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Evaluation of health hazards by exposure to

Ethylbenzene

and proposal of a health-based quality criterion for ambient air

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Evaluation of health hazards by exposure to Ethylbenzene and proposal of a health-based quality criterion for ambient air

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Preface

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to ethylbenzene and proposal of a health based quality criterion for ambient air. This resulted in 2006 in the present report, which was prepared by Elsa Nielsen and Ole Ladefoged, Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research, i.e. the present Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark.

The report has been subjected to review and discussion and has been endorsed by a steering committee consisting of representatives from the following Danish authorities:

The Danish Working Environment Authority,
The Danish Ministry of Food, Agriculture and Fisheries (The Faculty of Agricultural Sciences),
The Danish Veterinary and Food Administration,
The National Board of Health, Denmark,
The Danish Environmental Protection Agency

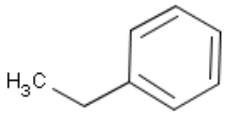
The Danish Environmental Protection Agency
Copenhagen, September 2013.

1 General description

1.1 Identity

Molecular formula: C_8H_{10}

Structural formula:



Molecular weight: 106.16

CAS-no.: 100-41-4

Synonyms: Ethylbenzol
Phenylethane

1.2 Physical / chemical properties

Description: Colourless liquid with a gasoline-like odour.

Purity: Typical min. 99.5% (w/w). Typical impurities: < 0.3% (w/w) benzene and toluene; < 0.2 % total xylenes; max. 1-3 mg/kg total chlorides (as chlorine) and max 4 mg/kg total organic sulphur.

Melting point: - 94.95°C

Boiling point: 136.2°C

Density: 0.8670 g/ml (at 20°C)

Vapour pressure: 9.3 mmHg (1240 Pa) (at 20°C) (WHO 1996);
15 mmHg (2000 Pa) (at 20°C) (A&H 1986)

Concentration of saturated vapours: 54000 – 87000 mg/m^3 (calculated) (at 20°C and 760 mmHg)
54000 mg/m^3 (at 25°C and 760 mmHg) (A&H 1986).

Vapour density: 3.7 (air = 1)

Conversion factor:	1 ppm = 4.41 mg/m^3 (at 20°C and 760 mmHg) 1 mg/m^3 = 0.23 ppm
Flash point:	12.8°C; 15°C (closed cup); 23°C (WHO 1996); 21°C (ATSDR 1997).
Flammable limits:	0.8-6.7 (v/v% in air) (ATSDR 1997)
Autoignition temp.:	432°C (ATSDR 1997)
Solubility:	Water: 152 mg/L (at 20°C) Miscible with organic solvents.
$logP_{octanol/water}$:	3.13
Henry's constant:	1.17 (atm x m^3) / mole (= 887 Pa x m^3/mol) (at 20°C)
pK_a-value:	-
Stability:	-
Incompatibilities:	Reacts with strong oxidants; attacks plastic and rubber.
Odour threshold, air:	10.1 mg/m^3 (Amoore & Hautala 1983) About 2 mg/m^3 (WHO 1996) 0.27-0.4 mg/m^3 (WHO 1996a)
Odour threshold, water:	0.029 mg/L (Amoore & Hautala 1983) About 0.1 mg/L (WHO 1996) 0.002-0.13 mg/l (WHO 1996a)
References:	WHO (1996), Amoore & Hautala (1983), Merck Index (1996), A&H (1986).

1.3 Production and use

Ethylbenzene is present in crude oil. It is present in refined oil products. It is produced by incomplete combustion of natural materials making it a component of forest fires and cigarette smoke. (WHO 1996).

Ethylbenzene is manufactured by alkylation from benzene and ethylene.
The estimated yearly production in the USA is 5.3 million tonnes (1993), and in 1983 it was approximately 3 million tonnes in Western Europe (WHO 1996). North America and Western Europe had production capacities of 7048 and 5157 thousand tonnes, respectively, in 1995 (IARC 2000).

Ethylbenzene is almost exclusively (> 99%) used as an intermediate for the production of styrene monomer. Less than 1 % of the ethylbenzene produced is used as a paint solvent or as an intermediate for the production of diethylbenzene and acetophenone (IARC 2000).

Ethylbenzene is a constituent (15-20%) of commercial xylene ("mixed xylenes"), and hence used as a component of solvents, as a diluent in paints and lacquers, and as a solvent in the rubber and chemical manufacturing industries (WHO 1996).

Ethylbenzene has been added to motor and aviation fuels because of its anti-knock properties. Estimates of ethylbenzene in gasoline have ranged from <1-2.7%. (IARC 2000).

1.4 Environmental occurrence

Ethylbenzene may occur naturally, as it has been found in orange peel, parsley leaves, dried legumes, and other foodstuffs (IARC 2000).

From physico-chemical properties it can be predicted that, when ethylbenzene is released into air, the major part remains in the atmosphere and only small amounts are found in water, soil and sediment (WHO 1996).

1.4.1 Air

Ethylbenzene levels in air at rural sites are generally less than 2 µg/m^3. Mean levels of ethylbenzene ranging from 0.74 to 100 µg/m^3 have been measured at urban sites. (WHO 1996).

Ethylbenzene was not detectable in some rural samples, while those taken on busy urban streets contained levels up to 99 µg/m^3 (ATSDR 1997).

In Southampton, United Kingdom the mean ethylbenzene levels on urban roads were 30.3 µg/m^3 compared with 15.1 µg/m^3 for suburban areas. Two metres from the exhaust of a stationary idling vehicle, the mean ethylbenzene level was 137 µg/m^3. (Bevan et al. 1991 – quoted from WHO 1996).

1.4.2 Water

The levels of ethylbenzene found in surface waters are generally less than 0.1 µg/L in non-industrial areas. In industrial and urban areas, ethylbenzene concentrations of up to 15 µg/L have been reported. Concentrations in uncontaminated groundwater are generally less than 0.1 µg/L, but are much higher in contaminated groundwater. (WHO 1996).

In Canadian treated potable water, levels of ethylbenzene ranged from <1 to 10 µg/L (Otson et al. 1982 – quoted from WHO 1996).

Ethylbenzene was detected in 8 out of 945 samples of finished (undefined) water from groundwater supplies; the levels ranged from 0.74 to 12 µg/L (Westrick et al. 1984 – quoted from WHO 1996).

The physical-chemical properties of ethylbenzene indicate that only small amounts should be found in sediment (WHO 1996). In a study of the Tees Estuary, United Kingdom, levels of ethylbenzene between 1 and 5 µg/kg were found in river sediment from a heavily industrialized area (WHO 1996). In the U.S., ethylbenzene levels in sediments are generally less than 0.5 µg/kg (WHO 1996).

1.4.3 Soil

Ethylbenzene can be released to soils from many sources, including spillage of gasoline and other fuels, leaking underground storing tanks, leaching from landfill sites and disposal of solvents and household products such as paint, cleaning and degreasing solvents, varnishes and pesticides (ATSDR 1997).

The soil acts only as a reservoir. The soil concentration is controlled almost entirely by the rate at which it can evaporate (WHO 1996).

1.4.4 Foodstuffs

There are few data on concentrations of ethylbenzene in foodstuffs. It has been identified as a trace component in the volatiles from honey, jasmine, papaya, olive oil and cheese flavour. Trace quantities of ethylbenzene have been detected in split peas (13 µg/kg), lentils (5 µg/kg) and beans (mean, 5 µg/kg; maximum, 11 µg/kg) 0 and in beans, in split peas and in lentils (Lovegren et al. 1979 – quoted from IARC 2000, WHO 1996). Concentrations of ethylbenzene in orange peel (23.6 µg/kg dry weight) and in parsley leaves (257 µg/kg dry weight) have been reported (Górna-Binjul et al. 1996 – quoted from IARC 2000).

Mean concentrations of ethylbenzene found in freshwater fish samples ranged from 2.45 to 49.6 µg/kg in muscle tissue and from 1.81 to 46.3 µg/kg in liver tissue from turbot in the Canadian Arctic. In white fish muscle tissue samples, levels ranged from 7.46 to 104 µg/kg (Lockart et al. 1992 – quoted from WHO 1996, IARC 2000).
Ethylbenzene was detected in 43 of 138 fish samples at 16 of 42 sites in Japan with concentrations ranging from 1.0 to 9.8 µg/kg wet weight (Environment Agency of Japan 1989 – quoted from WHO 1996).

Migration of ethylbenzene from polystyrene into various foods has been reported with levels in foods from < 2.5 – 6 µg/L (sour milk beverages) up to 89 – 153 µg/kg (noodle curry) (ECETOC 1986 – quoted from IARC 2000).

Concentrations of ethylbenzene were determined in olives and olive oils exposed to gasoline vapours (air concentrations of 7-88 µg/m^3) from gasoline-powered engines either on the tree or during storage. The levels in oil from olives 3 days after storage ranged from 15-55 µg/kg and from 6-60 µg/kg in pressed oil. (Biedermann et al. 1996 – quoted from IARC 2000).

1.5 Environmental fate

1.5.1 Air

Ethylbenzene is being degraded primarily by photo-oxidation and biodegradation. Volatilisation to the atmosphere is rapid. Atmospheric oxidation of ethylbenzene is rapid and proceeds via free-radical chain processes. The most important oxidant is the hydroxyl radical, but ethylbenzene is also reactive with other species found in the atmosphere, such as alkoxy radicals, peroxy radicals, ozone and nitrogen oxides. Estimates for the half-life of ethylbenzene in the atmosphere have been made from smog chamber experiments and from knowledge of the reaction rate constant for reaction with hydroxyl radicals. (WHO 1996).

An atmospheric half-life of around 15 hours has been estimated for ethylbenzene (Callahan 1970 – quoted in WHO 1996). Another report gave a figure of 51% loss of ethylbenzene due to reaction with hydroxyl radicals in one day (12 sunlight hours) (Singh et al. 1981 and 1983 – quoted from WHO1996).

The photo-oxidation reaction of ethylbenzene in the atmosphere may contribute to photochemical smog formation (WHO 1996).

1.5.2 Water

In surface water, transformations of ethylbenzene may occur through two primary processes, photo-oxidation and biodegradation. Although ethylbenzene does not directly absorb light wavelengths that reach the troposphere, it is capable of undergoing photo-oxidation in water through an indirect reaction with other light absorbing molecules, a process known as sensitised photolysis. The compounds 1-methylphenyl ketone, 1-phenylethanol and benzaldehyde were identified from laboratory photo-oxidation with acetophenone used as a sensitiser, and in the environment similar degradation is expected to occur. Biodegradation in aerobic surface water will compete with sensitised photolysis and transport processes such as volatilisation. Slow degradation of ethylbenzene has been reported in anaerobic aquifer materials known to support methanogenesis, although a long acclimation period or lag time was required. Less than 1% of the initial concentration of ethylbenzene remained after 120 weeks, indicating that, given sufficient time, ethylbenzene will be essentially completely biodegraded. (ATSDR 1997).

The contrast between biodegradation rates in the presence or absence of oxygen has been demonstrated by a biofilm reactor study. In the aerobic biofilm column, 99% of the ethylbenzene initially present was degraded within a 20-minute detention time, while under methanogenic (anaerobic) conditions, only 7% was degraded within a 2-day detention time. (Bouwer & McCarty 1984 – quoted from ATSDR 1997).

1.5.3 Soil

Soil bacteria have been shown to be capable of using ethylbenzene as a sole carbon source. Microbial oxidative degradation has been shown to proceed via hydroxylation of the aromatic ring to give 2,3-dihydroxy-1-ethylbenzene (Gibson et al. 1973 – quoted from WHO 1996).

A similar intermediate has been postulated in the degradation of ethylbenzene by *Pseudomonas* sp. NCIB 10643 cultures. The 2,3-dihydroxy intermediate was suggested to undergo further degradation by meta cleavage of the aromatic ring. (Smith & Ratledge 1989 – quoted from WHO 1996).

1.5.4 Bioaccumulation

Ethylbenzene has an octanol-water partition coefficient of 3.13 (log value), which indicates that bioaccumulation of ethylbenzene could take place. Using this partition coefficient, an estimated bio concentration factor (BCF) of 2.16 (log value) can be calculated (Bysshe 1982 – quoted from WHO 1996).

In goldfish, a measured BCF of 1.19 (log value) has been reported (Ogata et al. 1984 – quoted from WHO 1996).

When the manila clam *(Tapes semidecussata)* was exposed to ethylbenzene at a concentration of 0.08 mg/L in water containing other petroleum hydrocarbons, the concentration found in the tissue was 0.37 mg/kg after 8 days. Depuration occurred rapidly after exposure ceased, tissue concentrations being below the limit of detection (< 0.13 mg/kg) after 15 days. (Nunes & Benville 1979 – quoted from WHO 1996).

The low measured BCF values indicate that biomagnification of ethylbenzene through the aquatic food chain is unlikely. No aquatic food chain magnification was predicted from model calculations and empirical observations. (WHO 1996).

1.6 Human exposure

The general population is exposed to ethylbenzene via inhalation of ambient and indoor air, intake of contaminated foodstuffs and drinking water, and from use of consumer products.

Ethylbenzene is ubiquitous in rural and urban atmospheres with very low levels being reported in rural areas (generally less than 2 µg/m^3), while those on busy urban streets are much higher with levels of up to about 100 µg/m^3. Even higher levels have been reported in the surroundings of gasoline stations and near to the exhaust of a stationary idling vehicle. (WHO 1996, IARC 2000, ATSDR 1997).

The range of measured indoor air levels overlaps with those measured outdoors, but when outdoor and indoor levels are compared for a specific house, higher levels of ethylbenzene are usually found indoors (IARC 2000).
In indoor air, tobacco smoke is a major source of exposure to ethylbenzene (Wallace et al. 1987 – quoted from ATSDR 1997, IARC 2000). In a study carried out in the US, indoor air concentrations (geometric means) of ethylbenzene in homes with smokers were 8.3 µg/m^3 in the fall and winter, significantly higher than those in homes without smokers, 5.1 µg/m^3; the levels of ethylbenzene during the spring and summer in homes with smokers and in homes without smokers were the same, 3.5 µg/m^3 (IARC 2000).

Information on exposure from food is limited but is not expected to be a significant source of ethylbenzene for the general population (ATSDR 1997). Levels of ethylbenzene measured in dried legumes ranged from 5 to 13 µg/kg, and in fish up to about 100 µg/kg (IARC 2000).

Concentrations in uncontaminated groundwater are generally less than 0.1 µg/L, but are much higher in contaminated groundwater (WHO 1996).

Ethylbenzene is almost exclusively (> 99%) used as an intermediate for the production of styrene monomer. However, ethylbenzene has also been detected in consumer products. The highest mean concentrations were 7.2% in automotive products, 2.4% in paint-related products, and 1.0% in fabric and leather treatment products. (IARC 2000).

2 Toxicokinetics

2.1 Absorption

2.1.1 Inhalation

Volunteers (number not given) were exposed to 99, 185, 198 or 365 mg/m^3 ethylbenzene for 8 hours. Sixty four percent of the inhaled ethylbenzene was taken up by the respiratory tract. (Bardodej & Bardodejova 1966 – quoted from WHO 1996).

In another study, six volunteers were exposed under controlled conditions for 8 hours to 18, 34, 80, 150 or 200 mg/m^3. The retention of ethylbenzene in the lungs (difference in concentration between inhaled and exhaled air) was 49% (± 5%) independent of the exposure concentration. (Gromiec and Piotrowski 1984 – quoted from WHO 1996).

Volunteers that were exposed to 430 mg/m^3 or 870 mg/m^3 of "industrial xylene" (containing 40% ethylbenzene and 60% xylenes) for 2 hours, took up about 60% of the inhaled concentration, independent of concentration. If the workload increased during exposure, the retention dropped to 50%. (Åstrand et al. 1978 – quoted from WHO 1996).

Male Harlan-Wistar rats were exposed to ^{14}C-labelled ethylbenzene at a concentration of 1000 mg/m^3 for 6 hours. Assuming a ventilation rate of 100 ml/minute, each rat had an estimated retention of 36 mg ethylbenzene, of which 44% was absorbed. (Chin et al. 1980 – quoted from WHO 1996).

Blood concentrations of ethylbenzene in rats after a 2-hour inhalation period were proportional to its concentration in the atmosphere (Freundt et al. 1989 – quoted from IARC 2000).

After exposure for 6 hours to an atmosphere containing 600 ppm (2600 mg/m^3) ethylbenzene, peak blood levels of ethylbenzene in rats occurred at the end of exposure, falling rapidly thereafter (Elovaara et al. 1990 – quoted from IARC 2000).

2.1.2 Oral intake

Ethylbenzene appears to be rapidly and well absorbed from the gastro-intestinal tract in rats since more than 80% of the administered radioactively labelled compound was recovered in the urine within 48 hours (Climie et al. 1983 – quoted from WHO 1996).

Toxicity studies in various animal species show indirectly that ethylbenzene is absorbed after oral administration (Wolf et al. 1956, NTP 1992 – both quoted from WHO 1996).

2.1.3 Dermal contact

One human subject was exposed for 2 hours to ethylbenzene vapour at concentrations ranging from 650 to 1300 mg/m^3 in an exposure chamber. The exposed skin accounted for 90-95% of the total skin area. Clean breathing air was provided by means of a gas-tight respirator. The mandelic acid concentration in urine, before, during and up to 6 hours after exposure, was within physiological limits (approximately 2.7 mg/L). The authors concluded that the skin is not a relevant route of entry into the body for ethylbenzene vapours. (Gromiec & Piotrowski 1984 - quoted from WHO 1996).

The possible absorption of liquid ethylbenzene across human skin has also been studied. Ethylbenzene (0.2 ml = 174 mg) was applied in a watch glass tightly fixed on the forearm. The exposed skin area was 17.3 cm^2. On the basis of the quantity of ethylbenzene not recovered, the mean absorption rate for seven people was calculated to be 28 mg/cm^2 per hour (range 22-33 mg cm^2 per hour). (Dutkiewicz & Tyras 1967 – quoted from WHO 1996).

The penetration rate of ethylbenzene through excised rat skin has been determined in a penetration chamber. One ml of ethylbenzene was applied to 2.55 cm^2 skin. After a 6-hour application period, the penetration rate was found to be about 0.99 nmoles/cm^2 per min (6 µg/cm^2 per hour) (Tsuruta 1982 - quoted from WHO 1996).

Percutaneous absorption of ethylbenzene has been studied in hairless mice (11 animals). ^{14}C-ring-labelled ethylbenzene (in a volume of 5 µl) was injected into a chamber glued onto the back skin (0.8 cm^2), and the animals were housed in metabolism cages for 4 hours. About 95.2% of the nominal dose was recovered. The absorption rate was calculated to be 0.037 (± 0.0315) mg/cm^2 per min (2.2 ± 1.9 mg/cm^2 per hour). (Susten et al. 1990 – quoted from WHO 1996).

Dermal absorption of volatile organic chemicals from aqueous solutions has been studied in male Fischer-344 rats. Animals were exposed (3.1 cm^2 dorsal shaved skin) for 24 hours to 2 ml (in a glass exposure cell) of one-third saturated, two-thirds saturated, or a fully saturated solution of ethylbenzene. Blood samples were obtained at 0, 0.5, 1, 2, 4, 8, 12 and 24 hours. The peak blood level (exposure to neat ethylbenzene) was 5.6 mg/L. The level reached a maximum within 4 hours and then either remained at about the same level for the duration of the exposure or decreased. The blood levels were directly related to the exposure concentrations. (Morgan et al. 1991 – quoted from WHO 1996).

According to Barber et al. (1992 – quoted from WHO 1996), the data concerning skin permeability of ethylbenzene in humans is not consistent with the animal data. The reliability of the estimated fluxes of ethylbenzene through human skin must be questioned because they are many times higher than the measured fluxes through rat skin, whereas from studies of *in vitro* percutaneous absorption it is generally accepted that rat skin is more permeable than human skin (mean ratio about 3) for several chemicals.

2.2 Distribution

2.2.1 Inhalation

Twelve male volunteers were exposed for 2 hours to 100 or 200 ppm “industrial xylene” (consisting of 40.4% ethylbenzene, 49.4% *m*-xylene, 8.8% *o*-xylene and 1.4% *p*-xylene). The amount of ethylbenzene taken up correlated with the amount

of body fat. The concentration of ethylbenzene was unchanged in subcutaneous adipose tissue from 30 min to 22 hours after exposure and ranged from 4 to 8 mg/kg. (Engström & Bjurström 1978 – quoted from A&H 1986).

The ethylbenzene concentration in the subcutaneous fat of workers in a styrene polymerisation plant varied from 0.1 to 0.7 mg/kg in 21 of 25 workers. The level of exposure to ethylbenzene was reported to be below 17.4 mg/m^3. The 25 workers were exposed to a variety of other chemicals as well. (Wolff et al. 1977 – quoted from WHO 1996).

When rats were exposed to 1000 mg/m^3 ^{14}C-ring-labelled ethylbenzene for 6 hours, 0.2% of the radioactivity was found 42 hours later in the tissues, mainly in the liver, gastrointestinal tract, fat and the carcass (Chin et al. 1980 – quoted from WHO 1996).

After exposure to an atmosphere containing 600 ppm (2600 mg/m^3) ethylbenzene for 6 hours, peak blood levels of ethylbenzene in rats occurred at the end of the exposure, declining rapidly thereafter. Ethylbenzene was detected in brain, liver, kidney and adipose tissue; the time courses of concentrations were broadly similar to those in blood, but there was considerable retention in adipose tissue. (Elovaara et al. 1990 – quoted from IARC 2000).

Twenty male Wistar rats were exposed to 215, 1290 or 2580 mg ethylbenzene/m^3 for 6 hours/day, 5 days/week for up to 16 weeks. The amount of ethylbenzene in peri-renal fat was 8.5, 167.7 and 262.2 mg/kg fat at the three exposure levels, respectively. (Engström et al 1985 – quoted from WHO 1996).

2.2.2 Oral intake

No data have been found.

2.2.3 Dermal contact

No data have been found.

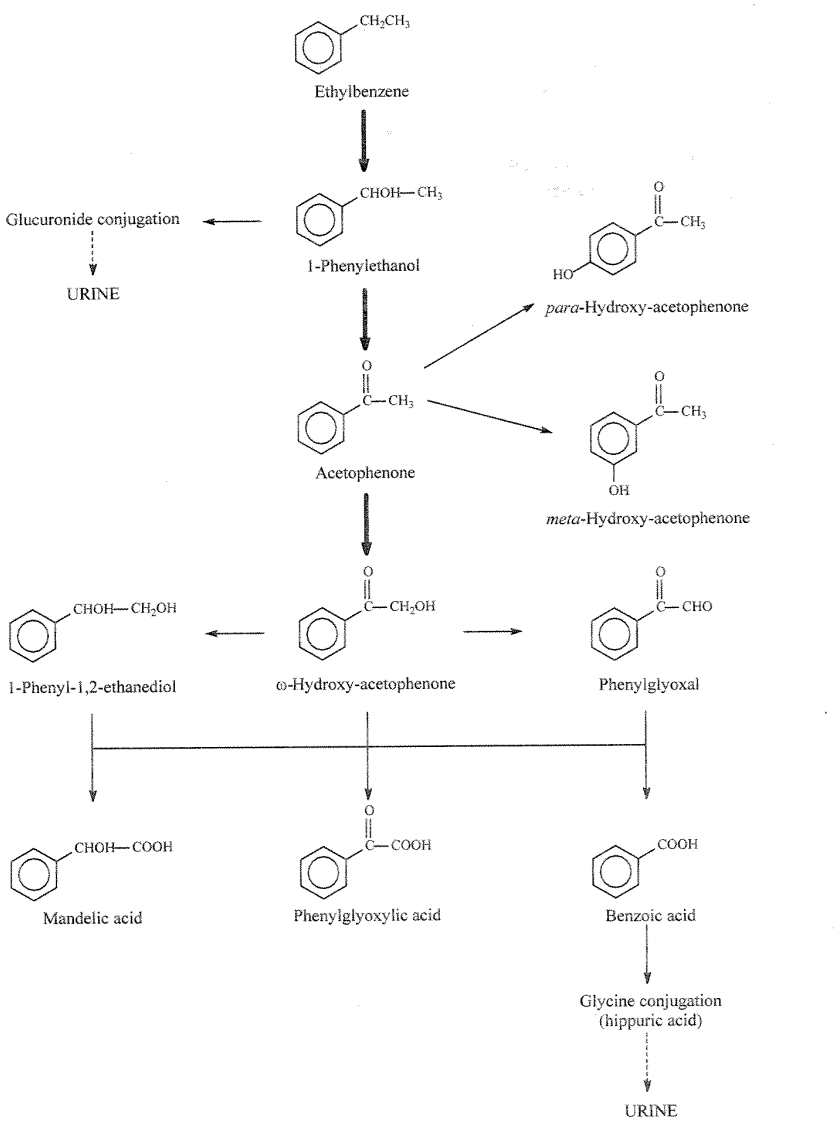
2.3 Elimination

Metabolic pathways of ethylbenzene based on urinary metabolites have been proposed for humans and for rats, see Figure 1.

The main metabolic pathway is oxidation of the side chain, both in humans and in animals. However, it has been demonstrated that there are both qualitative and quantitative inter-species differences in the metabolites produced. (Engström et al. 1984, Engström 1984 – both quoted from WHO 1996).

In humans, the major pathway in the metabolism of ethylbenzene is oxidation of the α-carbon, yielding 1-phenylethanol (also called α-methylbenzyl alcohol) as the primary metabolite. The α-carbon of ethylbenzene is a prochiral centre and hydroxylation thus yields a chiral product. The mandelic acid excreted in human urine following exposure to ethylbenzene is predominantly the R-enantiomer. (IARC 2000).

Figure 1. Metabolism of ethylbenzene. Reproduced from IARC (2000).



Four male volunteers were exposed to 655 mg/m^3 ethylbenzene for 4 hours. Mandelic acid (71.5%) and phenylglyoxylic acid (19.1%) were the main metabolites; smaller amounts of 1-phenylethanol, *p*-hydroxyacetophenone, *m*-hydroxyacetophenone, 1-phenyl-1,2-ethandiol, 4-ethylphenol, omega-hydroxyacetophenone and acetophenone were also found. Ring oxidation accounted for 4.0%. Simultaneous exposure to 150 ppm *m*-xylene did not alter the urinary metabolite pattern, but it delayed excretion and decreased the amounts of metabolites excreted (Engström et al. 1984 – quoted from WHO 1996).

Mandelic acid (64%) and phenylglyoxylic acid (25%) were found to be the main urinary excretion products in volunteers after an 8-hour exposure to 99-365 mg/m^3 ethylbenzene (Bardodej & Bardodejová 1970 – quoted from WHO 1996).

In several animal species, the metabolic transformation proceeds to benzoic acid, leading to excretion of hippuric acid after conjugation with glycine. This conjugate is generally one of the main urinary metabolites, together with mandelic acid, in rats and dogs (Chin et al. 1980 – quoted from WHO 1996).

Hydroxylation of the aromatic nucleus is a minor pathway. In the rabbit, this pathway accounts for less than 2% of the ethylbenzene absorbed. (Kiese & Lenk 1974 – quoted from WHO 1996).

Ethylbenzene is metabolised by the microsomal cytochrome P-450 enzyme system. Although specific isozymes have not been unequivocally identified, enzyme induction studies suggest that CYP2B1/2, CYP1A1/2 and CYP2E1 may be involved. (WHO 1996).

2.3.1 Inhalation

In humans, ethylbenzene is mainly excreted in the urine as mandelic and phenylglyoxylic acids (Bardodej & Bardodejová 1970, Åstrand et al. 1978, Engström et al. 1984, Gromiec & Piotrowski 1984 – all quoted from WHO 1996). Only up to 5% of retained ethylbenzene is estimated to be exhaled without transformation (Åstrand et al. 1978 – quoted from WHO 1996).

The elimination half-lives of ethylbenzene in exhaled air and urine have been estimated to be 0.5-3 hours and 8 hours, respectively (Wolff 1976 – quoted from WHO 1996).

Male Harlan-Wistar rats exposed to ^{14}C-ring-labelled ethylbenzene (1000 mg/m^3) for 6 hours excreted 82% of the radioactivity in the urine, 8.2% in expired air (0.03% as CO_2) and 0.7% in faeces. After 42 hours, 0.2% remained in the tissues. The remaining 8.3% could not be accounted for. (Chin et al. 1980 – quoted from WHO 1996).

Groups of 20 male Wistar rats were exposed to 215, 1290 or 2580 mg/m^3 for 6 hours/day, 5 days/week, for up to 16 weeks. Urinary excretion of selected metabolites was measured in weeks 2, 5 and 9. A significant dose-related percentage decrease of phenylglyoxylic acid and hippuric acid plus benzoic acid was found. A corresponding increase of 1-phenylethanol and omega-hydroxyacetophenone excretion was also noted. The total amount of metabolites in urine collected during the 24 hour period after onset of exposure remained, however, constant at each exposure level throughout the study. (Engström et al. 1985 – quoted from WHO 1996).

2.3.2 Oral intake

Following a single oral dose of 318 mg/kg b.w. of ethylbenzene administered to rabbits, the main urinary metabolites found were hippuric acid and methylphenylglucuronic acid, which together represented 60-70% of the dose, while mandelic acid and phenaceturic acid were minor metabolites (El Masry et al. 1956 – quoted from WHO 1996).

2.3.3 Dermal contact

No data have been found.

2.4 Mode of action

No information has been found with respect to the mode of action for ethylbenzene on a molecular basis. Ethylbenzene has irritative and narcotic properties and the anaesthetic and other typical CNS depressive effects have been explained as typical effects for solvents with a high solubility in fats (A&H 1986).

Potential factors underlying the carcinogenic activity of ethylbenzene were examined in F344 rats and in B6C3F1 mice inhaling 750 ppm (about 3300 mg/m^3) ethylbenzene vapour 6 hours/day, 5 days/week, for one or four weeks. Target tissues (kidneys of rats, and livers and lungs of mice) were evaluated for changes in organ weights, mixed function oxygenases (MFO), glucuronosyl transferase activities, S-phase DNA synthesis, apoptosis, alpha2µ-globulin deposition, and histopathology.
In male rats, increased kidney weight was accompanied by focal increases in hyaline droplets, alpha2µ-globulin, degeneration, and S-phase synthesis in proximal tubules. In female rats, only decreased S-phase synthesis and MFO activities occurred.
In mice, increased liver weights were accompanied by hepatocellular hypertrophy, mitotic figures, S-phase synthesis, and enzyme activities. S-phase synthesis rates in terminal bronchiolar epithelium were elevated and accompanied by loss of mixed function oxygenases activity.
According to the authors, these data, considered with the general lack of ethylbenzene genotoxicity, suggest a mode of action dependent upon increased cell proliferation and altered population dynamics in male rat kidney and mouse liver and lungs. A similar response in the kidneys of female rats appears to require a longer exposure period than was employed. (Stott et al. 2003).

A histopathological re-evaluation of the renal tubules from the kidneys from the NTP (1999) carcinogenicity study in F 344 rats (see section 4.5.1.1) revealed no evidence of renal tubule injury or increased mitotic activity that would support sustained cytotoxicity/cell regeneration as a mode of action for tumour development. An absence of granular casts and linear papillary mineralisation discounted the possibility of alpha2µ-globulin nephropathy as the primary underlying basis in male rats, even though sub-chronic studies revealed a modest accumulation of hyaline droplets in proximal tubules. Based on the close association of atypical tubule hyperplasia and renal tumours with chronic progressive nephropathy, it was concluded by the authors that chemically induced exacerbation of chronic progressive nephropathy was the mode of action underlying the development of renal tumours, a pathway that is considered to have no relevance for extrapolation to humans. (Hard 2002).

3 Human toxicity

3.1 Single dose toxicity

3.1.1 Inhalation

In a review of studies from the 1930s, it was stated that exposure to 21500 mg/m^3 ethylbenzene for a few seconds resulted in intolerable irritation of nose, eyes and throat. A few seconds of exposure to 4300 mg/m^3 initially resulted in eye irritation, which diminished after a few minutes of exposure. (Nielsen & Alarie 1982 – quoted from WHO 1996).

In a study on ethylbenzene metabolism in 4 male volunteers, exposure above 100 ppm (430 mg/m^3) for 8 hours resulted in complaints of fatigue, sleepiness, and headache, and irritation of the eyes and respiratory tract were reported (Bardodej & Bardodejová 1970 – quoted from WHO 1996).

3.1.2 Oral intake

No data have been found.

3.1.3 Dermal contact

A dermal maximization test conducted on 25 volunteers with a concentration of 10% ethylbenzene in petrolatum produced no skin sensitisation (ECETOC 1986 – quoted from WHO 1996).

3.2 Repeated dose toxicity

In a long-term study (~ 20 years) of about 200 ethylbenzene production workers exposed to an undefined concentration, none of the workers showed changes in haematological parameters or serum enzyme levels as measure of liver function (Barodej & Cirek 1988 – quoted from IARC 2000).

3.3 Toxicity to reproduction

No data have been found.

3.4 Mutagenic and genotoxic effects

No data have been found.

3.5 Carcinogenic effects

Between 1964 and 1985, some 200 ethylbenzene-production workers (in Czechoslovakia) were monitored twice a year for mandelic acid excretion. The mean age of the workers exposed to ethylbenzene was 36.6 years and their mean length of employment was 12.2 years. The authors stated that the cancer incidence among chemical workers in the industrial complex (of comparable age and length of employment) not engaged in ethylbenzene production was about 3 times the national average, whereas in the group of ethylbenzene production workers, no tumours had been reported over the previous 10 years. (Bardodej & Cirek 1988 – quoted from IARC 2000).
According to IARC (2000), no precise figures were given to substantiate the assertions. In addition, co-exposure to benzene was present, and the age of the workers and the length of follow-up were not sufficient for a proper evaluation of cancer risk in relation to exposure to ethylbenzene.

4 Animal toxicity

4.1 Single dose toxicity

4.1.1 Inhalation

The following LC-values have been reported for male rats: LC_{10} of 17200 mg/m^3 (1 hour), LC_{50} of 17200 mg/m^3 (4 hours), and LC_{100} of 34400 mg/m^3 (1 hour) (Smyth et al. 1962 – quoted from WHO 1996).

The minimum narcotic concentration reported in male rats was 9370 mg/m^3 (Molnár et al. 1986 – quoted from WHO 1996). Following exposure to 400 ppm (1760 mg/m^3) for 4 hours, male rats showed activation in motor behaviour (Molnár et al. 1986 – quoted in ATSDR 1997).

A respiratory depression of 50% (RD_{50}) was recorded in male mice at 1432 ppm (6300 mg/m^3) for 5 minutes (De Ceaurriz et al. 1981 – quoted from ATSDR 1997), and at 4060 ppm (17900 mg/m^3) for 30 minutes (Nielsen & Alarie 1982 – quoted from ATSDR 1997).

Male mice exposed to 2000 ppm ethylbenzene (8800 mg/m^3) for 20 minutes showed lachrymation and palpebral ocular closure and changes in a number of functional observations (e.g., changed posture, disturbed gait, decreased mobility) (Tegeris & Balster – quoted from ATSDR 1997).

In guinea-pigs, inhalation of ethylbenzene for 8 minutes at a concentration of 4300 mg/m^3 caused eye irritation; slight nasal irritation was recorded following a 3-minute exposure. At 8600 mg/m^3, exposure for one minute, both eye and nasal irritation was recorded. (Cavender 1993 – quoted from WHO 1996).

4.1.2 Oral intake

Oral LD_{50}-values for rats of 3500 mg/kg b.w. and 4700 mg/kg b.w. have been reported (Wolf et al. 1956, Smyth et al. 1962 – both quoted from WHO 1996).

4.1.3 Dermal contact

A dermal LD_{50}-values of 77400 mg/kg was reported in rabbits (Smyth et al. 1962 – quoted from WHO 1996).

4.2 Irritation

4.2.1 Skin irritation

Undiluted ethylbenzene has been shown to produce moderate irritation when applied to the uncovered skin of rabbits (Smyth et al. 1962 – quoted from WHO 1996).

4.2.2 Eye irritation

Two drops of undiluted ethylbenzene placed in the eyes of rabbits resulted in slight conjunctival irritation, but no effects on the cornea (Wolf et al. 1956 – quoted from WHO 1996).

A slight conjunctival irritation with some reversible corneal injury was reported in rabbits (Smyth et al. 1962 – quoted from WHO 1996).

4.2.3 Respiratory irritation

No data have been located.

4.3 Sensitisation

No data have been found.

4.4 Repeated dose toxicity

4.4.1 Inhalation

4.4.1.1 Rat

Six male rats (Sprague Dawley) were exposed for 6 hours/day during 3 consecutive days to 8600 mg/m^3 ethylbenzene. The animals were killed 16-18 hours after the last exposure. Small increases in dopamine and noradrenaline levels and turnover in various parts of the hypothalamus and the median eminence were reported. Ethylbenzene was also found to produce selective reduction in prolactin and corticosterone secretion and selective increase in dopamine turnover within the dopamine-cholecystokinin-8-immuno-reactive nerve terminals of the nucleus accumbens (posterior part). (Andersson et al. 1981 – quoted from WHO 1996).

Rats were exposed to ethylbenzene at 0, 300, 400, or 550 ppm (0, 1323, 1765, or 2425 mg/m^3) for 8 hours/day for 5 consecutive days. Three to six weeks after the exposure, auditory function was tested. In addition, outer hair cell (OHC) loss was quantified by histological examination. At 300 ppm, ethylbenzene had no effects on auditory function. At 400 ppm, auditory thresholds were increased by15 and 16 dB at 12 and 16 kHz, respectively, and at 550 ppm by 24, 31, and 22 dB at 8, 12, and 16 kHz, respectively. Distortion product otoacoustic emissions amplitude growth with stimulus level was affected only after 550 ppm at 5.6, 8, and 11.3 kHz. OHC loss was found in two of the five examined locations in the cochlea. At 400 ppm, 25% OHC loss was found at the 11- and 21-kHz region. The highest concentration evoked 40% and 75% OHC loss at the 11- and 21-kHz location, respectively. Thus, the mid-frequency region of rats is affected after exposure to relatively low concentrations of ethyl benzene (400-550 ppm). According to the authors, these results indicate that ethyl benzene is one of the most potent ototoxic organic solvents known today. (Cappaert et al. 2000 – quoted from BIOSIS Previews 2003).

A 4-week inhalation study was performed with Fischer-344 rats. Five rats of each sex per group were exposed to ethylbenzene for 6 hours/day, 5 days per week, at exposure levels of 0, 426, 1643 or 3363 mg/m^3. At the two highest exposure levels,

sporadic lachrymation and salivation, and significantly increased liver weights were seen. At the highest exposure level, there was a small increase in leukocyte counts and, in males, a marginal increase in platelet counts. No changes were seen in mortality pattern. No changes in gross or microscopic pathology were noted in any of over 30 tissues examined at the highest concentration. (Cragg et al. 1989 – quoted from WHO 1996).

In a 13-week study performed by the National Toxicology Program in the U.S, groups of 10 rats (F-344/N) of each sex were exposed for 6 hours (plus 10 minutes to reach 90% of the target chamber concentration) per day, 5 days per week for 92 (female rats), or 93 (male rats) days, at ethylbenzene concentrations of 0, 440, 1100, 2200, 3300 or 4400 mg/m^3. Increased absolute and relative liver and kidney weights were observed in male rats at the two highest dose levels, and increased absolute liver and kidney weights in female rats at the three highest dose levels. No chemically related histopathological changes were observed in any of the tissues examined. (NTP 1992 – quoted from WHO 1996).

Groups of five male rats (Wistar) were exposed for 6 hours/day, 5 days/week to ethylbenzene concentrations of 0, 215, 1290 or 2580 mg/m^3 for 2, 5, 9 or 16 weeks. At 2580 mg/m^3, liver cells showed a slight proliferation of smooth endoplasmic reticulum, slight degranulation and splitting of rough endoplasmic reticulum, and enlarged mitochondria. Liver microsomal protein, but not cytochrome P-450, concentration was slightly increased. There was also an increase in NADPH-cytochrome-*c* reductase, 7-ethoxycoumarin-*O*-deethylase and UDPG-transferase activities in the liver. In the kidney, only the two latter enzymes showed dose-related increases. Urinary excretion of thioethers was measured to ascertain the generation of electrophilic intermediates during ethylbenzene metabolism. Excretion of thioethers increased in a dose-dependent manner, with some fluctuation over the course of 7 weeks, reaching about eight times the control level at 2580 mg ethylbenzene/m^3. However, there was no decrease in hepatic or renal levels of glutathione (GSH), indicating that the cells were able to maintain the intra-cellular homeostasis of GSH during exposure. (Elovaara et al. 1985 – quoted from WHO 1996).

Matched groups of 10-25 male and female Wistar rats were exposed 7 hours/day, 5 days/week, for up to 6 months. The exposure levels were 0, 1720 and 2580 mg/m^3 for 186 days, 5375 mg/m^3 for 214 days, or 9460 mg/m^3 for 144 days. Slightly increased liver and kidney weights were observed at 1720 mg/m^3 and 2580 mg/m^3, and slight histopathological changes (cloudy swelling) in liver and kidney at 5375 and 9460 mg/m^3. The no-observed-effect level was considered by the authors to be about half of the lowest concentration tested, i.e., 860 mg/m^3. (Wolf et al. 1956 – quoted from WHO 1996).

Groups of 50 male and 50 female rats (F 344/N) were exposed to ethylbenzene by inhalation in whole-body exposure chambers at concentrations of 0, 75, 250 or 750 ppm (0, 330, 1100, or 3300 mg/m^3) for 6 hours per day, 5 days per week for 104 weeks. Survival of male rats followed a negative trend, decreasing with increasing dose, and was significantly decreased at 750 ppm. The mean body weights of 250 and 750 ppm males were generally lower than those of control animals from week 20 until the end of the study (95% and 90% of the control group at week 104). Mean body weights of exposed females were generally less than those of the control animals during the second year of the study (94%, 95% and 95% of the control group at week 104). No clinical signs of toxicity were observed. In male rats exposed to 750 ppm, the incidences of renal tubule hyperplasia and of renal tubule proliferative lesions were significantly increased. The findings from an extended evaluation (step-section) of the kidneys revealed a significant increase in

the incidences of renal tubule hyperplasia in 750 ppm females as well. The severities of nephropathy were significantly increased in 750 ppm male (severity, grade 3.5 – 3 is moderate and 4 is marked) and in all exposed female rats (severity, grade 1.6 and 1.7 at 75 and 250 ppm, and grade 2.3 at 750 ppm – 1 is minimal and 2 is mild). Nephropathy was characterised by dilation of renal tubules with hyaline or cellular casts, interstitial fibrosis and mononuclear inflammatory cell infiltration, foci of tubular regeneration, and transitional epithelial hyperplasia of the renal papilla. The enhanced nephropathy was more severe in males than in females and involved most of the renal parenchyma. The incidence of interstitial cell hyperplasia in testes was significantly decreased in 750 ppm male rats. (NTP 1999). Neoplastic changes are addressed in section 4.5.1.1.

4.4.1.2 Mouse

$B6C3F_1$ mice (5/sex/group) were exposed to ethylbenzene for 6 hours/day, 5 days per week for 4 weeks, at exposure levels of 0, 426, 1643 or 3363 mg/m^3. At 1643 and 3363 mg/m^3, females showed increased absolute and relative liver weights. In males, an increased relative liver-to-brain weight ratio was seen. No changes in gross or microscopic pathology were noted in any of over 30 tissues examined at the highest concentration. (Cragg et al. 1989 – quoted from WHO 1996).

In a 13-week study performed by the National Toxicology Program in the U.S., groups of 10 mice ($B6C3F_1$) of each sex were exposed for 6 hours (plus 10 minutes to reach 90% of the target chamber concentration) per day, 5 days per week for 97 (female mice) or 98 (male mice) days, at ethylbenzene concentrations of 0, 440, 1100, 2200, 3300 or 4400 mg/m^3. Dose-related increases in absolute liver weight were seen in both sexes of mice exposed to the two highest dose levels. The relative kidney weight of female mice exposed to 4400 mg/m^3 was greater than that of the controls. No chemically related histopathological changes were observed in any of the tissues examined. (NTP 1992 – quoted from WHO 1996).

Groups of 50 male and 50 female $B5C3F_1$ mice were exposed to ethylbenzene by inhalation in whole-body exposure chambers at concentrations of 0, 75, 250 or 750 ppm (0, 330, 1100, or 3300 mg/m^3) for 6 hours per day, 5 days per week for 103 weeks. Survival and body weights of the exposed and control groups were similar. No clinical signs of toxicity were observed. The incidence of alveolar epithelial metaplasia increased in male mice with increasing exposure concentration and was significantly greater in 750 ppm males than that of controls. Alveolar epithelial metaplasia was also observed in one 750 ppm female. The incidence of eosinophilic foci in the liver was significantly increased in 750 ppm females. In 750 ppm male mice, the incidences of liver changes (syncytial alteration of hepatocytes, hepatocellular hypertrophy, and hepatocyte necrosis – minimal to mild in severity) were significantly increased; the incidence of syncytial alteration of hepatocytes was also significantly increased in 250 ppm male mice. The incidences of hyperplasia of the pituitary gland were significantly increased in 250 and 750 ppm females and the incidences of thyroid gland follicular cell hyperplasia were significantly increased in 750 ppm males and females. (NTP 1999). Neoplastic changes are addressed in section 4.5.1.2.

4.4.1.3 Rabbit

In eight male rabbits (New Zealand) exposed 12 hours daily for 7 days to 3220 mg/m^3 ethylbenzene, there was a marked depletion of striatal and tuberoinfundibular dopamine levels (Mutti et al. 1988 – quoted from IARC 2000).

A 4-week inhalation study was performed with rabbits (New Zealand White, 5/sex/group) at exposure levels of 0, 1643, 3363 and 6923 mg/m^3 for 6 hours/day, 5 days per week. At the highest exposure level, females gained weight more slowly than controls. No gross or microscopic organ changes were noted. (Cragg et al. 1989 – quoted from WHO 1996).

In inhalation experiments, matched groups of 1-2 rabbits of either sex or both sexes were exposed 7 hours/day, 5 days/week, for up to 7 months at exposure levels of 0, 1720, or 2580 mg/m^3 for 186 days, or 5375 mg/m^3 for 214 days. Histopathological effects in the testes, described as degeneration of the germinal epithelium, were seen in the 2580 mg/m^3 group only. The no-observed-effect level was considered by the authors to be about half of the lowest concentration tested, i.e., 860 mg/m^3. (Wolf et al. 1956 – quoted from WHO 1996).

4.4.1.4 Guinea-pig

In inhalation experiments, matched groups of 5-10 guinea-pigs of either sex or both sexes were exposed 7 hours/day, 5 days/week, for up to 7 months at exposure levels of 0, 1720, or 2580 mg/m^3 for 186 days, or 5375 mg/m^3 for 214 days. Slightly increased liver weights were noted in the 2580 mg/m^3 group only. At 5375 mg/m^3, a slight growth depression was noted. The no-observed-effect level was considered by the authors to be about half of the lowest concentration tested, i.e., 860 mg/m^3. (Wolf et al. 1956 – quoted from WHO 1996).

4.4.1.5 Monkey

In inhalation experiments, matched groups 1-2 rhesus monkeys of either sex or both sexes were exposed 7 hours/day, 5 days/week, for 6 months at exposure levels of 0, 1720, or 2580 mg/m^3 for 186 days. Slightly increased liver weights were noted in the 2580 mg/m^3 group only. At the same exposure level, histopathological effects in the testes, described as degeneration of the germinal epithelium, were noted. The no-observed-effect level was considered by the authors to be about half of the lowest concentration tested, i.e., 860 mg/m^3. (Wolf et al. 1956 – quoted from WHO 1996).

4.4.2 Oral intake

Groups of 10 Wistar female rats were given daily doses of ethylbenzene of 0, 13.6, 136, 408, or 680 mg/kg by stomach tube 5 days a week for 6 months. The two highest dosages induced slight increases in liver and kidney weights and slight cloudy swelling of parenchymal liver cells and of the tubular epithelium in the kidney. (Wolf et al. 1956 – quoted from WHO 1996).

4.4.3 Dermal contact

The application of undiluted ethylbenzene to the ear and to the shaved abdomen of rabbits for up to 20 times during a 4-week period resulted in moderate irritation. Erythema and oedema with superficial necrosis and exfoliation of large patches of skin were reported. (Wolf et al. 1956 – quoted from WHO 1996).

4.5 Toxicity to reproduction

4.5.1 Inhalation

4.5.1.1 Rat

In an inhalation study, rats (Wistar or Sprague-Dawley) were exposed to 430 or 4300 mg/m^3 ethylbenzene for 6-7 hours/day on gestation days 1 to 19. Maternal toxicity was observed in high-dose animals and included increased liver, kidney and spleen weights. There was an increased incidence of extra ribs at both exposure levels. (Hardin et al. 1981 – quoted from WHO 1996).

CFY rats were exposed by inhalation to ethylbenzene concentrations of 600, 1200 or 2400 mg/m^3 continuously (24 hours/day) from day 7 to day 15 of pregnancy. Maternal toxic effects (not specified) were reported as being moderate and dose-dependent. Skeletal growth retardation, extra ribs, and reduced foetal growth rate were reported at the highest concentration. (Ungváry & Tátrai 1985 – quoted from WHO 1996, IARC 2000).

The developmental toxicity of ethylbenzene was studied in Sprague-Dawley rats exposed at 100, 500, 1000 or 2000 ppm (440, 2200, 4400, or 8800 mg/m^3) for 6 hours/day, during days 6-20 of gestation. Maternal toxicity in form of reduction in maternal body weight gain was observed from 1000 ppm. Foetal toxicity in form of decreased foetal body weight occurred at concentrations from 1000 ppm. A significant increase in the mean percentage of foetuses per litter with skeletal variations was noted at 2000 ppm. No evidence of teratogenic effects was found up to 2000 ppm. (Saillenfait et al. 2003 – quoted from BIOSIS Previews 2003).

In rats (F 344/N) exposed to ethylbenzene at concentrations up to 782 ppm (about 3400 mg/m^3) for 4 weeks, no histopathological abnormalities in the testes were reported (Cragg et al. 1989 – quoted from ATSDR 1997).

In rats (F-344/N) exposed to ethylbenzene at concentrations of 0, 440, 2200 or 4400 mg/m^3 for 6 hours per day, 5 days per week, for 13 weeks, no changes in sperm or vaginal cytology were observed (NTP 1992 – quoted from WHO 1996).

4.5.1.2 Mouse

In CFLP mice exposed for 3 periods of 4 hours per day to 500 mg/m^3 of ethylbenzene on days 6-15 of pregnancy, maternal toxic effects (not specified) were reported as being moderate and dose-dependent. Ethylbenzene caused skeletal growth retardation, extra ribs and reduced foetal growth rate. (Ungváry & Tátrai 1985 – quoted from WHO 1996).

In B6C3F$_1$ mice exposed to concentrations up to 782 ppm (about 3400 mg/m^3) for 4 weeks, no histopathological abnormalities in the testes were reported (Cragg et al. 1989 – quoted from ATSDR 1997).

In mice (B6C3F$_1$) exposed by inhalation to ethylbenzene at concentrations of 0, 440, 2200 or 4400 mg/m^3 for 6 hours per day, 5 days per week, for 13 weeks, no changes in sperm or vaginal cytology were observed (NTP 1992 – quoted from WHO 1996).

No adverse effects on the reproductive tissues of male and female $B6C3F_1$ mice were observed in the NTP 2-year study at concentrations of up to 750 ppm (about 3300 mg/m^3) (NTP 1996 – quoted from ATSDR 1997).

4.5.1.3 Rabbit

In an inhalation study, rabbits (New Zealand White) were exposed to 440 or 4400 mg/m^3 ethylbenzene for 6-7 hours/day on gestation days 1 to 24. A significantly reduced number of live pups per litter was observed at both exposure levels, but the number of implantations per litter and the number of dead or resorbed foetuses per litter did not differ from those of the controls. According to the authors, the reduced number of live foetuses was not clear evidence of embryo- or foetotoxicity. (Hardin et al. 1981).

New Zealand White rabbits were exposed by inhalation continuously (24 hours/day) on days 7-20 of gestation to 500 or 1000 mg/m^3. At the highest concentration, maternal toxic effects (decreased weight gain) and reduction in the number of foetuses due to abortion were observed (Ungváry & Tátrai 1985 – quoted from WHO 1996, IARC 2000). According to IARC (2000), the low concentration led to lower foetal weight in the female offspring.

In New Zealand White rabbits exposed to concentrations of up to 1610 ppm (about 7100 mg/m^3) for 4 weeks, no histopathological abnormalities in the testes were reported (Cragg et al. 1989 – quoted from ATSDR 1997).

4.5.2 Oral intake

Acute oral exposure to 500 or 1000 mg/kg ethylbenzene resulted in decreased peripheral hormone levels and may, according to the authors, block or delay the oestrus cycle in female rats during the dioestrus stage. Decreased levels of hormones, including luteinising hormone, progesterone, and 17 β-oestradiol, were accompanied by uterine changes, which consisted of increased stromal tissue with dense collagen bundles and reduced lumen. No dose response was noted. (Ungvary 1986 – quoted from ATSDR 1997).

4.5.3 Dermal contact

No data have been found.

4.6 Mutagenic and genotoxic effects

4.6.1 *In vitro* studies

Ethylbenzene has been found consistently to be non-mutagenic in bacteria, yeast and insects. It did not cause chromosomal aberrations in mammalian cells. It has been found inactive in inducing sister chromatid exchanges in Chinese hamster embryo cells. It was positive *in vitro* in Syrian hamster embryo cells and caused cell transformations in these cells at the highest concentration tested. (Florin et al. 1980, Nestmann et al. 1980, Dean et al. 1985, Zieger et al. 1992, NTP 1999, Leddy et al. 1995, Nestmann & Lee 1983, Gibson et al. 1997, Kerckaert et al. 1996 – all quoted from IARC 2000).

In the TK+/- test in mouse lymphoma cells, there was a slight effect (trifluorothymidine resistance) at 80 mg/L (McGregor et al. 1988 – quoted from WHO 1996, IARC 2000).

Ethylbenzene had a marginal effect on sister chromatid exchange in human lymphocytes *in vitro* at 10 mmol/L (Norppa & Vainio 1983 – quoted from WHO 1996).

4.6.2 *In vivo* studies

There was no increased incidence of micronuclei in the peripheral blood of mice exposed to ethylbenzene orally (NTP 1992 – quoted from WHO 1996; NTP 1999 – quoted from IARC 2000).

There was no increased incidence of micronuclei in bone marrow erythrocytes of male mice exposed intraperitoneally to ethylbenzene (2 doses of 650 mg /kg b.w.) (Mohtashamipur et al 1985 – quoted from IARC 2000).

No excess of chromosomal aberrations in bone marrow cells was seen in rats after up to 18 weeks of exposure (6 hours/day, 5 days/week) to 300 ppm of a xylene mixture containing 18.3% ethylbenzene (Donner et al. 1980 – quoted from WHO 1996).

Ethylbenzene did not cause an increase in the spontaneous recessive-lethal frequency in the Drosophila recessive-lethal test, whereas an increase was noted following exposure to 1300 mg/m^3 of a xylene mixture containing 18.3% ethylbenzene (Donner et al. 1980 – quoted from WHO 1996, A&H 1986).

4.7 Carcinogenic effects

4.7.1 Inhalation

4.7.1.1 Rat

Groups of 50 male and 50 female rats (F 344/N) were exposed to ethylbenzene by inhalation in whole-body exposure chambers at concentrations of 0, 75, 250 or 750 ppm (0, 330, 1100, or 3300 mg/m^3) for 6 hours per day, 5 days per week for 104 weeks. Survival was significantly decreased in males at 750 ppm. In male rats exposed to 750 ppm, the incidences of renal tubule adenoma and adenoma or carcinoma (combined) were significantly greater than the control incidences. Also the incidence of renal tubule hyperplasia and renal tubule proliferative lesions was significantly greater in 750 ppm males. The findings from an extended evaluation (step-section) of the kidneys revealed a significant increase in the incidences of renal tubule adenoma and hyperplasia in 750 ppm males and females; the incidence of renal tubule adenoma or carcinoma (combined) was significantly increased in 750 males. The incidence of interstitial cell adenoma in the testis of 750 ppm males was significantly greater than that in the control group and slightly exceeded the historical control range for inhalation studies; the incidence of bilateral testicular adenoma was also significantly increased in 750 ppm males. NTP concluded "Under the conditions of this 2-year inhalation study, there was clear evidence of carcinogenic activity of ethylbenzene in male F344/N rats based on increased incidences of renal tubule neoplasms. The incidences of testicular adenoma were also increased. There was some evidence of carcinogenic activity of ethylbenzene

in female F344/N rats based on increased incidences of renal tubule adenomas." (NTP 1999). Non-neoplastic findings are addressed in section 4.2.1.1.

4.7.1.2 Mouse

Groups of 50 male and 50 female $B5C3F_1$ mice were exposed to ethylbenzene by inhalation in whole-body exposure chambers at concentrations of 0, 75, 250 or 750 ppm (0, 330, 1100, or 3300 mg/m^3) for 6 hours per day, 5 days per week for 103 weeks. Survival of the exposed and control groups were similar. Incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) in males increased with a positive trend and were significantly greater in 750 ppm males than those of the control group but were within the NTP historical control ranges. In addition, the incidence of alveolar epithelial metaplasia in 750 ppm males was significantly increased. In 750 females, the incidence of alveolar/bronchiolar adenoma was greater than that of the control group; the difference was not significant, but exceeded the historical control range. The incidences of hepatocellular adenoma and adenoma or carcinoma (combined) in females occurred with a positive trend and were significantly greater in 750 ppm females than those in the control group, but were within the NTP historical control ranges. In addition to liver neoplasms, the incidence of eosinophilic foci was significantly increased in 750 ppm females. NTP concluded "Under the conditions of this 2-year inhalation study, there was some evidence of carcinogenic activity of ethylbenzene in male $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar neoplasms. There was some evidence of carcinogenic activity of ethylbenzene in female $B6C3F_1$ mice based on increased incidences of hepatocellular neoplasms. (NTP 1999). Non-neoplastic effects are addressed in section 4.2.1.2.

4.7.2 Oral intake

In a carcinogenicity study, groups of Sprague Dawley rats (40 of each sex) were exposed to 500 mg/kg b.w. ethylbenzene by gavage (in olive oil), 4 or 5 days per week for 104 weeks. Survival was affected by treatment – recorded as an "intermediate reduction in animals" in both males and females. The first malignant tumour, a nephroblastoma, was observed after 33 weeks. The total number of malignant tumours was 31/77 in the exposed group at 33 weeks compared with an incidence of 23/94 in the control group. The authors concluded that ethylbenzene caused an increase in the incidence of total malignant tumours, although there was no increase in the incidence of any specific type of tumour. (Maltoni et al. 1985 – quoted from WHO 1996).

In another study, groups of 50 male and 50 female Sprague Dawley rats were administered 0 or 800 mg/kg b.w. ethylbenzene by stomach tube (in olive oil) 4 days per week for 104 weeks. This experiment was terminated at 123 weeks. Survival was affected by treatment – recorded as an "intermediate reduction in animals" in both males and females. In exposed animals, there was an increase in the incidence of tumours of the nasal cavity (type unspecified – 2% incidence in females *versus* 0% in controls), neuroesthesioepitheliomas (6% in males *versus* 0% in controls), and a borderline increase in oral cavity cancer (6% in females *versus* 2% in controls). (Maltoni et al. 1997 – quoted from IARC 2000).
According to IARC (2000), the lack of details with respect to number of animals with specific tumours, adjustments for survival, historical control data, and statistical analyses were noted for both studies.

4.7.3 Dermal contact

No data have been found.

5 Regulations

5.1 Ambient air

Denmark (C-value):	0.5 mg/m^3 (MST 2002).
WHO:	22 mg/m^3, a tentative guidance value based on a NOEL of 2150 mg/m^3 for increased liver weight in a 13-week study with rats and an uncertainty factor of 100 consisting of a factor of 10 for interspecies variability, 5 for intraspecies variability (effects seen in males only), and 2 for lack of chronic toxicity data. The NOAEL would be higher than 4300 mg/m^3 (highest concentration used) since the increase in liver weight was not associated with any histopathological findings. (WHO 1996).
US-EPA:	-

5.2 Drinking water

Denmark:	1 µg/l (alkyl benzenes) (MM 2001).
WHO:	300 µg/L, based on a NOAEL of 136 mg/kg b.w./day for hepatotoxicity and nephrotoxicity observed in a limited 6-month study in rats (administration 5 days/week), an uncertainty factor of 1000 consisting of 100 for intra- and interspecies variation and 10 for the limited database and short duration of the study, and allocating 10% of TDI to drinking water. (WHO 1996a).
US-EPA:	-

5.3 Soil

Denmark:	-
The Netherlands:	-

5.4 Occupational Exposure Limits

Denmark:	50 ppm (217 mg/m^3), Notation K (At 2005).
ACGIH:	100 ppm (435 mg/m^3), based on prevention of disagreeable irritation (ACGIH 1991).
Germany:	-

5.5 Classification

Ethylbenzene is classified for flammability (F;R11 – highly flammable) and for acute toxic effects (Xn;R20 – harmful by inhalation) (MM 2002).

5.6 IARC

Ethylbenzene is classified as possibly carcinogenic to humans (Group 2B). There is inadequate evidence in humans for the carcinogenicity of ethylbenzene. There is sufficient evidence in experimental animals for the carcinogenicity of ethylbenzene. (IARC 2000).

5.7 US-EPA

Inhalation reference concentration (RfC) of 1 mg/m^3, based on a NOAEC of 434 mg/m^3 for developmental toxicity in rats and rabbits and an uncertainty factor of 300 consisting of a factor of 10 to protect unusually sensitive individuals, 3 to adjust for interspecies conversion and 10 to adjust for the absence of multigeneration reproductive and chronic studies (IRIS 2004).

Oral reference dose (RfD) of 0.1 mg/kg b.w./day, based on a NOEL of 136 mg/kg b.w./day for liver and kidney toxicity observed in a 182-day study in rats (administration 5 days/week), an uncertainty factor of 1000 consisting of 100 for intra- and interspecies variation and 10 for extrapolation of a subchronic effect level to its chronic equivalent (IRIS 2004).

Regarding evidence for human carcinogenicity, ethylbenzene is classified in group D; not classifiable as to human carcinogenicity due to lack of animal bioassays and human studies (IRIS 2004).

6 Summary and evaluation

6.1 Description

Ethylbenzene is a colourless liquid with a (sweet) gasoline-like odour. The solubility in water is 152 mg/L and it is miscible with organic solvents. The reported vapour pressure is between 9 and 15 mmHg at 20°C. Odour thresholds of 0.27-0.4, about 2, and 10 mg/m^3 have been reported.

6.2 Environment

From physico-chemical properties it can be predicted that, when ethylbenzene is released into air, the major part remains in the atmosphere and only small amounts are found in water, soil and sediment.

Ethylbenzene levels in air at rural sites were generally less than 2 µg/m^3 while the mean levels of ethylbenzene at urban sites ranged from 0.74 to 100 µg/m^3.

Ethylbenzene is being degraded in the atmosphere primarily by photo-oxidation. The atmospheric oxidation of ethylbenzene is rapid and proceeds via free-radical chain processes. An atmospheric half-life of around 15 hours has been reported.

Ethylbenzene has an octanol-water partition coefficient of 3.13, which indicates that bioaccumulation of ethylbenzene could take place. However, the available data indicate that biomagnification of ethylbenzene through the aquatic food chain is unlikely.

6.3 Human exposure

The general human population is predominantly exposed to ethylbenzene via inhalation associated with the use of self service gasoline pumps or while driving a gasoline-powered motor vehicle especially in high traffic areas or in tunnels. In indoor air, tobacco smoke provides a general source of exposure to ethylbenzene. Exposure from food or drinking water is not expected to be a significant source of ethylbenzene.

6.4 Toxicokinetics

Ethylbenzene vapour is rapidly and well absorbed via inhalation. No data regarding absorption of ethylbenzene in humans are available, but data indicate that 50–65% of an inhaled concentration is retained in the lungs. In rats, about 44% was absorbed and blood levels reached maximum within 4-6 hours after inhalation. More than 80% of an orally administered dose to rats was excreted in urine within 48 hours indicating a rapid and complete absorption of ethylbenzene following oral administration.
In humans, skin appears not to be a relevant route of entry into the body for ethylbenzene vapour.

Following absorption, ethylbenzene is distributed in the body. In humans, a correlation was found between the amount of body fat and the amount of ethylbenzene taken up by volunteers during exposure by inhalation. In rats ethylbenzene was detected in brain, liver, kidney and adipose tissues with a considerable retention in the latter.

The metabolic pathways of ethylbenzene have been proposed for humans and for rats based on urinary metabolites, see Figure 1. The main metabolic pathway is oxidation of the side chain by the microsomal cytochrome P-450 enzyme system, both in humans and in animals. In humans, the main metabolites are mandelic (~70%) and phenylglyoxylic (~20 %) acids following exposure by inhalation. In several animal species, the metabolic transformation proceeds to benzoic acid, leading to excretion of hippuric acid after conjugation with glycine. This conjugate is generally one of the main urinary metabolites, together with mandelic acid, in rats and dogs. Hydroxylation of the aromatic nucleus is a minor pathway.

In humans exposed by inhalation, ethylbenzene is mainly excreted in the urine in the form of metabolites (up to about 90%). Only up to about 5% of retained ethylbenzene is estimated to be exhaled without transformation. The elimination half-lives of ethylbenzene in exhaled air and urine have been estimated to be 0.5-3 and 8 hours, respectively. Male rats exposed to ^{14}C-ring-labelled ethylbenzene (1000 mg/m^3) for 6 hours excreted 82% of the radioactivity in the urine, 8.2% in expired air (0.03% as CO_2) and 0.7% in faeces. After 42 hours, 0.2% remained in the tissues.

6.5 Human toxicity

6.5.1 Single dose toxicity

Studies from 1930s have reported that exposure to 21500 mg/m^3 ethylbenzene for a few seconds resulted in intolerable irritation of nose, eyes and throat and 4300 mg/m^3 for a few seconds initially gave eye irritation, which diminished after a few minutes of exposure. In a more recent study from 1970, concentrations above 430 mg/m^3 for 8 hours resulted in complaints of fatigue, sleepiness, headache, and irritation of the eyes and respiratory tract.

A dermal maximization test conducted on 25 volunteers with 10% ethylbenzene in petrolatum produced no skin sensitisation.

6.5.2 Repeated dose toxicity

Among 200 chemical workers engaged in ethylbenzene production, no tumours had been reported over a 10 years period, whereas a 3 times the national (Czechoslovakian) cancer incidence had been stated to occur among other chemical workers of comparable age and length of employment in the same industrial complex. No precise figures were given, and the age of the workers and the length of follow-up were not sufficient for a proper evaluation of cancer risk in relation to exposure to ethylbenzene. None of the workers showed changes in haematological parameters or serum enzyme levels as measure of liver function effects over a 20 year period. Co-exposure to benzene was also reported.

No data have been found regarding toxicity to reproduction or regarding mutagenic and genotoxic effects.

6.6 Animal toxicity

6.6.1 Single dose toxicity

An LC_{50} for rat of 17200 mg/m^3 (4 hours) has been reported. The minimum narcotic concentration reported in male rats is 9370 mg/m^3. Male mice exposed to 8800 mg/m^3 for 20 minutes showed changes in a number of functional observations (e.g., changed posture, disturbed gait, decreased mobility).

Oral LD_{50}-values in rats of 3500 and 4700 mg/kg b.w. have been reported, and a dermal LD_{50}-value of 77400 mg/kg b.w. in rabbits.

Undiluted ethylbenzene produced moderate skin irritation when applied to uncovered rabbit skin and conjunctival irritation in rabbit eye with no or reversible corneal injury reported.

Eye and nasal irritation was recorded in guinea-pigs exposed by inhalation to 4300 mg/m^3 for 8 minutes and to 8600 mg/m^3 for 1 minute. Male mice exposed to 8800 mg/m^3 for 20 minutes showed lachrymation and palpebral ocular closure. A respiratory depression of 50% (RD_{50}) was recorded in male mice at 17900 mg/m^3 for 30 minutes.

6.6.2 Repeated dose toxicity

A number of inhalation studies have been performed with rats, mice, rabbits, guinea-pigs, and monkeys with exposure durations ranging from a few days and up to 2 years.

Increased liver weight has been reported for rats and mice (from about 1640 mg/m^3 for 4 weeks, from about 3300 mg/m^3 for 13 weeks, and in rats from 1720 mg/m^3 for 186 days), and for guinea-pigs and monkeys (at about 2580 mg/m^3 for 186 days). Slight histopathological changes in the liver (cloudy swelling) were noted in one study of rats following exposure to about 5400 mg/m^3 for 214 days and about 9500 mg/m^3 for 144 days; no treatment-related liver effects were reported in the 2-year NTP rat study at exposure concentrations up to about 3300 mg/m^3. In the 2-year NTP study with mice, the incidences of liver changes were significantly increased in both sexes at 3300 mg/m^3 (females: eosinophilic foci in the liver; males: syncytial alteration of hepatocytes, hepatocellular hypertrophy, and hepatocyte necrosis – minimal to mild in severity), and in male mice at 1100 mg/m^3 (syncytial alteration of hepatocytes).

Increased kidney weight has been reported for rats (from about 3300 mg/m^3 for 13 weeks, and from 1720 mg/m^3 for 186 days), and for mice (at 4400 mg/m^3 for 13 weeks). Slight histopathological changes in the kidney (cloudy swelling) were noted in one study of rats following exposure to about 5400 mg/m^3 for 214 days and about 9500 mg/m^3 for 144 days. In the 2-year NTP study with rats, the incidences of renal hyperplasia were significantly increased in both sexes at 3300 mg/m^3, and of renal tubule proliferative lesions in males at 3300 mg/m^3, but not at 1100 mg/m^3. The severities of nephropathy were significantly increased in all exposed females and in males at 3300 mg/m^3; the nephropathy was more severe in males (graded minimal to moderate in controls and at 25 and 250 ppm, and moderate to marked at 750 ppm) than in females (graded minimal to mild in controls and in exposed animals) and involved most of the renal parenchyma.

In the 2-year NTP study with mice, the incidence of alveolar epithelial metaplasia increased in males with increasing exposure concentration and was significantly greater at 3300 mg/m^3 than that of controls. The incidences of hyperplasia of the pituitary gland were significantly increased in females from 1100 mg/m^3 and the incidences of thyroid gland follicular cell hyperplasia were significantly increased in both sexes at 3300 mg/m^3.

Histopathological effects in the testes, described as degeneration of the germinal epithelium, have been reported for rabbits and monkeys exposed to 2580 mg/m^3 for 186 days. In the 2-year NTP study with rats, the incidence of interstitial cell hyperplasia was significantly decreased in male rats at 3300 mg/m^3.

In one recent study of rats, exposure by inhalation at concentrations from about 1700 mg/m^3 for 5 days resulted in ototoxic effects in form of increased auditory thresholds and outer hair cell loss in the cochlea in the mid-frequency region.

In one study of rats, slight increases in liver and kidney weights and histopathological changes in liver parenchyma cells and in epithelium of the kidney tubes were reported after oral administration of ethylbenzene by gavage (5 days a week) for 6 months at dose levels from about 410 mg/kg to rats; the NOAEL was 136 mg/kg b.w./day.

Application of undiluted ethylbenzene to shaved abdominal skin of rabbits up to 20 times during a 4-week period resulted in irritation as evidenced by erythema and oedema with superficial necrosis and exfoliation of large patches of the skin.

6.6.3 Toxicity to reproduction

No studies regarding fertility and reproductive performance have been found.

Developmental toxicity studies in rats, mice and rabbits indicate that exposure of dams to ethylbenzene by inhalation can result in developmental toxicity in form of skeletal growth retardation, increased incidence of extra ribs, and reduced foetal growth at high concentrations (rats: from 2400 mg/m^3; mice: at 500 mg/m^3; and rabbits: from 400-500 mg/m^3) at which maternal effects also were noted. There was no indication of a teratogenic effect of ethylbenzene in rats, mice, or rabbits.

No changes in sperm or vaginal cytology were observed in rats or mice exposed to ethylbenzene at concentrations of up to 4400 mg/m^3 for 6 hours per day, 5 days per week, for 13 weeks; and no adverse effects on the reproductive tissues were observed in mice exposed at concentrations up to about 3300 mg/m^3 for 2 years.

No histopathological abnormalities were noted in the testes of rats and mice exposed to ethylbenzene at concentrations up to about 3400 mg/m^3 or of rabbits up to about 7100 mg/m^3 for 4 weeks.

6.6.4 Mutagenic and genotoxic effects

Ethylbenzene was not mutagenic in *in vitro* test with bacteria, yeast and insects. It did not cause chromosomal aberrations in mammalian cells or sister chromatid exchanges in Chinese hamster embryo cells, but had a marginal effect on sister chromatid exchange in cultured human lymphocytes at a relatively high concentration (10 mmol/L). It caused cell transformation in Syrian hamster embryo cells *in vitro* at the highest concentration tested.

When tested in *in vivo* tests, ethylbenzene did not increase the incidence of micronuclei in peripheral blood cells of mice exposed orally or in bone marrow erythrocytes of male mice exposed intraperitoneally. No excess of chromosomal aberrations was observed in bone marrow cells of mice after inhalation exposure for up to 18 weeks to a xylene mixture containing 18.3% ethylbenzene.

6.6.5 Carcinogenic effects

In the 2-year NTP inhalation study with rats, the incidences of renal tubule adenoma and adenoma or carcinoma (combined), and of interstitial cell adenoma in the testis and of bilateral testicular adenoma were significantly greater in males at 3300 mg/m^3 than the control incidences. For females, the incidences of renal tubule adenoma were significantly greater at 3300 mg/m^3.

In the 2-year NTP inhalation study with mice, the incidences of alveolar/ bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) were significantly greater in males at 3300 mg/m^3 than those of the control group but were within the NTP historical control ranges. In females, the incidence of alveolar/bronchiolar adenoma was greater at 3300 mg/m^3 than that of the control group; the difference was not significant, but exceeded the historical control range. The incidences of hepatocellular adenoma and adenoma or carcinoma (combined) were significantly greater in females at 3300 mg/m^3 than those in the control group, but were within the NTP historical control ranges.

In one oral carcinogenicity study with rats, the authors concluded that ethylbenzene caused an increase in the incidence of total malignant tumours, although there was no increase in the incidence of any specific type of tumour. In a later study performed by the same authors, there was an increase in the incidence of tumours of the nasal cavity, neuroesthesioepitheliomas, and a borderline increase in oral cavity cancer.

6.7 Evaluation

Ethylbenzene vapour is rapidly and well absorbed via inhalation and distributed widely in the body. One study in rats has reported that about 44% was absorbed. It is virtually completely metabolised to a range of metabolites, which are excreted predominantly in the urine.

Limited data are available to evaluate the toxic effects of ethylbenzene in humans. The effects observed relate to depression of the central nervous system (CNS) and to irritation of mucous membranes and eyes and have been reported to occur at concentrations above 430 mg/m^3 for 8 hours.

The acute toxicity of ethylbenzene in experimental animals is low with a reported LC_{50} for rat of 17200 mg/m^3 (4 hours). Symptoms of CNS depression have been reported for mice exposed to 8800 mg/m^3 for 20 minutes; the minimum narcotic concentration reported for rats is 9370 mg/m^3.
Eye and nasal irritation has been recorded at relatively high exposure concentrations (from about 4300 mg/m^3) and the RD_{50} recorded in male mice was 17900 mg/m^3 following exposure for 30 minutes.

There are no data available in order to evaluate the skin sensitising potential except for a dermal maximization test conducted on 25 volunteers in which 10% ethylbenzene in petrolatum produced no skin sensitisation.

A number of repeated inhalation studies have been performed with rats, mice, rabbits, guinea-pigs, and monkeys with exposure durations ranging from a few days and up to 2 years, see Table 6.7. Effects were observed in the liver, kidneys, lungs, pituitary gland, thyroid gland, and testes.
In the recent 2-year well-conducted NTP studies with rats and mice (NTP 1999), histopathological changes were observed in the kidney of rats and in the liver, lung, thyroid and pituitary gland of mice; organ weights were not evaluated in these studies. In mice a NOAEC of 330 mg/m^3 is considered based on histopathological changes in the pituitary gland of females and in the liver of males from 1100 mg/m^3. In rats, a NOAEC cannot be established as the severities of nephropathy were significantly increased in all exposed female rats from 330 mg/m^3 (the lowest concentration in the study); the severities of nephropathy were also increased in exposed males but only significantly at 3300 mg/m^3. The nephropathy was more severe in males (graded minimal to moderate in controls and at 330 and 1100 mg/m^3, and moderate to marked at 3300 mg/m^3) than in females (graded minimal to mild in controls and in exposed animals).
In the 13-week NTP studies with rats, increased relative liver and kidney weights were observed in males at exposure concentrations from 3300 mg/m^3; the NOAEC was 2200 mg/m^3.
In one very recent study of rats, ototoxic effects were observed following exposure concentrations from 1765 mg/m^3 for 5 days; the NOAEC was approximately 1320 mg/m^3.

No studies regarding fertility and reproductive performance have been found. In rats, mice and rabbits, developmental retardation and an increased incidence of skeletal variations has been reported after inhalation exposure during pregnancy at concentrations (rats: from 2400 mg/m^3; mice: at 500 mg/m^3; and rabbits: from 400-500 mg/m^3) at which maternal effects also were noted. In a study with rabbits, a significantly reduced number of live pups per litter was observed following exposure to 440 and 4400 mg/m^3 (6-7 hours/day on gestation days 1 to 24); however, as the number of implantations per litter and the number of dead or resorbed foetuses per litter did not differ from those of the controls, the authors concluded that the reduced number of live foetuses was not clear evidence of embryo- or foetotoxicity. No further details are available in the reference and thus, the relevance of this finding cannot be evaluated. There were no indications of a teratogenic effect of ethylbenzene. No changes in sperm motility or vaginal cytology were observed in rats or mice exposed to ethylbenzene at concentrations of up to 4400 mg/m^3 for 13 weeks; and no adverse effects on the reproductive tissues were observed in mice exposed at concentrations up to about 3300 mg/m^3 for 2 years.

Ethylbenzene has been tested for carcinogenicity in the 2-year NTP inhalation studies conducted with rats and mice. In mice, increased incidences of lung adenomas in males and of liver adenomas in females were observed. In male rats, increased incidences of renal tubule adenomas and carcinomas and in female rats of renal adenomas were observed. The incidences were only increased at the highest exposure concentration of 3300 mg/m^3; the NOAEC was 1100 mg/m^3.
The available data indicate that ethylbenzene is not a mutagenic or genotoxic substance.

Table 6.7. Repeated dose toxicity studies on ethylbenzene

Species / strain	Duration / exposure levels (mg/m^3)	Effects (mg/m^3)	NOAEC (mg/m^3)	LOAEC (mg/m^3)	Reference
Rat	5 days 8 hrs/day 0, 1323, 1765, 2425	2425: distortion product otoacoustic emissions amplitude growth with stimulus level affected ≥ 1765: ↑ auditory thresholds, outer hair cell loss	1323	1765	Cappaert et al. (2000)
F344 rat 5/sex/group	4 weeks 6 hrs/d, 5 d/wk 0, 426, 1643, 3363	3363: ↑ leucocyte counts, platelet counts (♂) ≥ 1643: lachrymation, salivation, ↑ w liver	426	1643	Cragg et al. (1989)
F344 rat 10/sex/group	13 weeks 6 hrs/d, 5 d/wk 0, 440, 1100, 2200, 3300, 4400	≥ 3300: ↑ w liver, kidney (♂, abs/rel) ≥ 2200: ↑ w liver, kidney (♀, abs)	2200	3300	NTP (1992)
Wistar rat 5/males/group	2, 5, 9 or 16 weeks 6 hrs/d, 5 d/wk 0, 215, 1290, 2580	2580: hist liver, ↑ enz liver, kidney	1290	2580	Elovaara et al. (1985)
Wistar rat 10-25/sex/group	7 hrs/d, 5 d/wk 0, 1720, 2580 (186 days) 5375 (214 days) 9460 (144 days)	≥ 5375: hist liver, kidney ≥ 1720: ↑ w liver, kidney	-	1720	Wolf et al. (1956)
F344 rat 50/sex/group	104 weeks 6 hrs/d, 5 d/wk 0, 330, 1100, 3300	3300: hist kidney (♂) ≥ 330: hist kidney (♀)	-	330	NTP (1999)
B6C3F1 mouse 5/sex/group	4 weeks 6 hrs/d, 5 d/wk 0, 426, 1643, 3363	≥ 1643: ↑ w liver (♀)	426	1643	Cragg et al. (1989)
B6C3F1 mouse 10/sex/group	13 weeks 6 hrs/d, 5 d/wk 0, 440, 1100, 2200, 3300, 4400	4400: ↑ w kidney (♀, rel) ≥ 3300: ↑ w liver (abs)	3300	4400	NTP (1992)
B6C3F1 mouse 50/sex/group	104 weeks 6 hrs/d, 5 d/wk 0, 330, 1100, 3300	3300: hist lung (♂), hist liver (♀), hist thyroid 1100: hist liver (♂), hist pituitary (♀)	330	1100	NTP (1999)
New Zealand white rabbit 5/sex/group	4 weeks 6 hrs/d, 5 d/wk 0, 1643, 3363, 6923	-	≥ 6923	-	Cragg et al. (1989)
Rabbit 1-2/group	7 hrs/d, 5 d/wk 0, 1720, 2580 (186 days) 5375 (214 days)	2580: hist testes	-	-	Wolf et al. (1956)
Guinea-pig 5-10/group	7 hrs/d, 5 d/wk 0, 1720, 2580 (186 days) 5375 (214 days)	5375: ↓ bw 2580: ↑ w liver	-	-	Wolf et al. (1956)
Rhesus monkey 1-2/group	7 hrs/d, 5 d/wk 0, 1720, 2580 (186 days)	2580: ↑ w liver	-	-	Wolf et al. (1956)

↓: reduced
↑: increased
♂ / ♀: male / female
bw / bwg: body weight / body weight gain
enz: enzyme activity / activities or levels
hist: histopathological changes
w: weight
abs / rel: absolute / relative

The potential factors underlying the carcinogenic activity of ethylbenzene have been studied in both rats and mice. For renal tumours in rats, one study has suggested a mode of action dependent upon increased cell proliferation and altered population dynamics in male rat kidney; a similar response in the kidneys of female rats appears to require a longer exposure period than was employed. However, another study concluded, based on the close association of atypical tubule hyperplasia and renal tumours with chronic progressive nephropathy, that chemically induced exacerbation of chronic progressive nephropathy was the mode of action underlying the development of renal tumours, a pathway that is considered to have no relevance for extrapolation to humans. For liver and lung tumours in mice, one study has suggested a mode of action dependent upon increased cell proliferation and altered population dynamics in mouse liver and lungs.
Overall, based on the available data, the carcinogenic activity of ethylbenzene is considered to be due to a non-genotoxic mode of action probably dependent upon increased cell proliferation and altered population dynamics in the target organs. Consequently, the NOAEC established for histopathological changes in these organs is considered to protect against development of tumours in these organs as well.

6.7.1 Critical effect and NOAEC

Based on the available data, the critical effect in humans following exposure to ethylbenzene is considered to be the effects observed in the liver, kidneys, lungs, pituitary gland, and thyroid gland in the 2-year NTP studies as well as the ototoxic effects observed after only 5 days of inhalation exposure. Furthermore, ethylbenzene has a potential for exerting irritative effects following inhalation of vapours or by direct contact.

For the purpose of estimating a health based quality criterion for ethylbenzene in ambient air, 330 mg/m^3 is considered as a LOAEC for histopathological changes in the kidney based on the increased severities of nephropathy (graded minimal to mild) observed in female rats from 330 mg/m^3 (NTP 1999). This LOAEC is considered to protect against the development of tumours in the target organs as well, as the carcinogenic activity of ethylbenzene is considered to be due to a non-genotoxic mode of action probably dependent upon increased cell proliferation and altered population dynamics in the target organs. The LOAEC is also considered to protect against the developmental toxicity observed in rats, mice and rabbits at slightly higher exposure concentrations at which maternal toxicity was observed as well, and against the irritative effects of ethylbenzene reported to occur in humans at concentrations above 430 mg/m^3 for 8 hours and in experimental animals at considerably higher concentrations.

6.7.2 Allocation

The general population is exposed to ethylbenzene via inhalation of ambient and indoor air, intake of contaminated foodstuffs and drinking water, and from use of consumer products.

In ambient air, levels of ethylbenzene in rural areas are generally less than 2 µg/m^3, while those on busy urban streets are much higher with levels of up to about 100 µg/m^3. Even higher levels have been reported in the surroundings of gasoline stations and near to the exhaust of a stationary idling vehicle. Assuming that an adult person weighing 70 kg inhales 20 m^3/day and 100% absorption of

ethylbenzene following inhalation, the systemic dose from inhalation of ambient air in rural areas will be around 0.6 µg/kg b.w./day, and up to around 29 µg/kg b.w./day in urban sites with heavy traffic.

Indoor air levels of ethylbenzene are usually higher than levels in ambient air. Indoor air concentrations of ethylbenzene in homes with smokers were 8.3 µg/m^3 in the fall and winter, significantly higher than those in homes without smokers, 5.1 µg/m^3; the levels of ethylbenzene during the spring and summer in homes with smokers and in homes without smokers were the same, 3.5 µg/m^3. Assuming that an adult person weighing 70 kg inhales 20 m^3/day and 100% absorption of ethylbenzene following inhalation, the systemic dose from inhalation of indoor air in homes without smokers may be up to around 1.5 µg/kg b.w./day, and up to around 2.4 µg/kg b.w./day in homes with smokers.

Levels of ethylbenzene measured in dried legumes ranged from 5 to 13 µg/kg, and in fish up to about 100 µg/kg. Assuming as a worst case situation that an adult person weighing 70 kg eats 1 kg of either fish containing 100 µg/kg of ethylbenzene or 1 kg of legumes containing 13 µg/kg of ethylbenzene and 100% absorption of ethylbenzene following oral intake, the systemic dose from intake of fish may be up to around 1.4 µg/kg b.w./day, and from dried legumes up to around 0.2 µg/kg b.w./day.

Concentrations in uncontaminated groundwater are generally less than 0.1 µg/L. Assuming that an adult person weighing 70 kg drinks 2 litres of drinking water per day and 100% absorption of ethylbenzene following oral intake, the systemic dose from intake of drinking water will generally be less than 0.003 µg/kg b.w./day.

Exposure to ethylbenzene from use in consumer products cannot be estimated based on the available data but is considered to be negligible.

Based on these considerations, the general population is predominantly exposed to ethylbenzene via inhalation of ambient air at urban sites with heavy traffic, and in the surroundings of gasoline stations and vehicles exhausts. Allocation of a certain fraction from other environmental media is therefore not warranted, i.e., the contribution of ethylbenzene from ambient air is considered as being 100%.

7 Quality criterion in air

The quality criterion in air (QC_{air}) is calculated based on the LOAEC of 330 mg/m^3 for histopathological changes (increased severities of nephropathy, graded minimal to mild) in the kidney of female rats exposed for 6 hours/day, 5 days/week for 2 years (NTP 1999). The LOAEC is adjusted to a continuous LOAEC of 59 mg/m^3.

$$QC_{air} = \frac{LOAEC}{UF_I * UF_{II} * UF_{III}} = \frac{59\ mg/m^3}{10 * 10 * 3}$$

$$= 0.2\ mg/m^3$$

The uncertainty factor UF_I accounting for interspecies variability is set to 10 assuming that humans are more sensitive than animals. The UF_{II} accounting for intraspecies variability is set to 10 reflecting the range in biological sensitivity within the human population. The UF_{III} is set to 3 because of using a LOAEC in stead of a NOAEC; a factor of 3 is considered sufficient as the only effect noted at the LOAEC (increased severities of nephropathy in female rats) was graded as being minimal to mild and is thus considered as being a rather conservative LOAEC and because the LOAEC is established based on 2 well-performed chronic toxicity studies using the relevant route of exposure, i.e., inhalation.

A quality criterion of 0.2 mg/m^3 has been calculated. A C-value of 0.2 mg/m^3 and placing in Main Group 2 is proposed.

For ethylbenzene, low odour thresholds in air ranging from 0.27-0.4 to 10 mg/m^3 have been reported. The proposed C-value of 0.2 mg/m^3 is considered to protect most individuals of the general population from experiencing adverse odour nuisance from ethylbenzene in the ambient air.

The C-value at present for ethylbenzene is 0.5 mg/m^3 and ethylbenzene is placed in Main Group 2 (MST 2002). A C-value of 0.2 mg/m^3 and placing in Main Group 2 is proposed.

7.1.1 C-value

0.2 mg/m^3, Main Group 2.

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Evaluation of health hazards by exposure to Ethylbenzene and proposal of a health-based quality criterion for ambient air

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to ethylbenzene. This resulted in 2006 in the present report which includes a health-based quality criterion for the substance in ambient air

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