Kendaia loam) in 31 days, compared to 11% mineralisation at the same time. Mineralisation did not occur in tests where the samples where sterilised, indicating that abiotic processes such as hydrolysis do not have a significant effect on the overall degradation of styrene. The rate of microbial mineralisation, however, was rapid in sewage, mineral soil (pH 7.23) and organic soil but much slower in aquifer sand, waterlogged soil and mineral soil of pH 4.87. In 30 hours, approximately 79% of styrene was reportedly sorbed to samples of mineral soils, but mineralisation was still rapid, suggesting that sorption is not necessarily a major limitation to the microbial transformation of this compound. The percentage of styrene mineralised per hour in soil was not found to vary greatly (i.e. 0.22-0.41% h^{-1}) for concentrations between 0.005 and 1.0 mg kg^{-1}. Between 1.0 and 5.0 mg kg^{-1} there appeared to be a reduction in the percentage mineralised per hour by a factor of about 3 (from 0.22 to 0.087% h^{-1}). The overall percentage of styrene mineralised after 30 days was also reduced, from 47% to 31% for these two concentrations. At the highest concentration tested (4000 mg kg^{-1}), the percentage mineralised per hour and the percentage mineralised after 30 days were 0.043% h^{-1} and 17%, respectively. This suggests high styrene concentrations may have adverse effects on soil microorganisms. In contrast, microbial mineralisation appeared to be more sensitive to changing styrene concentrations in aquifer sand. Only 1.09 and 1.51% of the styrene added to the aquifer sand at 0.02 mg kg^{-1} and 0.1 mg kg^{-1}, respectively, were mineralised in the test period (about 20 days). From these findings the authors concluded that styrene is likely to be rapidly destroyed by biodegradation in most aerobic environments although the rate of biodegradation will be slower at low concentrations in aquifers (probably due to the low biological activity in aquifers) and in environments at low pH. Sorption appeared to be the only other significant factor influencing the fate and behaviour of styrene in soil.

In another study, Fu *et al.* (1994) examined in more detail the desorption and biodegradation of sorbed styrene in soil and aquifer solids. In this study, approximately 40 and 18% of the styrene added to Lima loam and aquifer solids, respectively, were mineralised after 50 days. Measurements of equilibrium desorption under abiotic (i.e. sterile) conditions showed that only

7.2% and 5.7% of the sorbed styrene was present in aqueous solution at 150 hours (i.e. desorbed), respectively, but 61.0% and 66.7% of the compound were desorbed from the soil and aquifer solids, respectively, in only 16 hours under non-equilibrium conditions. In soil columns that had been repeatedly leached with water approximately 20% of the styrene which remained sorbed to the soil was mineralised within 49 days. Styrene added to an organic soil (32.9% organic matter) also mineralised extensively. However, the authors discovered that if the compound was maintained in the soil for increasingly long periods in the absence of microbial activity, the extent of biodegradability by subsequent addition of microorganisms became progressively lower. For example, when styrene was maintainted in sterile soil for 4 months, less than 3% was mineralised. In addition, the authors found that most of the freshly added styrene to soil could be recovered using acetonitrile, but after 123 days less than 4% could be recovered. This suggests that styrene which persists in soil for long periods of time may be abiotically transformed to a state which is resistant to biodegradation.

A1.1 Abiotic processes

Abiotic degradation processes such as hydrolysis and photolysis are not expected to be significant removal pathways for styrene in soil. Fu and Alexander (1992), for example, showed that mineralisation of styrene does not occur in sterilised soil samples where volatilisation is prevented. In this case, the only possible processes for degradation would have been abiotic (except volatilisation). Sorption, however, may play a more important role (see A1.3).

A1.2 Biodegradation

A1.2.1 Aerobic biodegradation

Studies carried out by Fu and Alexander (1992) and Fu *et al.* (1994) have indicated that aerobic biodegradation is an important mechanism for the removal of styrene from sub-surface layers of soil (volatilisation may account for the removal of up to 26% of styrene in surface layers) (see A1). From the various studies performed on the aerobic biodegradation of styrene it would appear that certain microorganisms which exist in soil (and sewage) are capable of degrading it over time. Fu and Alexander (1992), for example, showed that styrene concentrations of 0.005, 0.02, 0.1, and 1.0 mg kg^{-1} were 55, 62, 50 and 47% mineralised after 30 days, respectively. However, concentrations equal to or greater than 5.0 mg kg^{-1} were found to be increasingly less biodegradable, with only 17% mineralised at the highest concentration tested (4000 mg kg^{-1}). This suggests that high concentrations of styrene may inhibit microbial activity. Burback *et al.* (1994), for example, estimated a toxic concentration of 4.0 mM (approximately 417 mg l^{-1}) for soil mycobacterium exposed to styrene for 7 days in suspended media.

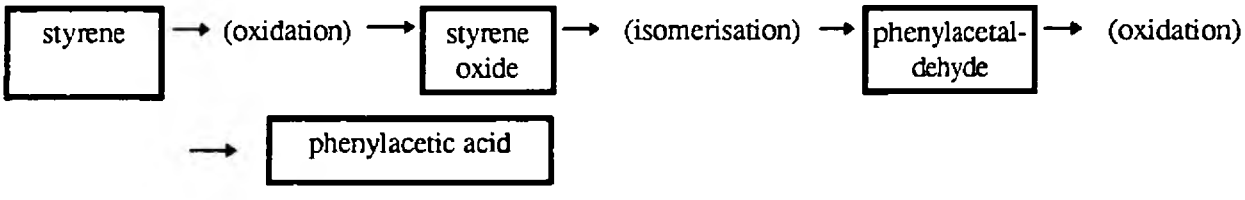
The rates and extent of mineralisation were markedly different among the soils studied by Fu and Alexander (1992). After 30 days, an initial concentration of 1.0 mg kg^{-1} (as ^{14}C), approximately 15%, 30% and 40% was mineralised in a silt loam (pH 4.87, 5.74% organic matter), Lima loam (pH 7.23, 7.5% organic matter) and organic soil (pH 7.50, 32.9 % organic matter), respectively, suggesting that biodegradation is reduced in acidic soils but enhanced in soils that are rich in organic matter. The effects of pH are probably explained by a reduction in microbial activity. Soils that are rich in organic matter are likely to support a wider variety of

microorganisms, hence increasing the potential for biodegradation. Fu and Alexander (1992) also reported that the mineralisation of styrene in soil was less extensive under waterlogged conditions, probably caused by a reduction in oxygen levels.

El Aalam *et al.* (1993) reported that the bacterium *Pseudomonas aeuginosa* degraded styrene at a specific activity of 293 mg g^{-1} h^{-1} under laboratory conditions. Using continuous-flow biofilm columns to simulate subsurface conditions (glass beads were used as media for biofilm formation), Bouwer and McCarty (1984) reported >99% removal of styrene from an influent concentration of 7.6 µg l^{-1} and with a minimum detention time of just 20 minutes. In this study, however, styrene was not the sole source of carbon and energy. Acetate was used as a primary substrate to support bacterial growth. This suggests that low concentrations of styrene do not necessarily require enriched cultures (i.e. microorganisms which can utilise styrene as a sole source of carbon and energy) to be degraded in soil. However, the removal of styrene in this test system may have partly been due to adsorption to the biofilm, and the relative proportion of styrene actually degraded is not reported.

Sielicki *et al.* (1978) investigated the microbial transformation of styrene and [^{14}C]styrene in soil and enrichment cultures. They proposed two different mechanisms responsible for the removal of styrene (and the unsaturated styrene dimer 1,2-diphenylbutane) in enrichment cultures: (i) a mixed population of microrganisms, capable of utilizing styrene as a sole source of carbon, oxidises this substrate to phenylethanol and phenylacetic acid, and (ii) the culture also mediates polymerisation of the monomer to low-molecular weight styrene oligomers. They explain the latter as probably being caused by the microbial breakdown of butylcatechol, an antioxidant polymerisation inhibitor present in commercial styrene. The resultant polymer material was reportedly metabolised by the microorganism cultures. In soil incubation studies, $^{14}CO_2$ evolution from applied 3[8-^{14}C]styrene was used to estimate microbial degradation. Approximately 90% of the labelled carbon was evolved from a 0.2% addition, and about 75% was lost from the 0.5% application over a 16-week period.

By using styrene as the sole source of carbon and energy (concentrations ranging from 10-500 µM), Hartmans *et al.* (1990) were able to isolate a number of aerobic styrene-degrading bacterial and fungal strains from soil and water samples. In one bacterial strain (*Xanthobacter* strain S5) styrene metabolism was investigated. At different times cell extracts were found to contain the enzymes styrene monooxygenase, styrene oxide isomerase and phenylacetaldehyde dehydrogenase. This led the authors to propose the following pathway for styrene degradation:



The pathway proposed by Hartmans *et al.* (1990) is in broad agreement with that suggested by Sielicki *et al.* (1978), although the latter indicated that oxidation to the alcohol (phenylethanol) rather than the aldehyde (phenylaldehyde) occurs prior to the formation of phenylacetic acid.

Hartmans *et al.* (1990) suggested that styrene oxide is probably an intermediate in the degradation of styrene by most organisms. However, they were unable to detect the

accumulation of styrene oxide in growing cultures or in suspensions of washed cells incubated with styrene. They therefore proposed that the isomerisation of styrene oxide was sufficiently rapid to prevent the persistence of styrene oxide. Burback *et al.* (1994) estimated a toxic concentration of >100 mM (approximately 12 020 mg l^{-1}) for soil mycobacterium exposed to styrene oxide in suspended growth media. This value is more than an order of magnitude lower than that determined for the styrene monomer (417 mg l^{-1}).

As already mentioned, Sielicki *et al.* (1978) found evidence of the styrene monomer being polymerised to low-molecular-weight styrene oligmers as well as being oxidised. The tests carried out suggested that this too is a microbially mediated reaction. They were able to demonstrate that the antioxidant polymerisation inhibitor TBC (4-tertiary butylcatechol) is readily oxidised by microorganisms in soil, hence explaining the uninhibited polymerisation of styrene. However, after the complete disappearance of the styrene monomer, due to direct oxidisation and/or polymerisation, microbial growth was found to continue. This was coupled with the loss of polymer material (styrene oligomer) in the mixed cultures being tested, indicating that the oligomer is ultimately broken down by microorganisms. The authors suggested that microbial oxidisation and fission of the oligomer leads to the formation of phenylacetic acid.

Higashimura *et al.* (1983) studied the effect of methyl substitution on the biodegradation of linear styrene dimers by two soil bacteria, *Alcaligenes* sp. (strain 559) and *Pseudomonas* sp. (strain 419). The two strains were found to readily decompose the styrene dimer I (1,3-diphenylbut-1-ene) (100% after 1 day's incubation) and both the styrene-methylstyrene codimers II and III. Codimers III were reported to have a methyl group on the benzene nucleus attached to the main-chain double bond, while the methyl group in codimers II was attached to the benzene ring by a saturated carbon. The methylstyrene homodimer remained intact (0% degradation after 5 days incubation).

A1.2.2 Anaerobic biodegradation

Methanogenic microbial consortia, originally enriched from an anaerobic sewage sludge with styrene as the sole carbon and energy source, were used by Grbic-Gblic *et al.* (1990) to study the transformation of styrene under strictly anaerobic conditions. Styrene was added to the test system as the substrate in a range of concentrations from 0.1 to 10 mmol l^{-1} (approximately 10.4 to 1040.0 mg l^{-1}), but no methane was detected during the total incubation period of 8 months. A styrene concentration of 1.0 mmol l^{-1} (as sole source of carbon, equivalent to 8.0 mmol carbon l^{-1}) was, however, found to be 99% degraded after 4 months. It was suggested that styrene is utilised rather than cometabolised since when a readily biodegradable substrate (yeast extract) was added, styrene degradation was completely inhibited. It was proposed that the faster growing yeast utilizers had out-competed the styrene-digesting microorganisms present in the consortia. The various analytical techniques used indicated that the transformation of styrene was initiated through an oxidation-reduction reaction and that the initial transformation was likely to have been caused by the addition of water across the double bond in the alkenyl side-chain to form phenylethanol. The authors reported that the process led eventually to the production of carbon dioxide, although several intermediates were identified, in particular benzoic acid and phenol. A tentative transformation pathway was proposed by Grbic-Galic *et al.* (1990) and this is summarized in Figure A1.

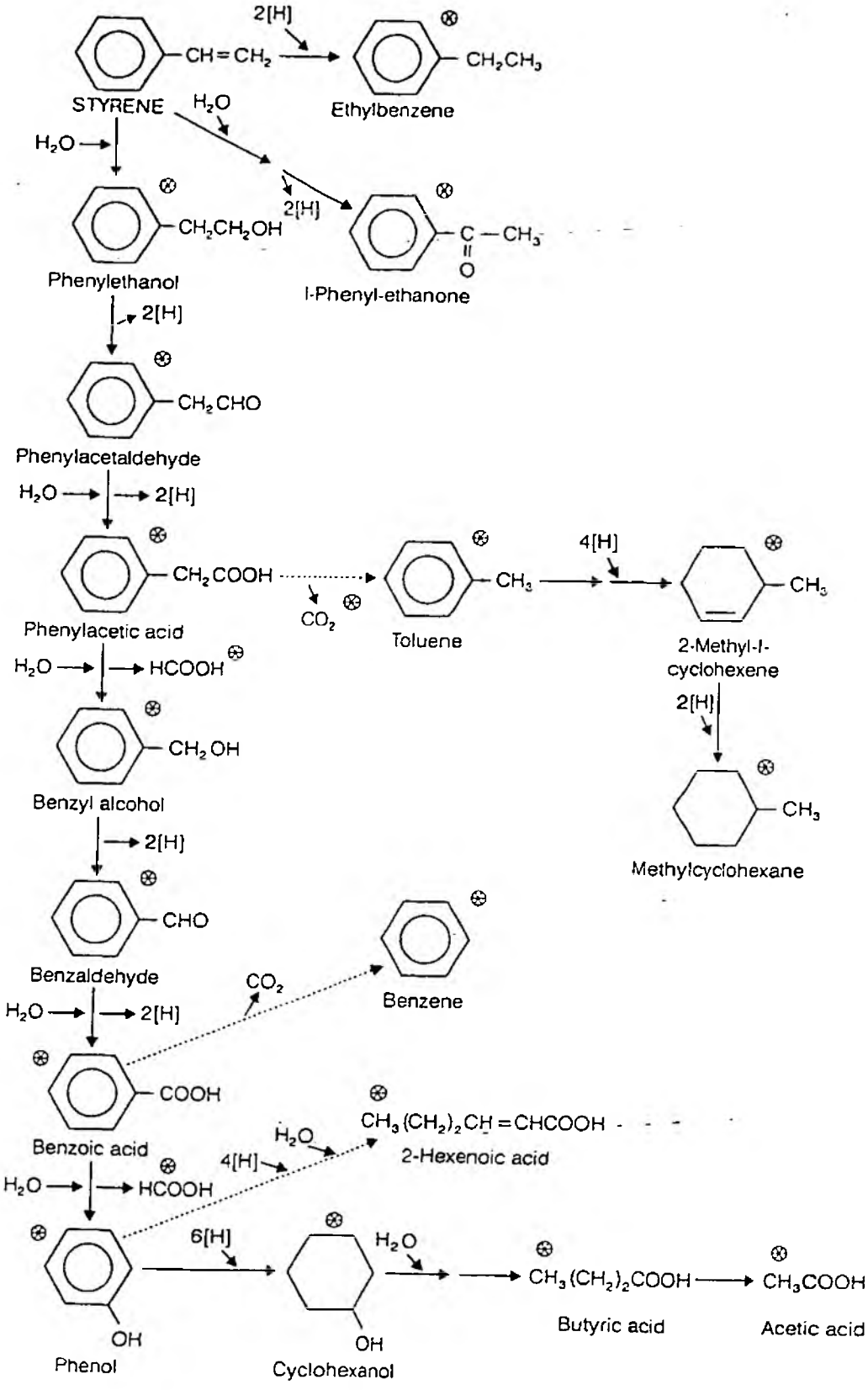


Figure A1 **Proposed tentative transformation pathway for styrene in anaerobic consortia (after Grbic-Galic *et al* 1990). (Reductive reactions which create some of the reduced electron and proton sink compounds (ethylbenzene, toluene, benzene) are shown. Compounds marked by an asterix were detected in culture fluids).**

The absence of methanogenic styrene degradation observed by Grbic-Galic *et al.* (1990) under anaerobic conditions was also demonstrated by Schink (1985). In the latter study, a variety or organic chemicals were tested under anaerobic conditions in enrichment cultures using mineral media inoculated with sewage sludge or sediment samples of limnic and marine origin. However, the type of biotransformation described by Grbic-Galic *et al.* (1990) was not investigated further.

Wilson *et al.* (1983) examined the degradation potential of styrene and a number of other volatile organic compounds in microcosms aimed at simulating subsurface soil conditions. Core samples of soil taken from two different sites just above (depths of 2.1 and 3.6 m, respectively) and below (depths of 3.6 and 4.8 m, respectively) the water table, and in such a way as to avoid contamination with surface microorganisms, were used in each microcosm. Samples from both sites were found to be about 90% sand (dry weight). Pore waters from the different microcosms were analysed after 0, 1, 3, 9 and 27 weeks of incubation. Abiotic controls were established by autoclaving some material prior to use in the microcosms. Styrene was found to degrade slowly in all samples. The percentage of initial concentration (600-800 $\mu g\ l^{-1}$) degraded per week for the two soil samples taken just above the water table were 2.3% (pH 4.3) and 3.8% (pH 7.8), respectively. For the samples taken just below the water table, these were 4.3% (pH 4.2) and 12.0% (total of 9 weeks incubation, pH 5.7), respectively. Very little degradation was observed in autoclaved samples, indicating that degradation of styrene in subsurface soil layers is mainly biological.

A1.3 Sorption

Adsorption of styrene in soil may occur to some extent. The log K_{oc} values of 2.43-2.74 reported for styrene (estimated from water solubility and K_{ow}, respectively) indicate that it has a moderate to low adsorption potential in soil. Studies carried out by Fu and Alexander (1992) and Fu *et al.* (1994) have confirmed that styrene does adsorb to soil particles (up to 79%), although the sorption processes do not appear to significantly affect the aerobic biodegradation of this compound within the upper soil layers. These studies are reviewed in more detail in Section A1.

A1.4 Volatility

A limited amount of information is available on the volatility of styrene from soil. However, the vapour pressure (5 mm Hg at 20 °C) and Henry's Law constant (2.3×10^{-3} - 5.2×10^{-3} atm $m^3\ mol^{-1}$) reported for this compound (see Table 2.1) suggest that it has a high potential for volatilisation. The extent to which volatilsation occurs is determined by a number of variable factors, including wind speed and soil depth. Fu and Alexander (1992) carried out a series of experiments to study the possible significance of volatilisation, in comparison to biodegradation and sorption (see A1 and A1.3), for the removal of styrene from different environmental samples, including aquifer sand, Lima loam (pH 7.23, 7.5% organic matter), Kendaia loam (pH 7.46, 5.3% organic matter), silt loam (pH 4.87, 5.7% organic matter), organic soil (pH 7.50, 32.9% organic matter) and primary sewage. Only 26% of styrene (initial concentration 1.0 mg kg^{-1}) was found to volatilise from a 1.5 cm depth of soil (Kendaia loam) in 31 days.

Table A1 Summary of fate and behaviour of styrene in soil (concentrations in mg kg^{-1} unless otherwise stated)

	Laboratory, field or modelled data	Media	Solvent used	pH	Temp (^{o}C)	Initial conc mg kg^{-1}	Final conc mg kg^{-1}	Duration	Half-life	Comments	Ref
Aerobic Biodegradation	L	lima loam[1]	N	7.2	22±2	1.0	0.45	30 days	ND	[^{14}C]styrene evolved as CO_2	1
	L	water-logged lima loam[1]	N	7.2	22±2	1.0	0.75	30 days	ND	[^{14}C]styrene evolved as CO_2	1
	L	lima loam[1]	N	7.2	22±2	0.005	2.25×10^{-3}	30 days	~6.5 days	0.32 % ^{14}C mineralised h^{-1}	1
	L	lima loam[1]	N	7.2	22±2	0.02	0.0096	30 days	~5 days	0.41 % ^{14}C mineralised h^{-1} Initial rate rapid, slower after 5 days (12% between days 5 and 30)	1
	L	lima loam[1]	N	7.2	22±2	0.1	0.05	30 days	~30 days	0.32 % ^{14}C mineralised h^{-1} Initial rate rapid, slower after 5 days (10% between days 5 and 30)	1
	L	lima loam[1]	N	7.2	22±2	1	0.53	30 days	>30 days	0.22 % ^{14}C mineralised h^{-1}	1
	L	lima loam[1]	N	7.2	22±2	5	3.45	30 days	>30 days	0.087 % ^{14}C mineralised h^{-1}	1

	Laboratory, field or modelled data	Media	Solvent used	pH	Temp (°C)	Initial conc mg kg^{-1}
	L	lima loam[1]	N	7.2	22±2	10
	L	lima loam[1]	N	7.2	22±2	50
	L	lima loam[1]	N	7.2	22±2	100
	L	lima loam[1]	N	7.2	22±2	500
	L	lima loam[1]	N	7.2	22±2	1000
	L	lima loam[1]	N	7.2	22±2	4000
	L	lima loam[1]	N	7.2	22±2	1
	L	silt loam [2]	N	4.9	22±2	1
	L	organic soil [3]	N	7.5	22±2	1
	L	aquifer sand [4]	N	6.9	22±2	0.02

Final conc mg kg^{-1}	Duration	Half-life	Comments	Ref
7.3	30 days	>30 days	0.076 % ^{14}C mineralised h^{-1}	1
37.0	30 days	>30 days	0.077% ^{14}C mineralised h^{-1}	1
80	30 days	>30 days	0.063 % ^{14}C mineralised h^{-1}	1
420	30 days	>30 days	0.04 % ^{14}C mineralised h^{-1}	1
840	30 days	>30 days	0.032 % ^{14}C mineralised h^{-1}	1
3320	30 days	>30 days	0.043 % ^{14}C mineralised h^{-1}	1
0.72	28 days	>28 days	mineral soil (neutral pH)	1
0.85	28 days	>28 days	acidic soil	1
0.72	28 days	>28 days	organic soil	1
0.0002	20 days	>20 days		1

	Laboratory, field or modelled data	Media	Solvent used	pH	Temp (°C)
	L	aquifer sand [4]	N	6.9	22±2
	L	aquifer sand [4]	N	6.9	22±2
	L	kendaia loam [5]	N	7.5	22±2
	L	lima loam [1]	N	7.2	22±2
	L	aquifer sand [4]	N	6.9	22±2
	L	lima loam [1]	N	7.2	22±2
	L	lima loam [1]	N	7.2	22±2
	L	lima loam [1]	N	7.2	22±2

Initial conc mg kg^{-1}	Final conc mg kg^{-1}	Duration	Half-life	Comments	Ref
0.1	0.0015	20 days	>20 days		1
1.0	0.9	30 days	>30 days		1
2	11% mineral-ised	30 days	>30 days	Loss attributed to mineralisation (26% also lost by volatilisation)	1
1	40% mineral-ised	50 days	>50 days		2
1	18% mineral-ised	50 days	>50 days		2
1	24% mineral-ised	50 days	ND	unleached	2
1	19% mineral-ised	50 days	ND	leached (% of remaining sorbed styrene)	2
1	13% mineral-ised	50 days	ND	leached (% of initial styrene)	2

	Laboratory, field or modelled data	Media	Solvent used	pH	Temp (^{o}C)	Initial conc mg kg^{-1}
	L	lima loam [1]	N	7.2	22±2	1
	L	organic soil [3]	N	7.5	22±2	1
	L	lima loam [1]	N	7.2	22±2	1
	L	organic soil [3]	N	7.5	22±2	1
	L	lima loam [1]	N	7.2	22±2	1
	L	organic soil [3]	N	7.5	22±2	1
	L	landfill soil [8]	190 ml kg^{-1} acetone	6.9	ND	2000

Final conc mg kg^{-1}	Duration	Half-life	Comments	Ref
28% mineral-ised	30 days	ND	unaged soil [7]	2
42% mineral-ised	30 days	ND	unaged soil [7]	2
4% mineral-ised	30 days	ND	aged for 30 days [7]	2
8% mineral-ised	30 days	ND	aged for 30 days [7]	2
<3% mineral-ised	30 days	ND	aged for 123 days [7]	2
<3% mineral-ised	30 days	ND	aged for 123 days [7]	2
95% decomposi-tion	16 weeks	7-8 weeks	[8-^{14}C]styrene evolved as CO_2	3

	Laboratory, field or modelled data	Media	Solvent used	pH	Temp (^{o}C)	Initial conc mg kg^{-1}
	L	landfill soil [8]	N	6.9	ND	5000
	L	sandy loam [8]	190 ml kg^{-1} acetone	7.0	ND	2000
	L	sandy loam [8]	N	7.0	ND	5000
	L	acidic sub-surface soil	N	4.3	17	0.6-0.8 mg l^{-1}
	L	acidic sub-surface soil	N	4.3	17	0.6-0.8 mg l^{-1}
	L	sub-surface soil	N	7.8	17	0.6-0.8 mg l^{-1}

Final conc mg kg^{-1}	Duration	Half-life	Comments	Ref
75% decomposi -tion	16 weeks	11 weeks	[8-^{14}C]styrene evolved as CO_2	3
87% decomposi -tion	16 weeks	2 weeks	[8-^{14}C]styrene evolved as CO_2	3
70% decomposi -tion	16 weeks	6 weeks	[8-^{14}C]styrene evolved as CO_2	3
2.3% decomposi -tion per week	27 weeks	~21.7 weeks	Soil sample taken just above the water table (2.1 m depth)	4
4.3% decomposi -tion per week	27 weeks	~11.6 weeks	Soil sample taken just below the water table (3.6 m depth)	4
3.8% decomposi -tion per week	27 weeks	~13.2 weeks	Soil samples taken just above the water table (3.6 m depth)	4

	Laboratory, field or modelled data	Media	Solvent used	pH	Temp (^{o}C)	Initial conc mg kg^{-1}
	L	sub-surface soil	N	7.8	17	0.6-0.8 mg l^{-1}
Sorption	L	lima loam [1]	N	7.2	22±2	1 mg l^{-1}
	L	lima loam [1]	N	7.2	22±2	1 mg l^{-1}
	L	lima loam [1]	N	7.2	22±2	1 mg l^{-1}
	L	lima loam [1]	N	7.2	22±2	10 mg l^{-1}
	L	lima loam [1]	N	7.2	22±2	10 mg l^{-1}
	L	lima loam [1]	N	7.2	22±2	10 mg l^{-1}
	L	lima loam [1]	N	7.2	22±2	100 mg l^{-1}
	L	lima loam [1]	N	7.2	22±2	100 mg l^{-1}
	L	lima loam [1]	N	7.2	22±2	100 mg l^{-1}

Final conc mg kg^{-1}	Duration	Half-life	Comments	Ref
12% decomposi -tion per week	9 weeks	~4.2 weeks	Soil samples taken just below the water table (4.8 m depth)	4
72% sorbed	6 hours	ND		1
81% sorbed	30 hours	ND		1
95% sorbed	78 hours	ND		1
64% sorbed	6 hours	ND		1
79% sorbed	30 hours	ND		1
85% sorbed	78 hours	ND		1
92% sorbed	6 hours	ND		1
96% sorbed	30 hours	ND		1
93% sorbed	78 hours	ND		1

	Laboratory, field or modelled data	Media	Solvent used	pH	Temp (°C)	Initial conc mg kg^{-1}
	L	organic soil [3]	N	7.5	22±2	1 mg l^{-1}
	L	organic soil [3]	N	7.5	22±2	1 mg l^{-1}
	L	organic soil [3]	N	7.5	22±2	1 mg l^{-1}
	L	organic soil [3]	N	7.5	22±2	10 mg l^{-1}
	L	organic soil [3]	N	7.5	22±2	10 mg l^{-1}
	L	organic soil [3]	N	7.5	22±2	10 mg l^{-1}
	L	organic soil [3]	N	7.5	22±2	100 mg l^{-1}
	L	organic soil [3]	N	7.5	22±2	100 mg l^{-1}
	L	organic soil [3]	N	7.5	22±2	100 mg l^{-1}
	L	organic soil [3]	N	7.5	22±2	1 mg l^{-1}

Final conc mg kg^{-1}	Duration	Half-life	Comments	Ref
93% sorbed	6 hours	ND		1
94% sorbed	30 hours	ND		1
96% sorbed	78 hours	ND		1
84% sorbed	6 hours	ND		1
90% sorbed	30 hours	ND		1
93% sorbed	78 hours	ND		1
91% sorbed	6 hours	ND		1
96% sorbed	30 hours	ND		1
93% sorbed	78 hours	ND		1
69% sorbed	6 hours	ND		1

	Laboratory, field or modelled data	Media	Solvent used	pH	Temp (^{o}C)	Initial conc mg kg^{-1}
	L	aquifer sand [4]	N	6.9	22±2	1 mg l^{-1}
	L	aquifer sand [4]	N	6.9	22±2	1 mg l^{-1}
	L	aquifer sand [4]	N	6.9	22±2	10 mg l^{-1}
	L	aquifer sand [4]	N	6.9	22±2	10 mg l^{-1}
	L	aquifer sand [4]	N	6.9	22±2	10 mg l^{-1}
	L	aquifer sand [4]	N	6.9	22±2	100 mg l^{-1}
	L	aquifer sand [4]	N	6.9	22±2	100 mg l^{-1}
	L	aquifer sand [4]	N	6.9	22±2	100 mg l^{-1}
Desorption/ leaching	L	lima loam [1]	N	7.2	22±2	1 mg l^{-1}
	L	aquifer sand [4]	N	6.9	22±2	1 mg l^{-1}

Final conc mg kg^{-1}	Duration	Half-life	Comments	Ref
75% sorbed	30 hours	ND		1
87% sorbed	78 hours	ND		1
57% sorbed	6 hours	ND		1
78% sorbed	30 hours	ND		1
85% sorbed	78 hours	ND		1
72% sorbed	6 hours	ND		1
84% sorbed	30 hours	ND		1
87% sorbed	78 hours	ND		1
61.0% desorbed	16 hours	ND	non-equilibrium desorption	2
66.7% desorbed	16 hours	ND	non-equilibrium desorption	2

	Laboratory, field or modelled data	Media	Solvent used	pH	Temp (°C)	Initial conc mg kg^{-1}	Final conc mg kg^{-1}	Duration	Half-life	Comments	Ref
	L	lima loam [1]	N	7.2	22±2	1	10% leached	3 leachings	ND	cumulative amount of styrene leached from a column of soil	2
	L	lima loam [1]	N	7.2	22±2	1	20% leached	6 leachings	ND	cumulative amount of styrene leached from a column of soil	2
	L	lima loam [1]	N	7.2	22±2	1	25% leached	9 leachings	ND	cumulative amount of styrene leached from a column of soil	2
	L	lima loam [1]	N	7.2	22±2	1	30% leached	12 leachings	ND	cumulative amount of styrene leached from a column of soil	2
	L	lima loam [1]	N	7.2	22±2	1	31% leached	15 leachings	ND	cumulative amount of styrene leached from a column of soil	2
Volatilisation	L	kendaia loam [5] (1.5 cm depth)	N	7.5	22±2	2	17% volatilised	10 days	ND		1
	L	kendaia loam [5] (1.5 cm depth)	N	7.5	22±2	2	24% volatilised	10 days	ND		1
	L	kendaia loam [5] (1.5 cm depth)	N	7.5	22±2	2	26% volatilised	30 days	ND		1

Notes to Table A1

L = Results from a laboratory study
ND = No data
[1] 7.5% organic matter
[2] 5.7% organic matter
[4] 0.4% organic matter
[5] 5.3% organic matter
[7] Styrene-contaminated soils were aged under sterile conditions for varying periods prior to microbial activation
[8] Landfill soil and sandy loam 0.35% and 2.0% organic matter, respectively

References

1. Fu and Alexander (1992)
2. Fu *et al.* (1994)
4.Wilson *et al.* (1983)

N = None or not applicable
[3] 32.9% organic matter
[6] 50-60 mg organic matter l^{-1}

3.Sielicki *et al.* (1978)

A2. FATE AND BEHAVIOUR IN WATER

Several studies have investigated the fate and behaviour processes which affect styrene in water (Fu and Alexander 1992, Watts and Moore 1988, Zoetman *et al.* 1980). The main process which is likely to influence the fate of styrene released to the aquatic environment is volatilisation, although sorption and aerobic biodegradation may also play minor roles. Styrene is generally resistant to hydrolysis and photolysis. The data available on the fate and behaviour of styrene in water are summarized in Table A3 at the end of this section.

Fu and Alexander (1992) carried out a series of experiments to study the possible significance of volatilisation, biodegradation and sorption of styrene in different environmental samples, including lake water, groundwater, deionised water and a variety of soils and sludges (see Table A1). Styrene was found to volatilise rapidly from shallow layers of lake water and deionised water, with 50% being lost in 1-3 hours and 6-7 hours, respectively (initial concentration 4.0 mg l^{-1}). In this particular test no UV-adsorbing compounds could be detected in solution after 40 hours. Aerobic mineralisation of styrene in lake water and aquifer sand was also found to occur but to a lesser extent. For example, at initial concentrations of 100 µg l^{-1} and 100 µg kg^{-1}, approximately 17% and 1.5% of ^{14}C was mineralised in lakewater and aquifer sand samples, respectively. The lack of biodegradation observed in aquifer sand samples is probably due to the low biological activity, in particular aerobic activity, usually found in this type of environment.

The study performed by Watts and Moore (1988) consisted of two stages: a field survey and the application of model predictions. In the field study, the River Clywedog was monitored for a number of volatile organic compounds, including styrene, at various points downstream of a sewage treatment plant outfall, the source of pollution. At the time of the study the effluent flow was 195 litres s^{-1} while the downstream river flow was 1351 litres s^{-1}. Samples were taken at points 30, 3500 and 6500 m downstream of the discharge, the last point being sampled approximately 7 hours after the initial effluent sample. The range of depths and widths of each sampling point were 25-45 cm and 720 cm, 20-40 cm and 710 cm, and 20-70 cm and 920 cm, respectively. A summary of the results of this survey is provided in Table A2.

Concentrations of styrene decreased further away from the discharge point. At the final sampling point, 6500 m downstream, 41% (19.1 ng l^{-1}) of the mean styrene concentration recorded 30 m downstream (46.5 ng l^{-1}) was present 7 hours after the first effluent sample was taken. The authors found that total organic carbon (TOC) and chloride remained fairly constant. This suggests that mechanical processess such as dilution or dispersion are not responsible for the reduction in styrene since TOC and chloride levels would have similarly been affected. Due to the time of year in which the study took place (February) microbial activity is likely to have been low. For this reason, and the fact that the flow-rate of the river was relatively high (thus only a short contact time with microorganisms would have been achieved), the authors did not attribute the removal of styrene to biodegradation. Since styrene is known to be generally resistant to hydrolysis and photolysis, the main removal process in this study was considered to be volatilisation. The overall half-life which was estimated from this field survey was 3.5 hours.

In the second stage of the study, Watts and Moore (1988) used the Mackay fugacity model level 1 (Mackay and Patterson 1986) to predict the environmental partitioning of styrene. The estimated percentages of total input present in the air, water, soil, sediment, suspended sediment and biota phases were calculated as 99.2%, 0.73%, 1.0 x 10^{-2} %, 5 x 10^{-2} %, 8 x 10^{-5} % and 2.0 x 10^{-5} %, respectively. The authors calculated a Henry's Law constant of 2.28 x 10^{-3} atm m^3 mol^{-1}, and using this value predicted a half-life for volatilisation of 3.4 hours, assuming a wind velocity of 3 m s^{-1}, river current velocity of 1 m s^{-1} and a mean river depth of 100 cm.

Table A2 Concentrations of styrene at different points along the River Clywedog (after Watts and Moore 1988)

Sample	Mean styrene concentration (ng l^{-1})	TOC (mg l^{-1})	Chloride (mg l^{-1})	pH	Temp. (°C)
Upstream	7.90 (±0.13)	2.25 (±0.33)	47.5 (±0.6)	7.7	5.5
Effluent	175 (±14)	18.8 (±0.4)	91.2 (±2.5)	7.1	9.3
30m downstream	46.5 (±6.0)	5.19 (±0.37)	61.2 (±1.2)	7.6	6.5
3500m downstream	22.4 (±12.7)	4.95 (±0.44)	55.9 (±0.3)	7.6	6.7
6500 downstream	19.1 (±3.6)	5.53 (±0.40)	56.4 (±0.5)	7.6	7.0

Similar observations on the removal of styrene were made in a subsequent study of the same river by Tynan *et al.* (1990), with 72% of the total styrene found in the effluent and 48% of that found 30 m downstream lost after 12 hours. However, in this particular study separate concentrations of sorbed and aqueous styrene were determined, and for most sampling sites the majority of styrene was found to be sorbed (mostly to suspended solids). For example, the percentage of sorbed styrene was estimated to be 83% and 79% for the effluent itself and just upstream of the effluent, respectively, and between 49% and 66% for the 5 sites sampled downstream. This is somewhat contradictory to the predictions made using the US EPA EXAMS II model (Burns *et al.* 1982) and by Watts and Moore (1988) using the Mackay fugacity model level 1, which identified volatilisation as the main mechanism for styrene removal. Theoretically, particulate sorbed compounds are unable to volatilise. No explanation could be found for this contradiction of results. Further information is therefore needed to assess the relationship between the sorption and volatilisation of styrene.

A half-life for styrene of 0.6 days (approximately 14.5 hours) was estimated by Zoetman *et al.* (1980) for a stretch of the River Rhine in the Netherlands, where the depth varied between 4 and 5 m. Water was reported to take about 24 hours to travel from the start to the end of the stretch in question and the concentration measured at the beginning of the test was 0.01 $\mu g\ l^{-1}$. However, it appears that only two sampling points were set up to monitor styrene, at the start and end of the study area. This half-life can therefore only be treated as a rough estimation.

A2.1 Abiotic processes

Styrene is not susceptible to abiotic degradation processes such as hydrolysis or photolysis. This is confirmed by a number of studies which have investigated the fate and behaviour of styrene in water (e.g. Fu and Alexander 1992, Watts and Moore 1988).

A2.2 Biodegradation

A2.2.1 Aerobic biodegradation

Fu and Alexander (1992) reported that the aerobic mineralisation of styrene in lake water and aquifer sand samples was limited under laboratory conditions. For example, at initial concentrations of 100 $\mu g\ l^{-1}$ and 100 $\mu g\ kg^{-1}$, approximately 17% and 1.5% of ^{14}C was mineralised in lakewater and aquifer sand samples, respectively. The lack of biodegradation observed in aquifer sand samples is probably due to the low biological activity, in particular aerobic activity, usually found in this type of sample. The authors also observed that the extent and rate of mineralisation of [^{14}C]styrene in lake water were concentration dependent, both decreasing with decreasing concentration. At the lowest concentration tested (2.5 $\mu g\ l^{-1}$) approximately 10% of [^{14}C]styrene was mineralised after 30 days. From their studies with lake water samples, the authors suggested that there is a threshold concentration below which microbial growth will not be supported and hence below which styrene persists. This putative threshold would have to be below the lowest concentration tested (2.5 $\mu g\ l^{-1}$). Further data are needed to confirm this.

A monitoring programme carried out on a Welsh river indicated that aerobic biodegradation was minimal due to the short contact time with microorganisms (the flow-rate of the river was high downstream of the discharge) and the time of year during which the study was conducted (microbial activity is relatively low in February). The rapid removal of styrene from the river (half-life of 3.5 hours) was attributed mainly to volatilisation. This was supported by model predictions made using the Mackay fugacity model level 1 (Mackay and Patterson 1986) (<1% of total input was predicted for each of the water, soil, sediment, suspended sediment and biota phases) (Watts and Moore 1988).

The BODs (biological oxygen demands) reported for styrene are somewhat contradictory. Bridié *et al.* (1979), for example, established a 5-day BOD and a COD (chemical oxygen demand) of 1.29 and 2.80 g $O_2\ g^{-1}$ styrene, respectively, which were found to be 42% and 91% of the ThOD (theoretical oxygen demand) (3.08 g g^{-1}), respectively. A BOD of 42% indicates that styrene is not readily biodegradable. However, when adapted innocula were used (acclimatisation period not specified), the BOD was found to be 80% of the ThOD. This suggests that a suitable period of acclimatisation is required before styrene can be effectively biodegraded in water. From this it might also be inferred that a relatively long contact-time is

needed with microorganisms for styrene to be significantly biodegraded. In most running waters, where volatilisation of styrene is likely to be particularly significant, this biological contact-time is probably not achievable. However, 5, 10, 15 and 20-day BODs of 65, 65, 78, and 87% of the ThOD were reported by Price *et al.* (1974) using non-acclimatised innocula in a freshwater dilution medium. In contrast to the findings of Bridié *et al.* (1979), these values indicate that styrene is readily biodegradable. Both studies utilised the same standard method for BOD determination (APHA 1971), using settled domestic wastewater innocula. However, Bridié *et al.* (1979) modified the procedure by inhibiting nitrification with the addition of allythiourea. This is the most likely reason for the marked discrepancy between the results of these two studies. Nitrification is the bacterial reduction of ammonia to nitrites and nitrates and as this is an aerobic process, unless it is inhibited, part of the BOD for styrene will be attributed to nitrification. The results of Bridié *et al.* (1979) are therefore likely to be a more accurate estimation. Price *et al.* (1974) also determined 5, 10, 15 and 20-day BODs of 8, 12, 21 and 80% of the ThOD in a synthetic saltwater medium (~28 g NaCl l^{-1}), suggesting that a greater period of acclimatisation (e.g. 15-20 days) is required for effective biodegradation to occur in the saltwater environment than in the freshwater environment.

Burback and Perry (1993) described styrene as a major groundwater pollutant in the United States. The ability of the bacterium *Mycobacterium vaccae* to degrade styrene was then investigated in suspension media in the laboratory. In this study it was discovered that *M. vaccae* was not able to grow with styrene (100 ppm *v/v*) as a sole source of carbon and energy. When tested in a suspension medium with propanol as the substrate, 50 ppm of styrene was, however, found to be approximately 50% catabolised after 100 hours (about 4 days). This seems to broadly agree with the conclusions drawn from Bridié *et al.* (1979). That is, a relatively long contact-time is required for styrene biodegradation to occur in aquatic media. When styrene (50 ppm) was present concomitantly with toluene, it was totally catabolised to styrene oxide in 72 hours. The fate of styrene oxide was, however, not examined. The authors also observed that the rate of toluene degradation was reduced by the presence of styrene.

A2.2.2 Anaerobic biodegradation

No information was found on the potential for anaerobic degradation of styrene in aquatic sediments. However, styrene is not expected to be associated to any large extent with anaerobic sediment layers.

A2.3 Sorption

The adsorption of styrene to soil has been demonstrated by several authors (e.g. Fu and Alexander 1992, Fu *et al.* 1994). However, few studies have examined the significance of styrene sorption to aquatic sediments (bedded or suspended sediments). The octanol-water partition coefficient for styrene (log K_{ow} 2.59-3.16) suggests that it has a moderate potential for adsorption. Studies carried out on the fate and behaviour of styrene in rivers indicate that volatilisation is the main route of removal from water, although volatilisation is dependent on variable factors such as wind speed and water depth, so in conditions which do not favour rapid volatilisation it is conceivable that a proportion of styrene will be adsorbed. Further data are needed to confirm this.

A2.4 Volatility

The vapour pressure (5 mm Hg at 20 °C) and Henry's Law constant (2.3 x 10^{-3} - 5.2 x 10^{-3} atm m^3 mol^{-1}) reported for this compound (see Table 2.1) suggest that it has a high potential for volatilisation. The extent to which volatilsation occurs is, however, determined by a number of variable factors, including wind speed and the width, depth and flow characteristics of a water body.

Volatilisation to the atmosphere appears to be the major process for the removal of styrene from natural waters. For example, Fu and Alexander (1992) found that it volatilised rapidly from shallow layers of lake water and deionised water, with 50% being lost in 1-3 hours and 6-7 hours, respectively (initial concentration 4.0 mg l^{-1}). In this particular test no UV-adsorbing compounds at all could be detected in solution after 40 hours. Using the Mackay fugacity model level 1 (Mackay and Patterson 1986), Watts and Moore (1988) predicted the environmental partitioning of styrene, with 98% of total input being attributed to the atmosphere. They also estimated a half-life for volatilisation of 3.4 hours, based on calculations from the Henry's Law constant. In a parallel field survey, Watts and Moore (1988) monitored the changing concentrations of styrene downstream of a sewage outfall (the source of 175 µg styrene l^{-1}) and calculated an overall half-life of 3.5 hours. Volatilisation was proposed as the main cause of styrene removal.

Table A3 Summary of the fate and behaviour of styrene in water

	Laboratory, field or modelled data	Media	Solvent used	pH	Temp (^{0}C)	Initial conc mg l^{-1}
Aerobic biodegradation	L	lake water [1]	N	7.5	ND	1
	L	lake water [1]	N	7.5	ND	1
	L	ground-water [2]	N	8.3	ND	1
	L	ground-water [2]	N	8.3	ND	1
Volatility	L	lake water [1]	N	7.5	room temp.	4
	L	lake water [1]	N	7.5	room temp.	4
	L	distilled water	N	ND	room temp.	4
	L	distilled water	N	ND	room temp.	4

Final conc mg l^{-1}	Duration	Half-life	Comments	Ref
31% mineral-ised	20 days	>20 days	[^{14}C]styrene evolved as CO_2 (losses due to volatilisation minimised by test design)	1
37% mineral-ised	30 days	>30 days	[^{14}C]styrene evolved as CO_2 (losses due to volatilisation minimised by test design)	1
35% mineral-ised	20 days	>20 days	[^{14}C]styrene evolved as CO_2 (losses due to volatilisation minimised by test design)	1
39% mineral-ised	30 days	>30 days	[^{14}C]styrene evolved as CO_2 (losses due to volatilisation minimised by test design)	1
50% volatilised	1-3 hours	1-3 hours		1
100% volatilised	40 hours	ND		1
50% volatilised	6-7 hours	6-7 hours		1
100% volatilised	40 hours	ND		1

	Laboratory, field or modelled data	Media	Solvent used	pH	Temp (^{o}C)	Initial conc mg l^{-1}	Final conc mg l^{-1}	Duration	Half-life	Comments	Ref
	F	river	N	7.1-7.6	5.5-9.3	1.75×10^{-4}	1.91×10^{-5}	7 hours	3.5	total styrene - monitored from sewage outfall to 6.5 km downstream	2
	M	N	N	N	N	N	N	N	3.4 hours	assuming wind velocity of 3 m s^{-1}, river current velocity of 1 m s^{-1}, and mean river depth 100 cm.	2
	F	river	N	ND	ND	2.05×10^{-4}	5.8×10^{-5}	12 hours	ND	total styene - monitored from sewage effluent to 6.4 km downstream	3
	F	river	N	ND	ND	3.5×10^{-5}	2.0×10^{-5}	12 hours	ND	aqueous styrene - monitored from sewage effluent to 6.4 km downstream	3
	F	river	N	ND	ND	1.7×10^{-4}	3.8×10^{-5}	12 hours	ND	sorbed styrene - monitored from sewage effluent to 6.4 km downstream	3
	F	river	N	ND	ND	1.0×10^{-5}	ND	ND	0.6 days	River Rhine, 4-5m depth	4

Notes:
L = Results from a laboratory study
F = Results from a field study
M = Results generated by a model
ND = No data
N= None or not applicable
[1] 50-60 mg organic matter l^{-1}
[2] 30.5 mg oganic matter l^{-1}

References:
1. Fu and Alexander (1990)
2. Watts and Moore (1988)
3. Tynan *et al.* (1989)
4.Zoetman *et al.* (1980)

A3. BEHAVIOUR IN SEWAGE TREATMENT PROCESSES

The BODs (biological oxygen demands) reported for styrene suggest that styrene will not be readily biodegraded by unacclimatised microorganisms (see A2). Bridié *et al.* (1979), for example, determined the BOD_5 as 42% of the theoretical oxygen demand (ThOD) using settled domestic wastewater inocula. However, using acclimatised inocula (duration of acclimatisation not specified) a BOD_5 of 80% ThOD was calculated. A similar test carried out by Price *et al.* (1974) determined 5, 10, 15 and 20-day BODs of 65, 65, 78 and 87% of ThOD using a freshwater medium and 8, 12, 21 and 80% using a saltwater medium, respectively. These results are, however, likely to have been somewhat overestimated as nitrification was not inhibited. Nevertheless, they suggest that acclimatisation in the order of 10-20 days is required. The BOD test is, however, only a fairly crude indication of a substance's potential to biodegrade. Details of studies which have investigated the behaviour of styrene during specific sewage treatment processes are described below.

The US EPA Risk Reduction Engineering Laboratory's Treatability database (RREL 1994) indicates that styrene is effectively removed from domestic and industrial wastewaters using activated sludge and sedimentation processes. For example, influent concentrations of up to 0.1 mg l^{-1} were 96.4% and 91.4% removed from domestic wastewaters by activated sludge and sedimentation, respectively (Canviro Consultants 1988, cited by RREL 1994). Used in combination, activated sludge and sedimentation were reported to remove >79% from an industrial effluent also containing 1.0 mg styrene l^{-1}.

The removal of styrene from wastewaters using biological filters is less certain. Influent styrene concentrations of up to 0.1 and 1.0 mg l^{-1} in highly polluted effluents (superfund effluents) were 8 and 0% removed, respectively. This could, however, be due to the presence of other pollutants which may have been toxic to microorganisms. Lapinskas (1993) reported the use of a submerged fixed-film reactor to treat groundwater polluted by a variety of volatile organic compounds, but in particular ethylbenzene, styrene and xylene. A flow rate of 250 m^3 d^{-1} was maintained through filter units packed with nonplasticised polyvinylchloride media for a period of 18 months. This test design also involved the artificial aeration of alternate columns from a compressed air source. The concentrations of styrene recorded throughout this study were not, however, reported, but reductions of >98.8% were consistently recorded for ethylbenzene (from which styrene is usually manufactured) with influent concentrations ranging from 14-152 mg ethylbenzene l^{-1} and an average residence time of 8.3 hours. The author recommended this approach as a viable alternative to activated sludge, steam or air stripping.

El Aalam *et al.* (1993) examined the degradability of styrene (solubilised in silicone oil) as a sole source of carbon and energy using a selected bacterial community in a two-phase aqueous-organic medium (80%:20% *v/v*). Preliminary studies with the mixed population in batch cultures indicated that the specific activity and maximum growth rate at optimal pH (pH 6) were 46 mg g^{-1} (biomass) h^{-1} and 0.15 h^{-1}, respectively. In pH-regulated chemostat cultures, styrene was degraded at dilution rates ranging from 0.05 to 0.2 h^{-1}. However, at 0.2 h^{-1}, only one strain of bacteria remained in the medium, *Pseudomonas aeruginosa*. This particular strain was found to degrade styrene at a specific activity of 293 mg g^{-1} (biomass) h^{-1}.

Studies on the aerobic biodegradation of styrene in soil suggest that high concentrations (e.g. concentrations equal to or greater than 5.0 mg kg^{-1}) become increasingly less biodegradable, although there is no information to show whether this is the case for aerobic biological treatment processes. Details of the information available on aerobic biodegradation in soil are given in Section A1.2.1.

Fu and Alexander (1992), for example, found that only 17% of [^{14}C]styrene mineralised at the highest concentration tested (4000 mg kg^{-1}). This suggests that high concentrations of styrene may inhibit microbial activity. Burback *et al.* (1994), for example, estimated a toxic concentration of 4.0 mM (approximately 417 mg l^{-1}) for the soil mycobacterium *Mycobacterium vaccae* exposed to styrene for 7 days in suspended media.

Sewage sludge with an initial styrene concentration of 1.3 mg g^{-1} was >99% removed by aerobic biological digestion after 90 days (RREL 1994).

There are very few data available on the effects of styrene on anaerobic sludge digestion. Information on the anaerobic degradation of styrene in soil indicate that non-methanogenic biotransformation of styrene to phenylethanol can occur, although probably quite slowly (see A1.2.2).

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APPENDIX B FRESHWATER TOXICITY AND BIOACCUMULATION

B1. FRESHWATER TOXICITY

The available toxicity data for styrene (see Table B.1) are limited to single-species laboratory tests, and in the majority of cases, static laboratory tests. Most of the reported studies have examined the effects of styrene to freshwater life after short-term exposure.

Styrene is highly volatile in aqueous media and exposure concentrations will therefore rapidly decrease during testing unless measures are taken to minimize the effects of volatilisation (e.g. covering test vessels, renewing test media). In the majority of studies reported no such measures appear to have been taken and very few have attempted to measure actual styrene concentrations during exposure. Styrene is also sparingly soluble in water (300 mg l^{-1} at 20 °C) but the majority of effects appear to have occurred within the solubility limit of styrene. A small number of toxicity studies did, however, use solvent carriers (e.g. acetone) to dissolve styrene in the test media.

Since most of the studies reported do not meet the ideal criteria for testing moderately soluble, volatile organic chemicals in the laboratory the overall quality of the freshwater toxicity dataset is questionable. It is conceivable that studies which have not measured or maintained exposure concentrations in some way (e.g. static tests) or which have not completely dissolved styrene in the test media, will have underestimated the toxicity.

Nevertheless, examination of the available toxicity data for freshwater organisms indicates that styrene is of moderate to low acute toxicity to those species tested, with the majority of effect concentrations above 5 mg l^{-1}.

The only study to have investigated chronic exposure was carried out using the crustacean *Pontoporeia affinis*. This study indicated that effects may occur at 2.3 mg l^{-1}, but the results of this test are not conclusive, in particular as the effects monitored were rather subjective (swimming activity).

B1.1 Bacteria

The limited amount of data available suggest that styrene is of moderately low acute toxicity to aquatic bacteria. Bringmann and Kuhn (1980), for example, determined the toxicity threshold for cell multiplication inhibition of 72.0 mg l^{-1} for *Pseudomonas putida* after 16 days exposure. In this study, bacteria cultured on agar were exposed to varying concentrations of styrene and the changing concentrations of bacterial suspensions were estimated by measuring the turbidity of the test cultures. The only other study reported investigated the effect of styrene on the reverse motility of the flagellate bacterium *Spirillum volutans* in suspension. An MEC_{90} (the minimum effective concentration of toxicant necessary to eliminate reversing motility in greater than 90% of bacteria cells within 5 minutes) of 636.0 mg l^{-1} was reported (Qureshi *et al.* 1982). However, it is unlikely that the effects on reverse motility of flagellate bacteria in the field will result in adverse effects on whole aquatic communities, so the ecological relevance of this endpoint is doubtful.

B1.2 Cyanobacteria

In the only toxicity test reported for cyanobaceria (blue-green algae), Bringmann and Kuhn (1978, cited by Chemical Information System 1995) recorded a toxicity threshold (cell multiplication inhibition) of 67.0 mg l^{-1} for *Microcystis aeruginosa*. However, the credibility of this particular test is unknown.

B1.3 Protozoans

The small number of studies reported all investigated the effects of styrene on cell multiplication of protozoans. The results of these studies suggest that protozoans are relatively tolerant to the effects of styrene, with reported effect concentrations greater than 100 mg l^{-1}.

Using the cell multiplication inhibition test, Bringmann and Kuhn (1980) determined a toxicity threshold for *Entosiphon sulcatum* of >256.0 mg l^{-1} (based on nominal concentrations). In this study, the various test compounds were dissolved into aqueous cultures of *E. sulcatum*, fed on the bacterium *Escherichia coli*. Cell multiplication inhibition was assessed by counting the number of protozoan cells before and after 72 hours exposure. However, in order to achieve the concentrations of styrene tested the authors would have needed to use a solvent carrier although there is no information to confirm that this was the case.

In what is likely to have been a similar test, Bringmann *et al.* (1980, cited by Chemical Information System 1995) recorded a toxicity threshold (cell multiplication inhibition) of 185.0 mg l^{-1} to *Uronema parduczi* Chatton-Lwoff, indicating low toxicity to this species.

B1.4 Algae

Algae appear to be relatively tolerant of styrene, with a toxicity threshold (cell multiplication inhibition) reported in excess of 200.0 mg l^{-1} for the green alga *Scenedesmus* quadricaudata (Bringmann and Kuhn 1980). In this test (see B1.1 and B1.3), algae were exposed to test concentrations in aqueous media and cell multiplication was measured by turbidity. However, no evidence was provided that styrene was dissolved in a solvent or that exposure concentrations were maintained.

The dataset for freshwater algae is particularly sparse (one species only) and those data which do exist are of limited relevance. Further data are needed to determine the likely levels of toxicity to algae.

B1.5 Macrophytes

There is no information on the effects of styrene on marginal or submerged aquatic macrophytes.

B1.6 Invertebrates

The acute toxicity dataset available for freshwater invertebrates comprises of data for two species of freshwater snail (*Lymnaea stagnalis* and *Amphomelania holandri*) and four species of crustaceans (*Daphnia magna*, *Asellus aquaticus*, *Gammarus fossarum* and *Pontoporeia*

affinis). Examination of the data indicates that styrene is of moderate to low acute toxicity to these species, with L(E)C_{50}s reported in the range 23-255 mg l^{-1}.

A number of different laboratories have examined the toxicity of styrene to the water flea (*Daphnia magna*). The range of acute effect concentrations reported is 23 mg l^{-1} (48-hour LC_{50}) to 300 mg l^{-1} (24-hour EC_{100}), indicating moderate to low acute toxicity to this species.

The toxicity tests carried out on freshwater snails (*L. stagnalis* and *A. hollandri*), freshwater shrimp (*G. fossarum*) and water louse (*A. aquaticus*) were performed under similar conditions by the same laboratory (Erben and Pisl 1993). These species appear to be no more sensitive to styrene than *D. magna*, although the acute LC_{50}s reported for these species suggest the following order of decreasing sensitivity: *G. fossarum*, *A. aquaticus*, *A. holandri*, *L. stagnalis*. However, due a number of potential flaws in the study design, the accuracy of this study's results are questionable (see Section B1.6.1).

B1.6.1 Molluscs

The only available study examining the effects of styrene on molluscs suggests that the freshwater snails *Lymnaea stagnalis* and *Amphimelania holandri* are moderately sensitive to styrene (Erben and Pisl 1993). A semi-static test design (i.e. test media were periodically renewed, but the frequency of renewal was not specified) was used to maintain exposure concentrations, although analysis was not carried out to confirm that concentrations were infact maintained. For *L. stagnalis* LC_{50}s of 726.4, 538.0, 502.2, 457.4 and 421.5 mg l^{-1} were determined for 24-, 48-, 72-, 96- and 120-hours exposure, respectively. LC_{50}s of 128.2, 111.2, 102.2, 96.9 and 93.3 mg l^{-1} for the same respective durations of exposure, were reported for *A. holandri*.

Detailed examination of this study suggests that there were a number of potential flaws in the test design. Snails were collected from brooks and rivers on the outskirts of Zagreb in Croatia and then transferred to laboratory aquaria. The snails were allowed to acclimatise in the laboratory for only 24 hours prior to testing, and while they appear to have been acclimatised in water taken from local natural waters, the actual exposure tests were carried out using tap water. If acclimatisation is inadequate test animals will be placed under additional stress at the onset of testing, which may result in inaccurate estimations of chemical-specific toxicity.

As already mentioned, tests were carried out under semi-static conditions. However, the higher styrene concentrations tested (maximum 627.9 mg l^{-1}) far exceeded the water solubility limit of styrene (water solubility is reported to be 300 mg l^{-1} at 20 °C, Verscheuren 1983). In fact, the authors reported that a film of styrene appeared on the surface of the animals' bodies and on the surface of the test media in some tests. To prevent the latter from significantly reducing concentrations of dissolved oxygen in the test media, the authors aerated each test system, but, aeration would have also increased the rate of volatilisation of styrene from the water.

It is difficult to interpret the results of this particular study. On one hand, due to the apparent inadequacy of the acclimatisation period it could be argued that the LC_{50}s calculated are an overestimate of actual styrene toxicity because extraneous stress was applied to the test system. However, aerating each test vessel would undoubtedly have led to reduced test concentrations of styrene, suggesting that the LC_{50}s were somewhat underestimated. In

addition, the high concentrations of styrene tested make it impossible to differentiate between indirect effects (e.g. "smothering") and direct effects or to assess the bioavailability of the test substance. It is therefore not possible to use these results in the derivation of the EQS with any real certainty.

Regardless of its potential flaws, however, this study does highlight a number of potential concerns for the release of high styrene concentrations to the freshwater environment, such as those which might result from accidental spillages or leakages. In shallow, slow-flowing waters, where dilution is limited and natural aeration low, indirect toxic effects on aquatic invertebrates may occur rapidly as a result of films forming on body surfaces, i.e. "smothering" (e.g. disruption of gaseous exchange from respiratory surfaces) and eventually by the formation of insoluble surface films (reducing natural aeration from the atmosphere).

B1.6.2 Crustaceans

Acute toxicity tests are reported for three species of crustaceans, the water flea *Daphnia magna*, the shrimp *Gammarus fossarum* and the water louse *Asellus aquaticus*. A review of the data indicates that styrene is of moderate to low acute toxicity to these species. A wide range of effect concentrations are reported: 48-hour LC_{50} 23.0 mg l^{-1} (*D. magna*); 24-hour EC_{100} 300.0 mg l^{-1}(*D. magna*); 48-hour LC_{50} 57.4 mg l^{-1} (*G. fossarum*); 48-hour LC_{50} 68.2 mg l^{-1} (*A. aquaticus*).

A number of different laboratories have examined the acute toxicity of styrene to the water flea (*Daphnia magna*). The range of acute effect concentrations reported is 23-300 mg l^{-1}, indicating moderate to low acute toxicity to this species. The wide variability in results obtained by the different laboratories is probably due more to differences in laboratory conditions than to natural intraspecies variability. All of the studies reported appear not to have measured concentrations of styrene during exposure nor appear to have minimized the effects of volatilisation in any way (e.g. closed vessels, semi-static conditions).

LeBlanc (1980) used a static test method based on US EPA protocols (US EPA 1975) to examine the acute toxicity of styrene to *D. magna*. Some of the quality criteria required by current standard static, acute *Daphnia* tests (e.g. OECD 1981) appear to have been met by LeBlanc (1980), except the concentration of styrene was not measured at any time during or after the exposure period and no information was given to indicate whether closed vessels were used or not. The reported 24- and 48-hour LC_{50}s of 27.0 and 23.0 mg l^{-1}, respectively, are therefore likely to be underestimated values. LeBlanc also observed no discernible effects on *D. magna* at a concentration of 6.8 mg l^{-1}.

Qureshi *et al.* (1982) carried out an unaerated, static test with young daphnids (<24 hours old) and determined a 48-hour LC_{50} of 59.0 mg l^{-1}. As in the case of LeBlanc (1980), no information was given to indicate whether the test vessels employed by Qureshi *et al.* (1982) were covered or not. They did, however, analyse the actual concentrations of styrene at the start of each test (t=0), but because concentrations were measured neither during nor at the end of exposure, the LC_{50} reported is effectively based on nominal concentrations.

Bringmann and Kuhn (1978) and Bringmann and Kuhn (1982) reported EC_0, EC_{50} and EC_{100} values (the effects measured were lethality and immobilisation, respectively) of 130.0, 255.0 and 300.0 mg l^{-1} and 105.0, 182.0 and 300.0 mg l^{-1}, respectively.

The effects of styrene on the water louse (*Asellus aquaticus*) and the freshwater shrimp (*Gammarus fossarum*) were investigated in a semi-static laboratory test (Erben and Pisl 1993) (see B1.6.1). LC_{50}s were determined by the authors after 24-, 48-, 72-, 96- and 120-hour exposure by probit analysis and it appears that both species are of similar sensitivity to styrene. The LC_{50}s reported range between 48.4 (120-hours) and 88.8 (24-hours) mg l^{-1} for *A. aquaticus*, and 50.2 (120-hours) and 64.6 (24-hours) mg l^{-1}.

B1.7 Fish

Information is available on six species of non-salmonid freshwater fish (only three of which are indigenous to the UK) and one species of salmonid fish (rainbow trout, *Oncorhynchus mykiss*). The majority of tests appear to have adopted static conditions but the most sensitive values presented in Table B1 were obtained from flow-through studies. The advantage of flow-through studies over static tests is that for most substances they are more likely to maintain constant exposure concentrations. Due to the volatile nature of styrene, however, confirmation of test concentrations with analysis is still important.

The majority of effect concentrations recorded for non-salmonid fish range from 25.0-75.0 mg l^{-1}. However, the most reliable study reported is a flow-through study with fathead minnow (*Pimephales promelas*). From this test a 96-hour LC_{50} of 4.02 mg l^{-1} (based on measured concentrations) was determined (Gieger *et al.* 1990). This is similar to the results of the only flow-through study conducted with salmonid fish (*Oncorhychus mykiss*). A 96-hour LC_{50} of 5.9 mg l^{-1} was reported, although in this case the LC_{50} was based on nominal concentrations (Abram and Collins 1981).

There are no data on the chronic toxicity of styrene to fish. The longest exposure duration tested was 168 hours (7 days) with *O. mykiss*. The LC_{50} calculated after this length of exposure (flow-through test, nominal concentrations) was 4.9 mg l^{-1}, which is not significantly different from the LC_{50}s calculated after shorter durations (e.g. 12-, 24-, 48- and 96-hours).

B1.7.1 Non-salmonid fish

The non-salmonid fish that have been tested are fathead minnow (*Pimephales promelas*), bluegill sunfish (*Lepomis macrochirus*), red killifish (*Oryzias latipes*), golden orfe (*Leuciscus idus melanotus*), goldfish (*Carassius auratus*) and guppy (*Poecilia reticulata*).

Pickering and Henderson (1966) investigated the toxicity of styrene to *P. promelas*, *L. macrochirus*, *C. auratus* and *P. reticulata* and reported 96-hour LC_{50}s of 46.4, 25.1, 64.7 and 74.8 mg l^{-1}, respectively in soft water (20 mg $CaCO_3$ l^{-1}) at pH 7.5. When *P. promelas* was tested at a higher water hardness (360 mg $CaCO_3$ l^{-1}) and pH (8.2), the authors found no significant difference in the LC_{50}s calculated (96-hour LC_{50} 59.3 mg l^{-1}).

Mattson (1976, cited by Chemical Information System 1995) found that using acetone to dissolve styrene in the test media had no marked effects on the toxicity results of a static laboratory test with fathead minnows (*P. promelas*).

In the only flow-through study conducted for non-salmonid fish, Gieger *et al.* (1990) reported a 96-hour LC_{50} of 4.02 mg l^{-1} (based on measured concentrations) for *P. promelas*. However, since this species is a warm water fish found mainly in North America, it is not suitable for the derivation of Environmental Quality Standards (EQSs).

B1.7.2 Salmonid fish

Data on the toxicity of styrene to salmonid fish are very sparse, and infact are restricted to one species, rainbow trout (*Oncorhynchus mykiss*). This limited dataset does suggest that styrene is of moderate acute toxicity to *O. mykiss*, with effect concentrations ranging from 2.5-8.8 mg l^{-1}.

Qureshi *et al.* (1982) studied the effects of styrene on juvenile *O. mykiss* in an unaerated static test with relatively hard water (135 mg $CaCO_3$ l^{-1}) and determined a 24-hour LC_{50} of 2.5 mg l^{-1}. The only other study reported to have considered the effects of styrene on juvenile *O. mykiss* is a flow-through study conducted with hard water (270 mg $CaCO_3$ l^{-1}) for a maximum duration of 168 hours (7 days) (Abram and Collins 1981). In this study, 12-, 24-, 48, 96- and 168-hour LC_{50}s of 8.8, 6.5, 6.3, 5.9 and 4.9 mg l^{-1} were determined, respectively. Although test concentrations were not measured at any time during this study, acetone was used to dissolve styrene into the test solution (maximum acetone concentration used was approximately 2400 mg l^{-1}). It is not known, however, what effect the use of acetone will have had on maintaining the concentrations of styrene applied to the test system. The authors reported that solvent control vessels were not included in the test design. Although the concentrations of acetone applied are comfortably below reported acute toxicity data for rainbow trout (e.g. 96-hour LC_{50}s of 8000 and 10 000 mg l^{-1} in hard and soft water, respectively, Bathe *et al.* 1975, Shumway and Paelensky 1973, respectively), the use of solvents may in some cases add another source of stress to the system, so the inclusion of solvent controls is important.

B2. BIOACCUMULATION

There is no experimental evidence to suggest that styrene is accumulated by freshwater organisms. Based on the *n*-octanol-water partition coefficients (log K_{ow}) reported (e.g. 2.59-3.16), it might be argued that styrene has the potential to moderately bioaccumulate.

Ogata *et al.* (1984) exposed goldfish (*Carassius auratus*) to varying measured concentrations of styrene (0.017-0.8 mg l^{-1}) in order to determine the *n*-octanol-water partition coefficient (log K_{ow}) and bioconcentration factor (BCF) for styrene. Water and tissue concentrations were measured by gas chromatography (GC) but the duration of exposure was not reported. The BCF reported for styrene was 13.5, indicating that bioaccumulation is infact not significant. The log K_{ow} determined by Ogata *et al.* (1984) was 2.59.

Sabljic (1987) predicted a fish BCF of 6.76 using a model developed from a database of 20 chlorinated benzenes, biphenyls, diphenyloxides and similar compounds. The validity of the

equation used in this model is uncertain since the dataset on which it was based is relatively small. However, the result seems to agree with the findings of Ogata *et al.* (1984).

Table B1 Toxicity of styrene to freshwater life

Species	Life stage	Test type	Analysis	Temp (°C)
BACTERIA				
Pseudomonas putida	ND	S	N	25
Spirillum volutans	ND	S	N[1]	20-22
CYANO-BACTERIA				
Microcystis aeruginosa	ND	S	N	27.0
ALGAE				
Scenedesmus quadricauda	ND	S	N	ND
PROTOZOANS				
Chilomonas paramaecium	ND	ND	N	20.0
Entosiphon sulcatum	ND	S	N	25

Hardness ($mg\ l^{-1}\ CaCO_3$)	pH	Exposure time	Conc ($mg\ l^{-1}$)	Effect	Ref
ND	ND	16 h	72.0	Toxicity threshold (cell multiplication inhibition)	1
ND	ND	5 m	636.0	MEC_{90}[2]	8
ND	7.0	ND	67.0	Toxicity threshold	14
ND	ND	72 h	>200.0	Toxicity threshold (cell multiplication inhibition)	1
ND	ND	48 h	>100.0	≥5% decrease in cell count	16
ND	ND	72 h	>256.0	Toxicity threshold (cell multiplication inhibition)	1

Species	Life stage	Test type	Analysis	Temp (°C)
Uronema parduczi	ND	S	N	ND
MOLLUSCS				
Lymnaea stagnalis (freshwater snail)	ND	S	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22
Amphimelania holandri (freshwater snail)	ND	S	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22

Hardness ($mg\ l^{-1}\ CaCO_3$)	pH	Exposure time	Conc ($mg\ l^{-1}$)	Effect	Ref
ND	ND	ND	185.0	Toxicity threshold (cell multiplication inhibition)	2
300-400	7.0-8.0	24 h	726.4	LC_{50}	7
300-400	7.0-8.0	48 h	538.0	LC_{50}	7
300-400	7.0-8.0	72 h	502.2	LC_{50}	7
300-400	7.0-8.0	96 h	457.4	LC_{50}	7
300-400	7.0-8.0	120 h	421.5	LC_{50}	7
300-400	7.0-8.0	24 h	128.2	LC_{50}	7
300-400	7.0-8.0	48 h	111.2	LC_{50}	7
300-400	7.0-8.0	72 h	102.2	LC_{50}	7
300-400	7.0-8.0	96 h	96.9	LC_{50}	7
300-400	7.0-8.0	120 h	93.3	LC_{50}	7

Species	Life stage	Test type	Analysis	Temp (°C)
ARTHROPODS: CRUSTACEANS				
Daphnia magna (water flea)	ND	S	N	ND
	ND	S	N	ND
	ND	S	N	ND
	<24 h	S	N	20-22
	<24 h	S	N	20-22
	<24 h	S	N	20-22
	<24 h	S	N	22±1
	<24 h	S	N	22±1
	<24 h	S	N	22±1
	<24 h	S	N[1]	15±1

Hardness (mg l^{-1} $CaCO_3$)	pH	Exposure time	Conc (mg l^{-1})	Effect	Ref
ND	ND	24 h	105.0	EC_0 immobilisation	12
ND	ND	24 h	182.0 (168.0-197.0)	EC_{50} immobilisation	12
ND	ND	24 h	300.0	EC_{100} immobilisation	13
70.0	7.6-7.7	24 h	130.0	LC_0	13
70.0	7.6-7.7	24 h	255.0	LC_{50}	13
70.0	7.6-7.7	24 h	300.0	LC_{100}	13
173±13	8.0± 0.2	24 h	27.0 (20-35)	LC_{50}	10
173±13	8.0± 0.2	48 h	23.0 (18-29)	LC_{50}	10
173±13	8.0± 0.2	48 h	<6.8	no discernible effect concentration	10
ND	7.8-8.1	48 h	59.0 (41.0-85.0)	LC_{50}	8

Species	Life stage	Test type	Analysis	Temp (°C)
Gammarus fossarum (freshwater shrimp)	ND	SS	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22
Asellus aquaticus (water louse)	ND	S	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22
	ND	S	N	20-22
FISH: NON-SALMONIDS				
Pimephales promelas (fathead minnow)	1.0-2.0g	S	N	25
	1.0-2.0g	S	N	25
	1.0-2.0g	S	N	25
	1.0-2.0g	S	N	25

Hardness ($mg\ l^{-1}\ CaCO_3$)	pH	Exposure time	Conc ($mg\ l^{-1}$)	Effect	Ref
300-400	7.0-8.0	24 h	64.6	LC_{50}	7
300-400	7.0-8.0	48 h	57.4	LC_{50}	7
300-400	7.0-8.0	72 h	53.8	LC_{50}	7
300-400	7.0-8.0	96 h	52.0	LC_{50}	7
300-400	7.0-8.0	120 h	50.2	LC_{50}	7
300-400	7.0-8.0	24 h	88.8	LC_{50}	7
300-400	7.0-8.0	48 h	68.2	LC_{50}	7
300-400	7.0-8.0	72 h	58.3	LC_{50}	7
300-400	7.0-8.0	96 h	52.9	LC_{50}	7
300-400	7.0-8.0	120 h	48.4	LC_{50}	7
20	7.5	24 h	56.7	LC_{50}	3
20	7.5	48 h	53.6	LC_{50}	3
20	7.5	96 h	46.4	LC_{50}	
360	8.2	24 h	62.8	LC_{50}	3

Species	Life stage	Test type	Analysis	Temp (°C)
	1.0-2.0g	S	N	25
	1.0-2.0g	S	N	25
	30 d (0.1g,)	F	A	21.3
	juvenile (4-8 w)	S	N	18-22
	juvenile (4-8 w)	S	N	18-22
	juvenile (4-8 w)	S	N	18-22
	juvenile (4-8 w)	S	N	18-22
	juvenile (4-8 w)	S	N	18-22
	juvenile (4-8 w)	S	N	18-22
	juvenile (4-8 w)	S	N	18-22
	juvenile (4-8 w)	S	N	18-22

Hardness (mg l^{-1} $CaCO_3$)	pH	Exposure time	Conc (mg l^{-1})	Effect	Ref
360	8.2	48 h	62.8	LC_{50}	3
360	8.2	96 h	59.3	LC_{50}	3
52.8	7.2	96 h	4.02	LC_{50}	11
ND	ND	1 h	100.0	LC_{50}	16
ND	ND	1 h	40.0[3]	LC_{50}	16
ND	ND	24 h	32.0	LC_{50}	16
ND	ND	24 h	30.0[3]	LC_{50}	16
ND	ND	48 h	32.0	LC_{50}	16
ND	ND	48 h	29.0[3]	LC_{50}	16
ND	ND	72 h	32.0	LC_{50}	16
ND	ND	72 h	29.0[3]	LC_{50}	16

Species	Life stage	Test type	Analysis	Temp (°C)
	juvenile (4-8 w)	S	N	18-22
	juvenile (4-8 w)	S	N	18-22
Lepomis macrochirus (bluegill sunfish)	1.0-2.0g	S	N	25
	1.0-2.0g	S	N	25
	1.0-2.0g	S	N	25
Carrasius auratus (goldfish)	1.0-2.0g	S	N	25
	1.0-2.0g	S	N	25
	1.0-2.0g	S	N	25
Carrasius auratus (goldfish)	ND	ND	ND	ND
	3.3g	S	A	20±1
	ND	S	ND	ND
Leuciscus idus melanotus (golden orfe)	ND	S	ND	ND

Hardness (mg l^{-1} $CaCO_3$)	pH	Exposure time	Conc (mg l^{-1})	Effect	Ref
ND	ND	96 h	32.0	LC_{50}	16
ND	ND	96 h	29.0[3]	LC_{50}	16
20	7.5	24 h	25.1	LC_{50}	3
20	7.5	48 h	25.1	LC_{50}	3
20	7.5	96 h	25.1	LC_{50}	3
20	7.5	24 h	64.7	LC_{50}	3
20	7.5	48 h	64.7	LC_{50}	3
20	7.5	96 h	64.7	LC_{50}	3
ND	ND	24 h	26.0	LD_{50}	4
ND	6.0-8.0	24 h	26.0	TL_m	6
ND	ND	24 h	25.0	TL_m	15
ND	ND	'acute'	17.0	LC_{50}	9
			66.0	LC_{50}	9

Species	Life stage	Test type	Analysis	Temp (°C)	Hardness (mg l^{-1} $CaCO_3$)	pH	Exposure time	Conc (mg l^{-1})	Effect	Ref
Poecilia reticulata (guppy)	0.1-0.2g	S	N	25	20	7.5	24 h	74.8	LC_{50}	3
							48 h	74.8	LC_{50}	3
Poecilia reticulata (guppy)							96 h	74.8	LC_{50}	3
Oryzias latipes (red killifish)	ND	SS	ND	20±1	40	7.2	96 h	30.0	LC_{50}	11
FISH: SALMONIDS										
Oncorhynchus mykiss (rainbow trout)	0.5-3.0g	S	N[1]	15±1	135	7.8-8.1	24 h[4]	2.5 (1.8-3.4)	LC_{50}	8
	juvenile (0.22g)	F	N	15±1	270	7.0-7.3	12 h	8.83[5]	LC_{50}	5
	juvenile (0.22g)	F	N	15±1	270	7.0-7.3	24 h	6.5 (5.3-7.8)	LC_{50}	5
Oncorhynchus mykiss (rainbow trout)	juvenile (0.22g)	F	N	15±1	270	7.0-7.3	48 h	6.3 (5.3-7.3)	LC_{50}	5
	juvenile (0.22g)	F	N	15±1	270	7.0-7.3	96 h	5.9 (4.8-7.1)	LC_{50}	5
	juvenile (0.22g)	F	N	15±1	270	7.0-7.3	168 h	4.9 (4.5-5.3)	LC_{50}	5

Notes to Table B1

S: = Static | SS= Semi-static | F:=Flow-through
m:=minutes | h:=hours | d:=days
A:=Analysed | N=Nominal | ND:=no data
95% confidence limits are given in parentheses where available.
[1]Concentrations were only measured at the start of the tests and are therefore considered to be nominal concentrations.
[2]MIC$_{90}$ is the minimum effective concentration of toxicant necessary to eliminate reversing motility in greater than 90% of bacteria cells within 5 minutes.
[3]Solvent carrier used (i.e. acetone).
[4]Cited as a 96-hour LC$_{50}$ but the maximum exposure period tested was 24 hours.
[5]Insufficient data to determine 95% confidence limits

References to Table B1:

1. Bringmann and Kuhn (1980)
2. Bringmann *et al.* (1980)
3. Pickering and Henderson (1966)
4. Verscheuren (1983)
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APPENDIX C SALTWATER TOXICITY AND BIOACCUMULATION

C1. SALTWATER TOXICITY

The toxicity data available for saltwater species are extremely sparse. In this case, a 24-hour median tolerance limit (TLm) of 68.0 mg l^{-1} was recorded for the brine shrimp (*Artemia salina*) under static laboratory conditions (Price et al. 1974). No further details of the test design were provided by the authors. Qureshi *et al.* (1982) performed the Microtox test with styrene, using the luminescent bacteria *Photobacterium phosphoreum*, and determined a 5 minute EC_{50} of 5.4 mg l^{-1}. There is a paucity of data relating to the sensitivity of marine species, limited to three studies looking at short term exposure:

1. Price *et al.* (1974) reported a 24-hour median threshold limit (Tlm) of 68 mg l^{-1} for the brine shrimp (*Artemia salina*) under static conditions (no further study details were reported).

2. Qureshi *et al.*(1982) performed a MICROTOX test with styrene, using the luminescent bacteria *Photobacterium phosphoreum*, and determined a 5 minute EC_{5} of 5.4 mg l^{-1}.

3. Heitmuller *et al.* (1981) reported 24, 48, 72 and 96 hour LC_{50}s as 9.1 mg l^{-1} with a no observed effect concentration (NOEC) of 5.1 mg l^{-1}. The tests were carried out according to EPA guideline under static conditions and results reported as nominal values.

The only study to have investigated chronic exposure was carried out using the species *Pontoporeia affinis* (Lindstrom and Lindstrom 1980, cited by Cambridge Scientific Abstracts 1995). However, the endpoint used in this study appears to have been fairly subjective. At concentrations from 2.3 to 23 mg l^{-1}, an immediate increase in swimming activity was observed, while at concentrations ranging from 35 to 46 mg l^{-1}, immediate cessation of swimming activity was observed, although the crustaceans recovered within a few days. In both instances the total duration of exposure was 40 days. Further details of the test design were not available

C2. BIOACCUMULATION

No information exists on the bioaccumulation potential of styrene in the saltwater environment, although it is not expected to differ significantly from the freshwater environment (see B2, Appendix B). Based on the water partition coefficient (log K_{ow} 2.95-3.16), it might be argued that styrene has the potential to moderately bioaccumulate.

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APPENDIX D MAMMALIAN TOXICOLOGY

This section deals with the mammalian toxicology of styrene from a drinking water perspective although the most usual route of exposure is by inhalation.

Styrene has been detected in drinking water at less than 1 $\mu g\ l^{-1}$ in the US and at 1 $\mu g\ l^{-1}$ in the Scheldt river of the Netherlands. A survey by WRc identified styrene in 4/14 drinking water samples examined (Fawell and Hunt 1988). Styrene is a relatively volatile chemical (5 mm Hg at 20 °C) and thus, if released to raw waters, it is not expected to remain in high concentration for long.

Studies on humans and in experimental animals have shown that styrene is rapidly and very well absorbed following administration by gavage. It has been suggested that as much as 90% of the styrene intake is absorbed and distributed throughout the body, in particular to the lipid-rich areas such as the adipose tissue. Elimination from these areas is rather slower than that for other tissues with a half-life estimated at between 2 and 4 days. An important point to note is that whilst the metabolites eliminated by humans and laboratory animals are qualitatively the same, they are quantitatively very different.

Styrene is metabolised in the liver and a variety of other tissues and organs by the cytochrome P450 mixed function oxidase system. The major metabolite produced is styrene 7,8 oxide. This metabolite undergoes further oxidation to styrene glycol as a result of the actions of the enzyme styrene epoxide hydrolase. Further metabolism of styrene glycol can take place and this is followed by elimination.

Beliles et al. carried out a 2 year chronic toxicity study incorporating a 3 generation reproduction study in rats (Beliles *et al.* 1985). The study involved groups of male and female rats that were given doses of styrene of 0, 125 or 250 mg styrene per litre of water. To calculate the approximate daily dose of styrene taken by the male and female rats, the actual styrene concentration in drinking water is multiplied by the respective mean weight of water consumed per rat unit body weight per day. Using this procedure, the dose levels of 125 $mg\ l^{-1}$ and 250 $mg\ l^{-1}$ equate to 7.7 and 14 $mg\ kg^{-1}$ body weight for the male rats and 12 and 21 mg/kg body weight for the female rats (Beliles et al. 1985). The World Health Organisation identified a No Observed Adverse Effect Level (NOAEL) of 7.7 $mg\ kg^{-1}$ body weight (WHO 1993).

Styrene 7,8 oxide is a very reactive intermediate metabolite. It has been shown to be a directly acting mutagen. Styrene itself has only been shown to be mutagenic in the presence of metabolic activation (WHO 1983).

There is some evidence that styrene is carcinogenic. However, the data set and quality of the studies are poor and thus, the International Agency for Research on Cancer have classified styrene as being " possibly carcinogenic to humans where there are insufficient data in human studies and limited data in animal studies" i.e. Group 2B in their classification system (IARC 1987).

Taking the more conservative value of 7.7 mg kg^{-1} body weight identified in the 2 year chronic study by Beliles et al. (1985), and allocating an uncertainty factor of 1000 to account for inter- and intra-species variation and also the fact that the intermediary metabolite styrene 7,8 oxide is carcinogenic and directly mutagenic, WHO in their revised drinking water guidelines (1993) established a Tolerable Daily Intake of 7.7 $\mu g\ kg^{-1}$ body weight. Assuming a 60 kg adult drinking 2 litres of water per day, and allocating 10 % of the TDI to be due to drinking water, a rounded guideline figure of 20 $\mu g\ l^{-1}$ was established.

A factor to consider when assessing the acceptable level of a chemical in drinking water is the aesthetic properties of the chemical (i.e. taste, odour or colour). In the case of styrene, reported threshold odour concentrations in the literature range from 3.2 - 2600 $\mu g\ l^{-1}$ (Verschueren 1983, Amoore and Hautala 1983, Van Gemert and Nettenbreijer 1977, Alexander et al. 1982). The odour threshold established by WRc is approximately 37 $\mu g\ l^{-1}$ for styrene of 99% purity and dissolved in still, bottled water equilibrated at 40 °C. Styrene has also been reported to have a bitter taste and threshold concentrations of 80 and 160 $\mu g\ l^{-1}$ have been reported in the literature (Alexander et al. 1982). The WRc established taste threshold concentration is about 94 $\mu g\ l^{-1}$ for styrene of 99% purity and dissolved in still, bottled water equilibrated at 25 °C.

In conclusion, at the levels generally detected in drinking water, styrene does not present a significant health hazard. The World Health Organisation have suggested a health-based guideline value of 20 $\mu g\ l^{-1}$. However, at concentrations below this, there may be aesthetic problems with the drinking water.

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APPENDIX E SPECIES CITED

Scientific name	Common name	Taxonomic group	Water
Amphimelania holandri	Freshwater snail	Mollusc	F
Asellus aquaticus	Water louse	Arthropod - crustacean	F
Daphnia magna	Water flea	Arthropod - crustacean	F
Carassius auratus	Goldfish	Fish	F
Cilomonas paramaecium		Protozoan	F
Entosiphon sulcatum		Protozoan	F
Gammarus fossarum	Freshwater shrimp	Arthropod - crustacean	F
Lepomis macrochirus	Bluegill sunfish	Fish	F
Leuciscus idus melanotus	Golden orfe	Fish	F
Lymnaea stagnalis	Snail	Mollusc	F
Microcystis aeruginosa	Blue-green alga	Cyanobacterium	F
Poecilia reticulata	Guppy	Fish	F
Pontoporeia affinis		Arthropod - crustacean	F
Oncorhynchus mykiss	Rainbow trout	Fish	F
Oryzias latipes	Red killifish	Fish	F
Pimephales promelas	Fathead minnow	Fish	F
Photobacterium phosporeum	Microtox test bacterium	Bacterium	S
Pseudomonas putida		Bacterium	F
Scenedesmus quadricauda	Green alga	Alga	F
Spirillum volutans		Bacterium	F
Uronema parduczi Chatton-Lwoff		Protozoan	F

APPENDIX F ABBREVIATIONS USED

EC_{50}	=	concentration of toxicant causing adverse effects (e.g. immobilisation, growth, reproduction)on 50% of test population
EQS	=	environmental quality standard
F	=	freshwater
LC_{50}	=	concentration of toxicant causing 50% lethality in test population
LOEC	=	lowest observable effect concentration
MEC_{90}	=	minimum effective concentration of toxicant necessary to eliminate reverse motility in greater than 90% of bacteria cells within 5 minutes
ND	=	no data
NOEC	=	no observable effect concentration
S	=	saltwater
TLm	=	median tolerance limit