RISK ASSESSMENT

Ethylbenzene

CAS-No.: 100-41-4

EINECS-No.: 202-849-4

Draft of November 2008

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Information on the rapporteur
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0 OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT

CAS No. 100-41-4

EINECS No. 202-849-4

IUPAC Name Ethylbenzene

Overall results of the risk assessment:

( ) i) There is need for further information and/or testing

(x) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(x) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Summary of conclusions:

Environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (ii) applies to

all production sites as well as all production and processing sites to *Surface water*, *Waste water treatment plants* and the *Terrestrial compartment*. It also applies to biotic effects of ethylbenzene in the *Atmosphere*.

Conclusion (iii) applies to

Atmosphere (indirect effects of ethylbenzene)

Conclusion (iii) applies to the contribution of the commercial product ethylbenzene to the formation of ozone. In the context of the consideration of which risk reduction measures that would the most appropriate, it is recommended that under the relevant Air Quality Directives a specific in-depth evaluation be performed. Such an evaluation should focus on the contribu-

tion of isolated as well as non-isolated ethylbenzene to the complex issue of ozone and smog formation and the resulting impact on air quality.

Human Health

Workers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Conclusion (iii) applies to dermal and combined exposure of scenario 2 (use of paints, lacquers, inks containing 20% ethylbenzene) after repeated dose toxicity and regarding developmental toxicity. On the background of systemic effects after repeated exposure, dermal exposure should be controlled to levels in the range of 1.7 mg/kg/day or 120 mg/person/day (critical exposure level for systemic effects of repeated dose toxicity). If the exposure is reduced to this level, dermal risks from other endpoints, as developmental toxicity would similary and effectively be mitigated too.
Concerning inhalation exposure, the critical exposure level is 9.3 mg/m^3. The exposure values of scenario 1 (production and processing) and scenario 2 (use of paints, lacquers, inks containing 20% ethylbenzene) are below this value, thus reaching no concern.

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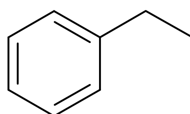
1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 100-41-4
EINECS Number: 202-849-4
IUPAC Name: Ethylbenzene

Molecular formula: $C_8 H_{10}$

Structural formula:



Molecular weight: 106 g/mol

Synonyms: Ethylbenzol

Benzene, ethyl-

Phenylethane

.alpha.-Methyltoluene

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1.2 PURITY/IMPURITIES, ADDITIVES

The purity of Ethylbenzene was determined to be > 99.5 % by gas chromatography.

The following impurities have been identified and analyzed:

compound	**CAS-No.**
Benzene	71-43-2
Toluene	108-88-3
p-Xylene	106-42-3
m-Xylene	108-38-3
o-Xylene	95-47-6

Cumene	98-82-8
n-Propylbenzene	103-65-1
m-Ethyltoluene	620-14-4
p-Ethyltoluene	622-96-8
o-Ethyltoluene	611-14-3
tert-Butylbenzene	98-06-6
sec-Butylbenzene	135-98-8
Styrene	100-42-5
m-Diethylbenzene	141-93-5
p-Diethylbenzene	105-05-5
o-Diethylbenzene	135-01-3
Benzaldehyde	100-52-7
Acetophenone	98-86-2
Cyclohexylmethane	108-87-2
Ethylcyclohexane	1678-91-7
Methylbenzylalcohols	
Napthenes	
aliphatic compounds	
Unknowns	

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1.3 PHYSICO-CHEMICAL PROPERTIES

Vapour Pressure

The vapour pressure of a substance is defined as the saturation pressure above a solid or liquid substance. At the thermodynamic equilibrium, the vapour pressure of a pure substance is a function of the temperature only.

Vapour pressures in the range of 1 mm mercury column (1.333 hPa) at –9.8 °C to 760 mm (1013.25 hPa) mercury column at 136.2 °C are listed in the CRC Handbook of Chemistry and Physics (Lide, 1991-1992)

A value of 9.3 hPa at 20 °C is listed in the Auer Technikum (1988) which is a collection of common tables of several chemical compounds and is often used in laboratories. This value

has been used for further calculations. 16 hPa at 30 °C and 46 hPa at 50 °C are also mentioned in the Auer Technikum. These values are also in good agreement with the values mentioned in the CRC Handbook.

A value of 9.33 hPa at 20 °C is mentioned by Verschueren (1983) and Duve et al. (1976) which supports the value of the Auer Technikum (1988).

Moreover Verschueren (1983) mentions a value of 16 hPa at 30 °C. This value is in good agreement with the Auer Technikum and the CRC Handbook.

Water Solubility

The water solubility of a substance is specified by its saturation concentration in pure water at a certain temperature, preferably at 20 °C.

A water solubility of 160 mg/l at 25 °C is mentioned in the CRC Handbook of Chemistry and Physics (Lide, 1991-1992). This value is in very good agreement with other values and has been used for further calculations therefore.

A value of 152 mg/l at 20 °C is mentioned by Tewari et al. (1982) and Verschueren (1983) whereas the value of 186.7 mg/l at 25 °C has been mentioned by Crookes and Howe (1991).

Partition Coefficient

The partition coefficient (Pow) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two phase system consisting of two largely immiscible solvents. In the case of n-octanol and water:

$$\text{Pow} = \text{equilibrium } c_{\text{n-octanol}} : \text{equilibrium } c_{\text{water}}$$

The partition coefficient therefore is the quotient of two concentrations and is usually given in the form of its logarithm to base ten (logPow).

The logPow value at 25 °C was determined to be 3.13 by Tewari et al. (1982). This value was used for further calculations because the exact testing temperature was given. But nevertheless the value of 3.15 mentioned by Crookes and Howe (1991) and Verschueren (1983) correspond very well.

Table 1.1 **Summary of physico-chemical properties**

Property	Value	
Physical state	liquid at 25 °C	
Melting point	- 94.949 °C	Gerhartz (1987)
Boiling point (at 1013 hPa)	136.186 °C	Gerhartz (1987)
Relative density	0.8670 at 20 °C	Lide (1991-1992)
Vapour pressure	p(20 °C) = 9.3 hPa	Auer Technikum (1988)
Water solubility	160 mg/l at 25 °C	Lide (1991-1992)
Partition coefficient n-octanol/water (log value)	3.13 at 25 °C	Tewari et al. (1983)
Granulometry	not applicable (liquid)	
Conversion factors		
Flash point	23 °C	CHEMSAFE
Autoflammability	430 °C	CHEMSAFE
Flammability	highly flammable	CHEMSAFE
Explosive properties	not explosive	CHEMSAFE
Oxidizing properties	not oxidizing (structure)	
Viscosity		
Henry's constant	617 Pa m^3/mol	calculated
Surface tension	28.48 mN/m	Gerhartz (1987)
Index of refraction (nd)	1.49588 at 20 °C 1.49320 at 25 °C	Gerhartz (1987)

1.4 CLASSIFICATION

1.4.1 Current classification

- (Classification according to Annex I of the directive 67/548/EEC)

F R 11 Highly flammable

Harmful	R 20	Harmful by inhalation

1.4.2 Proposed classification

- (Classification according to Annex I)
  Xn, R 20

- (Proposal of the rapporteur)

The entry in Annex I of directive 67/548/EEC does not yet include all categories of danger and qualifying risk phrases that have to be assigned according to the data presented. It is therefore proposed to amend the entry. The substance should be classified and labelled **additionally** with:

R 36/37/38	Irritating to eyes, respiratory tract and to skin
R48/20	Harmful: Danger of serious damage to health by prolonged exposure through inhalation
R 65	Harmful: May cause lung damage if swallowed

Proposed classification based on GHS classification

H319	Eye Irrit.2
H335	STOT Single3
H315	Skin Irrit.2
H373	STOT Rep.2
H304	Asp.Tox.1

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

Ethylbenzene is naturally present in crude oil. Commercially it is mainly produced by alkylating benzene with ethene. Ethylbenzene is primarily used in the manufacture of styrene. Due to its use in fuels and solvents it is widely distributed in the environment.

2.1.1 Production processes

The majority of ethylbenzene (~ 90 %) is produced by catalytic reaction of benzene with ethene. This Friedel-Crafts-alkylation is carried out in a closed continuous system. No water is involved in the process; the benzene is dried prior to entering the reactor. Additionally to the commonly used aluminium chloride catalyst, often catalyst promoters like ethyl chloride or hydrogen chloride are present in the reactor.
As a variation to this liquid phase process the alkylation can take place in the vapour phase. In this case the reaction mixture is passed over a fixed bed catalyst, such as phosphoric acid or zeolite.
Several fractions are produced from the ethylbenzene reactor. These are either recirculated (excess benzene, light fraction of polyethylbenzene), passed on for further processing (toluene to lower olefins unit) or incinerated (light components, heavy fraction of polyethylbenzene). Ethylbenzene is further refined by closed continuous distillation. The solid waste (catalyst waste containing traces of ethylbenzene and waste clay) is treated by 3rd parties. (Shell NL, 2001)
Another production method is the fractionation of mixed xylene streams which is, however, employed to a much lesser extent. These streams occur in petroleum refineries during distillation of crude oil into petroleum products and contain ~ 80 % o-, m-, p-xylenes ("mixed xylene stream") and ~15 – 20 % ethylbenzene.

2.1.2 Production capacity

There are 9 producers in the EU; one company is a trader only. The production volume in the European Union can be estimated to be **~ 5.28 x 10^6 t/a**. No information on export from or import in the EU is available. The known volume to be processed is **~ 5.10 x 10^6 t/a**. The discrepancy to the production volume of 1.82 x 10^5 t/a might be due to inaccuracies from figures round up. A total market volume of ethylbenzene of **~ 5.28 x 10^6 t/a** is assumed for the risk assessment. The above figures are based on specific information given by industry in 2001-2004.

2.2 USES

2.2.1 Introduction

Ethylbenzene is primarily employed for the production of styrene. A small percentage is used in the production of other chemicals.

2.2.2 Scenarios

Table 2.1 and Table 2.2 give an overview over uses, quantities and the assigned ICs/UCs. **MC I** is allocated as main category (closed systems) for production and processing.

Table 2.1 **Quantity of ethylbenzene in different applications**

Application	Quantity used (tonnes/year)	Percentage of total use
Polymer/Chemical industry	5,253,600	99.5 %
Chemical industry	26,400	0.5 %

Table 2.2 **Use of ethylbenzene in Western Europe**

		IC	UC	%
Processing	Production of styrene	Chemical industry **(3)**	Intermediate **(33)**	99.5
	Production of other chemicals	Chemical industry: chemicals used in synthesis **(3)**	Intermediate **(33)**	0.5

2.2.2.1 Chemical industry - production of styrene

The majority of ethylbenzene is used for the production of styrene. The quantities given vary between 95 and 99.8 % of the total ethylbenzene volume (ECB, 2002). Some of the companies giving specific information on production and processing stated that 100 % of their produced ethylbenzene is processed to styrene. Since the exact percentage for the total tonnage is not known it is assumed that 99.5 % of the ethylbenzene produced is used as intermediate in the styrene production.

According to RAR Styrene (EU, 2000) there are two routes to manufacture styrene from ethylbenzene. Firstly, ethylbenzene can react with air to ethylbenzene hydroperoxide, followed by reaction with propene to styrene. Alternatively, ethylbenzene can be catalytically dehydrogenated.

Styrene is further processed to polystyrene which is used in large volumes in the automobile industry (replacing metal parts), in the building industry and for packaging. Furthermore, styrene is a raw material for the manufacture of synthetic rubber.

It can be referred to RAR Styrene (EU, 2000) for more detailed information on styrene and polystyrene.

2.2.2.2 Chemical industry – production of other chemicals than styrene

A small fraction of the total ethylbenzene serves as chemical intermediate, e.g. in the manufacture of acetophenone, cellulose acetate, diethylbenzene, propylene oxide. It is also used as solvent/reactant in unsaturated polyesters. Quantities involved in this application are assumed to be 0.5 % of the total amount, derived from information of the IUCLID data set (ECB, 2002).

2.2.2.3 Paint industry - use as solvent (intentional)

Ethylbenzene is known to be used as a solvent, both on its own and blended with xylenes. The solvents are applied in paints, lacquers, inks, rubber and agricultural industry.
There is no precise information on the amount of ethylbenzene formulated to solvents. It was assumed that about 1.5 % of the total quantity of ethylbenzene produced is applied in the solvent sector (intentional use of ethylbenzene as solvent). It was further assumed that the produced solvents consist of 15 % ethylbenzene. This value is a mean of data on the content of ethylbenzene in technical solvents, mainly on the German market (INFU, 2003). It is not clear if this content relates to ethylbenzene in “mixed xylene” solvent.
The European Council of Paint, Printing Inks and Artist's Colours (CEPE, 2000) had launched a survey among its members and supplied recently more detailed information about the results. A questionnaire was sent to all major paint manufactures plus most of the medium-sized companies as well as a number of small companies asking for information on the use of ethylbenzene in paints and solvents.
It became apparent that > 99 % of ethylbenzene used in paints is stemming from “mixed xylene” solvent. A more detailed description of this unintentional use of ethylbenzene can be found in 2.2.3.2.
The intentional use of ethylbenzene in paints seems not to be of relevance anymore. That might also be due to higher flammability and higher price of ethylbenzene compared to the “mixed xylene” solvent. In addition industry has emphasised their endeavour to develop solvent free systems and water based paints.
In summary it is concluded that the intentional use of ethylbenzene in the solvent sector is only marginal and is not further considered in the risk assessment.

2.2.3 Unintential uses

Some petroleum refineries isolate a reformate stream during the distillation of crude oil into petroleum products. Reformate is a blending stream used in the production of gasoline and has several CAS numbers. The final concentration of ethylbenzene in gasoline is approximately 2 % (by weight). CONCAWE estimate 20 million tons of reformate are produced annually for use in gasoline blending (CEFIC, 2004).
The reformate stream may be further processed to isolate a mixed xylene stream. “Mixed xylenes” contain generally about 80 % o-, m-, and p-xylenes and 15 to 20 % ethylbenzene (range of 10 – 45 % (CEFIC, 2004)). Ethylbenzene can be separated from the stream as means of production; however, this technology is economically not favourable.
The “mixed xylenes” (also called xylene-range aromatic solvent) is a commercial product (CAS: 1330-20-7). There are other processes which can be used to produce mixed xylene, e.g. gasoline pyrolysis and toluene disproportionation. “Mixed xylenes” is used mainly for blending into gasoline. The remainder is used as solvent to make coatings, agrochemicals and mis-

cellaneous products (car cleaning and household). Smaller applications are in the manufacture of perfumes, pesticides, pharmaceuticals as well as in the production of the individual isomers (ECETOC, 1986; Crookes and Howe, 1991; Lundberg et al., 1997).

For the risk assessment, the two main sources of ethylbenzene emissions stemming from unintentional uses of mixed xylenes are taken into account. Firstly, petrol-related emissions to the gas phase (2.2.3.1) are considered in the calculation of the regional background concentration. Secondly, a generic local scenario is calculated for the formulation and use of mixed xylenes as solvent (2.2.3.2).

It is known that ethylbenzene can be released during combustion processes other than combustion of gasoline, i.e. power stations and waste incinerators. Only very few measurements exist.
In BUA (1997), the quantity ethylbenzene released by coal-fired power stations is estimated to be 3 – 25 t/a for Germany. This amount is only 2 % of that from traffic (worst case).
It can be concluded that emissions from waste incineration and power stations are minor compared to releases by traffic, and will be neglected in this RAR.

2.2.3.1 Ethylbenzene in Petrol

The majority of the mixed xylenes produced is blended into gasoline (ECETOC, 1986). Ethylbenzene serves as a “anti-knock” agent (octane enhancer) (Fishbein, 1985). Although not strictly part of the risk assessment of ethylbenzene this application is discussed here since estimated emissions are used in the calculation of the background concentrations.
Leseman (1986) cites the following quantities of ethylbenzene in different types of fuels:

Table 2.3 **Quantity of ethylbenzene in different types of petrol (Leseman, 1986)**

	% ethylbenzene (by weight)
petrol, regular	1.5 – 3.1
petrol, unleaded	1.8 – 4.7
aviation fuel	0.77
kerosene	0.36 – 0.38
diesel No 2	< 0.2

Nowadays, regular petrol is practically phased out in favour of unleaded fuel.
Using a density of 750 kg/m^3 for petrol (BUA, 1998) and of 867 kg/m^3 for ethylbenzene (BUA, 1997), one can derive the respective volume fractions as 1.6 – 4.1 vol % for unleaded petrol. Concurringly, Fishbein (1985) has given a value of 4 vol % in reformate. Another study found 0.8 – 4.6 vol % ethylbenzene in common German engine fuels (DGMK, 1994).
Exhaust hydrocarbon emissions from vehicles are expected to be a mixture of unburned and partially burned fuel species. Ethylbenzene has been observed in exhaust gases of combustion engines. Various sources quote the content of ethylbenzene in these gases between 0.7-2 wt % for gasoline and between 0.55-0.7 wt % for diesel. A value of 2.3 wt % is given for vehicles thereby not differentiating between gasoline and diesel (cited in Crookes and Howe, 1991).

Annual consumption of petrol was about 120 million tons in the EU between 1990-1994 (see EU, 2001). According to data by IEA (2003) annual petrol consumption in the EU decreased slightly during the last years to about 111 million tons in 2001. Using an ethylbenzene content of ~ 2 wt %, given by DGMK (1994) and confirmed by CONCAWE (CEFIC, 2004), a quantity of **2.2 x 10^6 t/a** ethylbenzene can be roughly estimated to be present in petrol in the EU.

Ethylbenzene can be discharged during handling and combustion of gasoline (automobiles and aeroplanes). These petrol related emissions, e.g. from crude oil production, transport, exhaust gases, are estimated in Chapter 3.1.2.4.1 and taken into account for the calculation of the regional background concentrations.

2.2.3.2 Other uses of "mixed xylenes"

The remainder of the "mixed xylenes" is mainly used as solvent in spray paints, primers, paint removers, thinners, wood stains, varnishes and other finishes, and cleaners for automotive and household uses (OECD, 2002). A further quantity is applied for the production of the individual isomers of xylene.
CEPE (2004) gave more details on the uses of paints containing ethylbenzene. The majority of these paints are used as industrial paint in the following sectors: automotive, vehicle refinishes, marine, can coating, wood furniture, electrical insulating, printing inks. Only a minor part is applied in decorative/DIY area. Whereas brushing and roller coating are the main application techniques in the decorative sector, spraying, curtain coating, dipping, coil coating are further techniques used in industry. Most of the industrial paints are (aromatic) solvent-based. Over 60 % of the total paints volume in the decorative sector is used as aqueous suspensions.
Recently, the Hydrocarbon Solvent Producers Association *HSPA* (2004) provided information on the quantities of "mixed xylenes" as solvent in Europe. That information is representative of > 90 % of the quantity of hydrocarbon solvents produced in Western Europe.
HSPA estimates the quantity of mixed xylenes used as solvent in Western Europe at 2.5 - 3.5 x 10^5 t/a. The content of ethylbenzene varies according to the production process. About 82 % of mixed xylenes originate from catalytic reformate containing typically 10 – 15 % ethylbenzene. Approximately 11 % is produced by gasoline pyrolysis resulting in a ethylbenzene content of 40 – 45 %. A further route is by toluene disproportionation; the ethylbenzene content is not known.
The upper estimate of the total amount of ethylbenzene in mixed xylenes used as solvent can be calculated as follows:

$$350000 \text{ t} \times (0.82 \times 0.15 + 0.18 \times 0.45) = 71400 \text{ t}$$

A quantity of **71000 t/a** ethylbenzene in about 350000 t/a mixed xylenes is used further in the risk assessment.

Summarising the unintential uses, an additional quantity of ethylbenzene of **2.27 x 10^6 t/a** is recognised. About 97 % (2.2 x 10^6 t/a) of this quantity is present in petrol; the remaining 3 % (7.1 x 10^4 t/a) are assumed to be used as solvents in various fields of application.

A total tonnage of **7.55 x 10^6 t/a** ethylbenzene is assumed to be present in the EU resulting from the quantity produced (5.28 x 10^6 t) and the quantity arising from unintentional uses (2.27x 10^6 t).

2.2.4 Information from Product Registers

The SPIN (Substances in Preparations In the Nordic countries) database was searched for information on ethylbenzene in products on the national markets. The following data were found:

Table 2.4 **Ethylbenzene in consumer products according to SPIN for 2001**

country	number of preparations	tonnage
Norway	555	91,396
Finland	799	1,685
Denmark	1,589	366

Please note: The total amount of a substance in SPIN is the added quantity of the substance in all products, the export amount subtracted. That is to say that if a substance is registered first as the imported raw material and then as the final preparation, the quantity will be counted twice. Substances that are used for formulation of chemical products and that are imported, and most are in the Nordic countries, will thus be accounted for with maybe double the actual amount.

In addition, substances are registered in an interval tonnage range. The number obtained in SPIN and given in the table above represents the upper limit of that interval. Depending on how wide the interval is the given value might overestimate the true value.

Therefore, the given tonnage figures of Table 2.4 may not reflect the real situation.

Main industrial use categories are given as "Manufacture of coke, refined petroleum products and nuclear fuel" (Norway, 91266 t, 5 preparations), "Manufacture of chemicals and chemical products" (Finland, 1600 t, 44 preparations) and "Manufacture of fabricated metal products" (Denmark, 312 t, 600 preparations) as well as "Manufacture of other transport equipment" (Denmark, 192 t, 312 preparations). There was no more detailed information on the specifics of these industrial categories.
Specifying the use of these preparations, "Paints, lacquers and varnishes" (Norway: 375 preparations; Denmark: 914 preparations) and "Solvents" (Norway: 45 preparations; Denmark: 66 preparations) are identified as the main fields of application. Another significant technical use is as "Process regulator" (Norway: 80 preparations; Denmark: 123 preparations).

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3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

Ethylbenzene is industrially produced and mainly used in the production of styrene. Another application is in the solvent sector (unintentional use). Ethylbenzene also occurs naturally in crude oil. Due to its presence in petrol and its volatility ethylbenzene is widely distributed in the atmosphere.

3.1.2 Environmental releases

Environmental releases of ethylbenzene occur during its production and processing to styrene. Solvent related emissions take place during formulation and use of the solvent (unintentional use). As a constituent of fuel ethylbenzene can be emitted during storage and refuelling whereby evaporative losses may happen, and as constituent of traffic exhaust. Furthermore, ethylbenzene can be released from various sources during combustion processes, e.g. from waste incinerators and power stations, however, these are not considered further.

3.1.2.1 Release from production

There are 9 companies at 16 sites in the EU which manufacture ~ 5.28 x 10^6 t/a.
Production and processing of ethylbenzene are reported for 11 sites. At another 5 sites production only takes place.
The quantity produced/processed at one site ranges from ~ 10,000 to ~ 1.0 x 10^6 t/a. Unless specific information was provided by industry the following default emission factors were used (Table 3.1).

Table 3.1 **Generic release factors for production of ethylbenzene**

		water		**air**		**soil**	
alkylation of benzene (IC3/UC33)	90 %	ESD 3	0.003	A1.2	0.001 (1c)	A1.2	0.0001 (1c)
fractionating of xylenes (IC9/UC33)	10 %	A1.1	0.003	A1.1	0.001 (1c)	A1.1	0.0001

3.1.2.2 Release from processing/industrial use

About 99.5 % (~ 5.25 x 10^6 t/a) of the total ethylbenzene are processed to styrene which is subsequently polymerised.

Default release estimation has been carried out for sites without specific information using default emission factors summarised in Table 3.2.

Table 3.2 **Generic release factors for processing of ethylbenzene**

		water		air		soil	
production of styrene (IC3/UC33)	99.5 %	ESD 3	0.007	A3.3	0.0001 (1c)	A3.3	0.0001
production of other chemicals (IC3/UC33)	0.5 %	A3.3	0.007	A3.3	0.0001 (1c)	A3.3	0.0001

Summarising all specific information given by Industry up to now (i.e. 2004) shows that 6 of the 16 sites considered apply dry techniques (e.g. fixed bed catalyst) for production and/or processing. Four of these 6 sites use a dry production process but at the same time a wet processing technique. One company has two plants at one site, one wet-operating plant and one dry-operating plant. Only one company employs a completely dry process resulting in zero emissions to waste water.
One producer supplied the information that waste water from production as well as processing is steam stripped before going into the wwtp. In this process ethylbenzene concentrations are reduced from about 0.2 g/l (solubility limit) to 0.1 mg/l. The steam loaded with ethylbenzene is redirected into the working processes. Therefore, no emissions to the atmosphere are expected by the stripping procedure.

3.1.2.3 Release from disposal

Ethylbenzene is volatile. Therefore, ethylbenzene is found mainly in the atmosphere.

Various producers state that exhaust gases and solid waste from production and processing are incinerated. Expected products of the complete combustion are H_2O and CO_2. No further information is available. However, no relevant emissions of ethylbenzene are expected from the incineration plants. Please, refer to Chapter 2.2.3 for information on combustion.

3.1.2.4 Unintential releases of ethylbenzene

Ethylbenzene is a constituent of "mixed xylenes" which are used in petrol and as solvent. Due to the high vapour pressure the atmosphere is the main target compartment for ethylbenzene.

It is not possible to exactly quantify ethylbenzene emissions caused by unintential uses. The only known information on recent petrol consumption is the statistics supplied by IEA (2003). However, emissions will vary under different conditions. No exposure data are available concerning the application as solvent.

On the other hand, the discharge of ethylbenzene by these sources is substantial and significant. Therefore, releases for all identified sources are estimated and used for the calculation of the regional background concentrations.

3.1.2.4.1 Petrol-related emissions

Basis of this chapter is the assumption made in 2.2.3.1 that the annual consumption of petrol in the EU is about 111 million tons. The steps considered relevant in the production and use

of gasoline are listed in Table 3.3. Only emissions to the gas phase are considered since the atmosphere is by far the most relevant compartment.
Emissions from traffic exhaust were calculated using measured concentrations and an emission factor of waste gas (see Table 3.3). The concentrations were measured on engines not fitted with a catalytic converter. Catalytic converters are able to reduce emissions to 12 % (BUA, 1997). Exact information about the proportion of cars equipped with converters is only available for Germany. Here, 96 % of all vehicles are emission reduced cars (KBA, 2002). For Europe this number is assumed to be slightly smaller, i.e. 90 %.
Emission factors are taken from BUA (1997) and BUA (1998).

Table 3.3 **Estimates of petrol-related emissions to gas phase**

step	**parameter**	**value**	**emission [t/a]**
crude oil production	HC[1] emission factor content ethylbenzene in HC gas phase	(0.1 kg HC) / (t crude oil) [2) 3)] 0.38 % [4)]	42
petrol refining	HC[1] emission factor content ethylbenzene in HC gas phase	(0.3 kg HC) / (t petrol) [2)] 0.38 % [4)]	127
transport and storage	HC[1] emission factor content ethylbenzene in HC gas phase	(3.11 kg HC) / (t petrol) [4)] 0.38 % [4)]	1,312
refuelling	HC[1] emission factor content ethylbenzene in HC gas phase	(15 kg HC) / (t petrol) [4)] 0.38 % [4)]	6,327
traffic exhaust	waste gas emission ethylbenzene concentration in exhaust (no catalytic converter)	(11,800 m^3 waste gas) / (t petrol) [4)] 12.3 – 49.0 mg m^{-3} [4)] cars with converter (90 % of cars, reduction of emission to 12 %) cars without converter (10 % of cars) total	1,740 – 6,931 1,611 – 6,418 3,351 – 13,349
		total (max)	**21,157**

[1)] Hydrocarbon
[2)] BUA (1998)
[3)] As first approach it is set: tonnage (crude oil) = tonnage (petrol), i.e. calculations based on 111 x 10^6t.
[4)] BUA (1997)

3.1.2.4.2 Other sources

A quantity of 7.1 x 10^4 t/a ethylbenzene is assumed to be contained in about 3.5 x 10^5 t of "mixed xylene solvents". Diffuse entry of ethylbenzene is expected resulting from formulation and use of the solvents. In Table 3.4 the relevant release factors are shown.

Table 3.4 **Release factors for formulation and use of mixed xylene solvents (solvent-based)**

		water		air		soil	
formulation	IC3/UC48	A2.1	0.003	A2.1	0.005 (1c)	A2.1	0.0001
technical use	IC14/UC48	A3.15	0.02	A3.15	0.9	A3.15	0.001

3.1.2.5 Summary of releases

Intentional releases result from all identified and significant life cycle stages of ethylbenzene: production and processing as chemical intermediate. Unintentional releases occur by means of production and use of petrol as well as formulation and use of mixed xylene solvents.
Summaries of all regional and continental releases can be found in Table 3.15 and Table 3.16.

3.1.3 Environmental fate

3.1.3.1 Degradation in the environment

3.1.3.1.1 Atmospheric degradation

Direct photolysis is not expected to be an important removal process since ethylbenzene does not significantly absorb light at wavelengths > 290 nm.
Reaction with OH radicals is by far a more significant degradation pathway. Atkinson (1989) studied the reaction of ethylbenzene and OH radicals in air at 25 ^{o}C. A rate constant of 7.1 x 10^{-12} cm^3 $molecules^{-1}$ s^{-1} was obtained which corresponds to a half life of 2.3 d under atmospheric conditions (i.e. OH-radical concentration of 5 x 10^5 molec cm^{-3}). Ohta and Ohyama (1985) derived a rate constant of 6.8 x 10^{-12} cm^3 $molecules^{-1}$ s^{-1} at room temperature resulting in an atmospheric half life of 2.4 d. Another study reported a rate constant of 8.2 x 10^{-12} cm^3 $molecules^{-1}$ s^{-1} at 25 ^{o}C for the lower troposphere corresponding to $t_{1/2}$= 2.0 d (Ravishankara et al., 1978). A further value of 8.0 x 10^{-12} cm^3 $molecules^{-1}$ s^{-1} at 300 K agrees well (SINGH et al., 1981).
Atmospheric oxidation was also modelled by the Atmospheric Oxidation Program AOPWin v. 1.90 for comparative purposes. The model is based on structure-activity relationship (SAR) methods. It estimates the rate constant for the atmospheric gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The overall OH rate constant

was derived as 5.9 x 10^{-12} cm^3 $molecules^{-1}$ s^{-1} giving a half life of 2.7 d (OH-radical concentration of 5 x 10^5 molec cm^{-3}).
The half life of 2.3 d is used in the further risk assessment. This value is the most recent one and is within the range of the other values.

3.1.3.1.2 Aquatic degradation (incl. sediment)

Abiotic

Ethylbenzene is not expected to hydrolyse under typical environmental conditions.

Aerobic biodegradation

Many tests on the biological degradation of ethylbenzene are available. As ethylbenzene has a high volatility only closed tests are appropriate to determine the biodegradation. The most relevant test results for the risk assessment are presented below.
In a respirometric test on ready biodegradation according to EEC method performed with domestic activated sludge from a laboratory sewage treatment plant as inoculum, a biodegradation of 50 % after 28 days was obtained. The concentration of ethylbenzene was 87 mg/l. Biodegradation was measured as BOD. A repetition of the test resulted in a degradation of the test substance of > 60 % after 33 days for 4 of 6 test vessels. From the available degradation curve it can be concluded that the pass level was already reached after 28 d for these 4 parallels. However, for 2 test vessels the degradation was only about 25 % and 55 % respectively after 28 and 33 days. As the degradation in the parallel vessels differs by more than 20 %, the test has to be regarded as not valid according to the OECD guideline. (BASF AG, 1988).
As ethylbenzene is toxic to microorganisms at concentrations around 100 mg/l (cf. 3.2.1.1.4) and the test was performed at a concentration of 87 mg/l, the variable results obtained may be caused by toxic effects on the inoculum.
In a MITI I test performed with ethylbenzene in 3 replicates, in one replicate 100 % degradation was achieved while in the other 2 replicates 0 % degradation was found (CERI). These findings may also be explained by toxicity to microorganisms as the test substance concentration in the MITI I test was 100 mg/l.

In a recently performed CO_2-headspace test according to ISO 14593 a mean biodegradation of 79 % (measured as TIC/ThIC) was found after 28 days (BASF AG, 2003). At the end of the 10d window about 68 % biodegradation was obtained. The ethylbenzene concentration was 22 mg/l, equivalent to 20 mg/l TOC. As inoculum activated sludge from a laboratory wastewater treatment plant treating municipal sewage was used with a concentration of 4 mg/l. Aniline as reference substance was degraded by 88 % within 14 days. From this study it can clearly be concluded that ethylbenzene is readily biodegradable fulfilling the 10d window criterion.

In a MITI-II test (OECD 302C) a biodegradation of 81-126 % after 14 days was reached (CITI, 1992). From this study ethylbenzene can be classified as inherently biodegradable.
In addition, several biodegradation studies are available that have not been performed according to standardised methods. The most relevant studies for assessment purposes are described below.

The 5d BOD of ethylbenzene was determined by Babeu and Vaishnav (1987) using acclimated mixed microbial cultures. The ethylbenzene concentration was in the range of 0.4 to 3.2 ppm. A BOD_5/ThOD ratio of 0.29 was determined.
The 35d BOD of ethylbenzene was tested by ZoBell and Prokop (1966). An oxygen-saturated seawater medium was used that was inoculated with specific enrichment cultures of hydrocarbon-oxidizing bacteria. The concentration of ethylbenzene was about 2 mg/l. A biodegradation of 54 % was found.
Weber et al. (1987) studied the removal of ethylbenzene in a completely mixed batch reactor and a completely mixed flow reactor. A synthetic waste water with a background TOC of 100 mg/l was used in the experiments. Adapted activated sludge from a municipal wwtp served as inoculum. In the complete mixed batch reactor a removal of ethylbenzene of 82 % was reached. In the complete mixed flow reactor an influent concentration of ethylbenzene of between 50 and 150 µg/l (not exactly given) was reduced by > 99 %. 22 % of the influent concentration was removed by volatilisation and 78 % by primary degradation. Typical operating conditions of the flow reactor were a hydraulic retention time of 5.5 h, a solids residence time of 6 d and a MLSS concentration of 3500 mg/l.
The elimination of ethylbenzene in 6 different wastewater treatment systems was studied by Hannah et al. (1986). A 1:1 mixture of raw wastewater with secondary effluent from a wwtp was used as feed. This wastewater was spiked with 21 priority substances, among them ethylbenzene, providing a concentration of 100 µg/l for each substance. Among the examined wastewater treatment systems were a conventional activate sludge system and two lagoon systems (aerated and facultative) as representatives for a biological wastewater treatment. In addition, physical elimination by filtration and flocculation was studied. For ethylbenzene, the highest removal rates of 96 % and 93 % were obtained with the activated sludge system and the facultative lagoon, followed by the aerated lagoon (70 %).
In an industrial sewage treatment plant ethylbenzene was removed from an initial concentration of 29 µg/l by 78 %. The plant treats wastes from an organic chemicals manufacturing site by neutralisation and settling, followed by a combined powdered carbon-biological process. Ethylbenzene concentration was measured by GC/MS (Hutton and Du Pont, 1980).

As ethylbenzene is readily biodegradable, a degradation rate constant of 1 h^{-1} is used for the degradation of ethylbenzene in wwtp.
No tests are available that simulate the biodegradation of ethylbenzene in surface waters. Therefore, the rate constant and half-life for biodegradation in surface waters has to be derived based on the results from the available screening tests. For readily biodegradable substances a rate constant of $4.7 \cdot 10^{-2}$ d^{-1} that is equivalent to a half-life of 15 d is proposed by the TGD.

3.1.3.1.3 Degradation in soil and sediment

No tests are available that can be used for an assessment of the biodegradation of ethylbenzene in soil. Therefore, the rate constant and half-life for biodegradation in soil has to be based on the results from the available screening tests. For readily biodegradable substances with a Kpsoil < 100 $l \cdot kg^{-1}$ the TGD proposes a half-life of 30 d that corresponds to a rate constant of $2.31 \cdot 10^{-2}$ d^{-1}.

For sediments a half-life of 300 d, equivalent to a rate constant of $2.31 \cdot 10^{-3}$ d^{-1} can be derived according to the TGD.

3.1.3.1.4 Summary of environmental degradation

Direct photolysis or hydrolysis is not expected. Under atmospheric conditions ethylbenzene has a half live of 2.3 d (rate constant of 7.1×10^{-12} cm^3 $molecules^{-1}$ s^{-1}) due to reaction with the OH radical ($Conc_{[OH]}$ of 5×10^5 molec cm^{-3}).
Ethylbenzene has been classified as readily biodegradable in surface water and wwtp. The following table shows the derived rate constants and half lives in different compartments.

Table 3.5 Rate constants and half lives due to biodegradation of ethylbenzene

	Rate constant	**Half live**
Surface water	4.7×10^{-2} d^{-1}	15 d
wwtp	1 h^{-1}	-
Soil	2.31×10^{-2} d^{-1}	30 d
Sediment	2.31×10^{-3} d^{-1}	300 d

3.1.3.2 Distribution

The equilibrium distribution in the environment was quantified by EQC Model Level I (v. 1.0) based on chemical-physical properties. Using the properties given in Chapter 1 the following distribution in different compartments was derived (Table 3.6).

Table 3.6 Distribution in the environment, Mackay Level I

	%
Air	99.45
Water	0.45
Soil	0.05
Sediment	0.05

3.1.3.2.1 Adsorption

The organic carbon-water partitioning coefficient Koc was derived according to TGD ("predominantly hydrophobics") using a log Kow of 3.13. Further partitioning coefficients can be found in Table 3.7 (see Appendix I for details).

Table 3.7 **Partition coefficients between different media**

	unit	**value**
K_{oc}	$l\ kg^{-1}$	43 1.8
Kp_{susp}	$l\ kg^{-1}$	43 .1
Kp_{sed}	$l\ kg^{-1}$	21 .6
Kp_{soil}	$l\ kg^{-1}$	8. 6
$K_{soil\text{-}water}$	$m^3\ m^{-3}$	13 .2
$K_{susp\text{-}water}$	$m^3\ m^{-3}$	11 .7
$K_{sed\text{-}water}$	$m^3\ m^{-3}$	22 .4

The order of magnitude of Koc (log Koc = 2.64) indicates a moderate mobility in soil.
A measured log $K_{sed\text{-}water}$ of 1.01 (Foc_{sed} = 4 %) corresponds to a $K_{sed\text{-}water}$ of 10.2 m^3/m^3 (Vowles, 1987). Only the solute phase was analysed. Loss of compound can not be excluded, e.g. by volatilisation. A log Koc of 2.47 was experimentally derived by Szabo (1992) whereby analysis was based on above cited log $K_{sed\text{-}water}$ of 1.01.
Partitioning coefficients as shown in the table above are used in the risk assessment.

3.1.3.2.2 Volatilisation

Ethylbenzene has a water solubility of 160 mg/l and a relatively high vapour pressure (930 Pa). A Henry's Law constant (H) of 617 Pa m^3 mol^{-1} can be computed from these values indicating rapid volatilisation. The dimensionless K_{air_water} of 0.26 was derived from this Henry's Law constant (see Appendix I).

3.1.3.2.3 Distribution in wastewater treatment plants

Using a Koc of 432 l kg^{-1}, a Henry constant of 617 Pa m^3 mol^{-1} and a rate constant of 1 h^{-1}, the following distribution of ethylbenzene in waste water treatment plants was calculated with Simple Treat 3.0.

Table 3.8 **Summary of distribution in STP**

	% of total
air	46.7
water	5.4
sludge	3.7
degraded	44.2
total removal	94.6

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3.1.3.3 Accumulation and metabolism

The log Kow of 3.13 indicates a potential for bioaccumulation. According to the TGD a BCF of 91 can be estimated from this value.
No bioaccumulation studies performed with pure ethylbenzene are available. In several studies the water-soluble fraction (WSF) of crude oil that contains ethylbenzene was tested.
Roubal et al. (1978) investigated the bioconcentration of ethylbenzene from the WSF of crude oil by Coho salmon (*Oncorhynchus kitutsch*) and starry flounder (*Platichthys stellatus*). The fish were exposed for 6 weeks (salmon) and 2 weeks (flounder) in a flow-through system to a mean WSF concentration of 0.9 mg/l containing a mean ethylbenzene concentration of 0.005 mg/l. At the end of the exposure periods, both species were transferred to clean sea water for 2 weeks to study the depuration. Bioconcentration factors for C2-substituted benzenes (related to dry weight) were 1.1, 2.4, 2 and 1 after 2, 3, 5 and 6 weeks of exposure for Coho salmon (muscle tissue). For the starry flounder the bioconcentration factors for C2-substituted benzenes in muscle tissues were determined to be 20 and 4 after 1 and 2 weeks of exposure. Depuration of the accumulated ethylbenzene to concentrations below the detection limit (0.05 mg/kg) occurred within 1 week for salmon and within 2 weeks for the flounder.
Manila clams (*Tapes semidecussata*) were exposed for 8 days in a flow-through system to the water-soluble fraction of crude oil containing a mixture of 6 monoaromatics (Nunes and Benville, 1979). The amount of aromatics in water was measured three times a day. The mean ethylbenzene concentration was 0.08 mg/l. Every 48 h a sub-sample of 10 test organisms was pooled and analyzed for aromatic content by GC. After 2 days of exposure the ethylbenzene concentration in the tissue (related to wet weight) was 0.34 mg/kg and after 8 days 0.37 mg/kg. After transfer of the clams into clean water, depuration of the ethylbenzene to concentrations below the detection limit (0.13 mg/kg) occurred within 7 days.
In a poorly documented study Ogata et al. (1984) determined a log BCF of 1.19 (BCF = 15) for ethylbenzene in goldfish. From the description of the study it is not clear whether the fish were exposed to pure ethylbenzene or to a mixture of alkyl benzenes. No information is given on the exposure duration or whether steady state conditions had been achieved. Therefore, the study is not regarded as valid and the result is not used for the risk assessment.
The validity of the available bioconcentration studies is limited as for all tests there is no information whether steady state was reached. In addition, the test organisms were not exposed to pure ethylbenzene but to a mixture of oil components. However, the available study results can be used as an indication that the bioaccumulation potential of ethylbenzene may be lower than predicted from the log Kow. The predicted BCF of 91 is used further in the risk assessment.

3.1.4 Aquatic compartment (incl. sediment)

3.1.4.1 Calculation of predicted environmental concentrations (PEC_{local})

All calculated PEC_{local} can be seen in Table 3.9. Site-specific dilution factors are used if known. However, the maximum dilution factor is 1000 for site-specific assessments according to the new TGD. If no specific information is available, the Emission Scenario Document of the new TGD (Part IV, Chapter 7) is used (IC3, dilution factor: 40).

Five sites (P3, P4, P5, PP5, PP7) are located at the sea. Dilution factor of 100 is used for sites situated at coastal zones. Please refer to Chapter 3.3.4.2 for results of marine assessment.

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Table 3.9 Data used in local aquatic exposure assessment

		site-specific information [1]	release factor to waste water [2] [kg/t]	$Clocal_{eff}$ (= PEC_{stp}) [3] [mg/l]	$Clocal_{water}$ [µg/l]	$PEClocal_{water}$ [4] [µg/l]
P1	production	effluent concentration, emission days	0.02	0.05	1.25	1.31
P2	production	emission factor, river flow rate, emission days	0	0	0	0.06
P3	production	waste water discharge rate, waste water concentration, emission days, direct discharge to sea	1×10^{-3}	-	0.075	0.081
P4	production	waste water discharge rate, waste water concentration, emission days, direct discharge to sea	$< 1 \times 10^{-5}$	-	0.019	0.025
P5	production	emission days, effluent concentration	3×10^{-3}	1×10^{-3}	0.010	0.016
PP1	production and processing	effluent concentration, dilution	2×10^{-5}	2×10^{-4}	1.83×10^{-3}	0.066

[1] If there was no site-specific information, calculations were done by rapporteur according to new TGD.

[2] Refers to maximum volume handled at that site (independent of process/es involved)

[3] Not given if known that there is no WWTP (either by specific information that there is no WWTP, or default assumption of no WWTP for known marine sites).

[4] $PEC_{regional}$ for marine sites as described in Chapter 3.3.4.2.

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PP2	production and processing	release for production, effluent concentration	0.05	14.0	14.0	14.1
PP3	production and processing	effluent discharge rate, municipal wwtp, emission days	1.5×10^{-3}	0.050	4.91	4.97
PP4	production and processing	effluent concentration, dilution, emission days	2×10^{-3}	1.0	9.99	10.06
PP5	processing	effluent discharge rate, emission days, direct discharge to sea	0.02	-	1.063	1.07
PP6	production and processing	effluent concentration, dilution, emission days	1.4×10^{-3}	0.01	0.031	0.095
PP7	production and processing	effluent concentration, effluent discharge rate	1×10^{-3}	0.062	0.620	0.630
PP8	production and processing	river flow rate, emission days, effluent concentration, dilution	0.09	0.02	0.122	0.19
PP9	production and processing	effluent concentration	5×10^{-4}	0.005	0.125	0.19
PP10	production and processing	WWTP influent concentration, dilution	3×10^{-4}	5×10^{-4}	0.023	0.09
PP11	production and processing	effluent concentration, elimination in WWTP, effluent discharge rate	4×10^{-3}	0.23	5.75	5.81
	other processing (generic)	-	7.0	0.665	16.6	16.7

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3.1.4.1.1 Calculation of PEC_{local} for production, or production and processing

There are 11 sites where production and processing takes place ("PP"), sole production takes place at 5 sites ("P"). Table 3.9 lists the calculated PECs and the respective data used in the calculation.
$PEC_{regional}$ as listed in Table 3.17 was taken into account in the calculation of the local PECs. Based on site-specific information a highest value of PEC_{local}= 1.31 µg l^{-1} for production is calculated.

Using the highest reported tonnage of 1 x 10^6 t/a the maximum value for production and processing (generically calculated) can be obtained:

PEC_{local}= 4.5 x 10^3 µg l^{-1} (T = 1 x 10^6 t/a (prod + proc), f_{prod} = 0.003, f_{proc} = 0.007, $T_{emission}$ = 300 d/a (prod + proc), $elimination_{STP}$: 94.6 %, dilution factor: 40).

Site-specific data for production and processing show a maximum PEC_{local} of

PEC_{local}= 14.1 µg l^{-1}

which is more than two orders of magnitude below the generic value.

3.1.4.1.2 Calculation of PEC_{local} for other processing

Ethylbenzene is used as chemical intermediate. Styrene production (99.5 %) was evaluated site-specifically (see 3.1.4.1.1). A small amount of ethylbenzene processed (0.5 %) could not be allocated to a specific site and was generically calculated giving a

PEC_{local}= 16.7 µg l^{-1} (T = 26 400 t/a, f = 0.007 (A 3.3), $T_{emission}$ = 300 d/a, f_{ms} = 0.2 (B 3.2), $elimination_{STP}$: 94.6 %, dilution factor: 40).

Results are shown in Table 3.9.

3.1.4.1.3 Calculation of PEC_{local} for disposal

No calculation was performed (see 3.1.2.3).

3.1.4.2 Measured data

Monitoring data are reported in the literature, particularly from government and public institutions.

3.1.4.2.1 Water

The following table summarises the measured concentrations between 1985 and 1994. Details can be found in the Appendix II.

Table 3.10 Measured ethylbenzene concentrations in water compartments (1985-1994)

Water compartment	Concentration range [µg/l]	Typical value [µg/l]	Remarks
Surface water	0.004 - 5	0.01	Measured in rivers: Rhine, Lippe, Emscher (D) and Brazo, Lower Tennessee (USA)
Sea water	<0.005 - 15	0.02	Measured in North Sea (D) and Gulf of Mexico; wide range of concentrations due to measurements at sites with and without direct anthropogenic influence
Rain	0.007 - 0.44	~ 0.03	Measured at various sites in CH, D and USA; concentrations depending on season
Snow	0.13 - 2.7	1.1	Measured in CH
Ground water	0.01 - 3320		Measured in D, NL, UK, I, CAN and USA; partly at highly polluted sites, e.g. beneath former paint factory or former harzardous waste site
Drinking water	<0.05 - 30	1 - 2	Measured in USA, CAN, D

More recent measurements in surface waters are available for Germany, The Netherlands and France.

Ethylbenzene was measured at 132 different points throughout Germany including large and small rivers (1 – 25 measurements per year and per point). The 90th percentile of the maximum measured concentration of all values was < 1 µg/l between 1999 – 2001 (UBA (D), 2003a; UBA (D), 2003b).

Ethylbenzene was monitored in The Netherlands (RIVM). Concentrations monitored at 7 surface water sites for the years 1998 – 2004 (5-27 measurements per year per site) were consistently below the analytical detection limit of 0.01 µg/l. Older data of the same sites from 1987-1994 shows that concentrations in those years were (with the exception of 1987) also below the detection limit, which was 0.1 µg/l in those years.

There are 3 measured concentrations in French surface waters (all 0.1 µg/l) for 2000 and 2002, however, no more detailed information is available regarding the sites (INERIS, 2004). In 2002, 88 measurements were below the detection limit (0.1 µg/l).

The Environment Agency (2004) provided monitoring data for various regions within the UK dating from 1990-2003. Although specific locations are identified (by name and number code), the regions are neither further described (e.g. reason for monitoring) nor exactly spatially defined. For 3 regions (NEast, NWest, Midlands) the data comprise about 7000 samples each over that period. Between 100 – 1000 samples each were provided by the other regions. The 90 percentile values are shown in the following table.

Table 3.11 Measured ethylbenzene concentrations in the UK in [µg/l] (90th percentile value) between different time periods

Region	1993 - 2003	2001 - 2003
Anglian	≤ 0.7	≤ 0.5
Midlands	≤ 10.0	≤ 10.0
N East	≤ 18.5	≤ 2.0
N West	≤ 10.0	≤ 10.0
Southern	≤ 10.0	≤ 1.92
S West	≤ 0.10	≤ 0.11
Thames	≤ 0.50	≤ 0.10
Wales	≤ 10 600.0	≤ 100.0

The data vary widely. Spot checks revealed that some points of measurements are in the direct vicinity of industrial sites which might be one reason for the large differences between the regions. In general, one can notice a decreasing trend in ethylbenzene concentration measured over the last years.

Due to the heterogeneity of monitoring data it is difficult to draw specific conclusions. Typical concentrations of ethylbenzene found in surface, sea and rain water are at or below 0.1 µg/l. The calculated $PEC_{regional}$ (surface water) of 0.06 µg/l falls in that range too.

3.1.4.2.2 Sediment

France provided monitoring data of ethylbenzene in sediment from 2000 to 2002 (INERIS, 2004). The 90th percentile of all values is 4.0 µg/kg (63 values; in addition, 100 values were below the detection limit in 2002). No further information regarding the specifics of the sites or results (e.g. wet or dry weight) is known.
There are two further measurements regarding ethylbenzene in sediment: 4.0 µg/kg from the lower Tennessee River (Goodley and Gordon, 1976) and 5.0 mg/kg from the USEPA STORET database (median, dry weight) (Staples et al., 1985). No further information about location or conditions are known and it is also not known if the value of 4.0 µg/kg is based on wet or dry weight, therefore, these values can not be assessed.

3.1.4.2.3 Sewage sludge

The Austrian EPA analysed sludge from municipal WWTP (UBA (A), 1995) within a project to investigate sewage sludge for organic, inorganic and biological parameters. There was only one out of 17 samples showing ethylbenzene < 4.29 µg/l (wet weight), in all other samples ethylbenzene could not be detected (detection limit: 1.20 µg/l).
There is a further Austrian study of a pilot unit of the main Vienna WWTP (UBA (A), 2000). The samples were taken over 4 days (24 h) in May/June 1999, and subsequently mixed to 4 day samples. Only a few selected chemicals/chemical classes were quantitatively determined. Other substances, like ethylbenzene, were screened for, and their concentrations roughly estimated. Concentrations of ethylbenzene in sewage sludge were between < 0.05 and 0.1 mg/kg (dry weight). Ethylbenzene could not be detected in the wastewater (detection limit

< 0.1 µg/l). Based on that the authors assume that ethylbenzene is formed in the WWTP as metabolite of other compounds.
The VOC concentrations of 12 digested sludges obtained from rural, urban and industrial wastewater treatment works in northwest England were investigated (Wilson et al., 1994). The percentage of industrial influent on the total influent flow varied between 1.4 to 45.6 %. Ethylbenzene was detected between 0.2 mg/kg dw (4.2 µg/l wet volume) and 9.0 mg/kg dw (338 µg/l wet volume). There was no apparent relationship between ethylbenzene concentration and percent of industrial influent, population served (capacity between 18 000 – 490 000 inhabitants) or solids content of sludge (1.7 – 6.5 %). Since values vary widely without specific information on the sites or origin of wastewater, these data can only be considered as indicative.
An American conference paper refers to measurements on municipal sludges done by EPA in 1980 (Naylor and Loehr, 1982). Combined sludges (i.e. a mixture of sludges generated by two or more wastewater treatment processes) of 13 treatment plants across the United States were analysed for priority pollutants. Ethylbenzene was detected in 12 of these sludges in concentrations between 1.0 – 51 mg/kg dw (median: 5.5 mg/kg dw), corresponding to 45 – 2100 µg/l wet volume (median: 248 µg/l wet volume).

None of the monitoring data available can be taken as representative since the studies are not described detailed enough. The American paper refers to measurements in the USA from over 20 years ago. The Austrian studies were done for screening purposes only. Therefore, the measured data can provide valuable additional information. However, the sludge concentration used further in the risk assessment is based on default calculations according to TGD (cf. 3.1.5).

3.1.4.3 Calculation of Predicted Environmental Concentration for Sewage Treatment Plants (PEC_{stp})

Since the PEC for micro-organisms in the STP (PEC_{stp}) equals the concentration in the effluent, the PEC_{stp} can be found in Table 3.9. The maximum value of effluent concentration is 14.0 mg l^{-1}, however there is a non-biological WWTP in place at that specific site. Therefore, the highest PEC_{stp} relevant for micro-organisms is 1.0 mg l^{-1}.

3.1.4.4 Calculation of Predicted Environmental Concentration for Sediment (PEC_{stp})

Using the highest aquatic exposure of PEC_{local_water} = 14.1 µg l^{-1} based on specific data (production and processing), K_{susp_water} = 11.69 m^3/m^3 and RHO_{susp} = 1150 kg/m^3 results in a

$$PEC_{local_sed} = \frac{K_{susp_water}}{RHO_{susp}} \cdot PEC_{local_water}$$

PEC_{local_sed} = 142.9 µg kg^{-1} (wet weight).

3.1.5 Terrestrial compartment

Ethylbenzene is expected to rapidly evaporate and therefore to occur mainly in the vapour phase (see Chapter 3.1.3.2). However, the log Kow = 3.13 (Koc = 432 l kg^{-1}) indicates a mod-

erate adsorptivity. According to the SimpleTreat 3.0 model about 3.7 % of the total ethylbenzene are directed to sludge in the STP.
It is generally assumed that only sludge from municipal STPs is applied to soil. It is known of just one producer (PP3) to discharge effluents into a municipal STP. The expected sludge concentration is

$$C_{sludge}(PP3) = 107\ mg/kg_{dw}.$$

This value is higher than all available measured data. The highest available value monitored is 51 mg/kg_{dw} (Naylor and Loehr, 1982). More recently obtained data show a range of 0.05 up to 9 mg/kg_{dw} (cf. 3.1.4.2.3). However, all available monitoring data have to be considered as not representative, since they are either only spot checks without detailed documentation, or over 20 years old. Therefore, the calculated C_{sludge} of 107 mg/kg_{dw} is used further in the risk assessment.

3.1.5.1 Calculation of PEC_{local}

Local soil scenarios are calculated as the sum of exposure through application of sludge and deposition. C_{sludge} of 107 mg/kg_{dw} results from production and processing with subsequent discharge of the effluents into a municipal WWTP.
A $PECregional_{nat\ soil} = 1.23 \times 10^{-5}\ mg/kg_{wwt}$ was obtained as regional concentration in natural soil (see Table 3.17).
The results of the calculation for site PP3 are shown in the following table. For comparison, the results of the calculations for PP1 (highest deposition flux) are also shown which only gets input via deposition (no sludge application).

Table 3.12 Data used in local terrestrial exposure assessment

	Sludge appli-cation	$DEPtotal_{ann}$ [mg/(m^2 d)]	$PEClocal_{soil}$* [mg/kg]	$PEClocal_{agr\ soil}$* [mg/kg]	$PEClocal_{grassl}$* [mg/kg]	$PEClocal_{soil_porew}$ [mg/l]	$PEClocal_{agr\ soil_porew}$ [mg/l]	$PEClocal_{grassl_porew}$ [mg/l]
PP3	yes	0.017	0.066	0.013	0.004	0.008	0.002	< 0.001
PP1	no	0.932	0.039	0.039	0.047	0.005	0.005	0.006

*Please refer to TGD, Chapter 2.3.8.5, Table 11 for characteristics of soil and respective endpoints.

Although the deposition rate assumed for PP1 is over 50 times higher than for PP3, the resulting $PEClocal_{soil}$(PP1) and $PEClocal_{soil}$(PP3) are of the same order of magnitude.
A model calculation for PP1 using the deposition flux of $DEPtotal_{ann}$ of 0.932 mg/(m^2 d) as well as sludge application (C_{sludge} of 107 mg/kg_{dw}) results in a $PEClocal_{soil}$ of 0.104 mg/kg pointing to the fact that both exposure routes (i.e. sludge application and deposition) may be significant for local soil concentration of ethylbenzene. It should be kept in mind that the $DEPtotal_{ann}$ used were derived from generic calculations.

3.1.5.2 Measured data

Monitoring data of ethylbenzene in soil are very scarce and can therefore not be considered representative. 76 mg/kg was measured at a contaminated site of a former asphalt production unit in Deventer/NL (VANDERHOEK et al., 1989). Measurements in the immediate vicinity of 8 petrol stations near Hamburg/Germany found ethylbenzene in concentrations between < 0.1 mg/kg (limit of detection) and 120 mg/kg (Stachel, 1993). For these values it is not known if they are based on wet or dry weight.

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3.1.6 Atmosphere

3.1.6.1 Calculation of PEC_{local}

All calculated PEC_{local} (annual average) as well as the total release to air and the average annual deposition rate can be seen in Table 3.13. Background concentrations were used as given in Table 3.17.

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Table 3.13 Data used in local atmospheric exposure assessment

		site-specific release information	direct release to air [t/a]	Clocal $_{air_ann}$ [mg/m^3]	PEClocal $_{air_ann}$ [mg/m^3]	total release to air (direct and from wwtp) [t/a]	DEPtotal $_{ann}$ [mg/(m^2 d)]
P1	production	release	2	1.5 x 10^{-3}	2.0 x 10^{-3}	3.6	0.003
P2	production	release, monitoring data	9	6.6 x 10^{-3}	7.0 x 10^{-3}	9	0.007
P3	production	release	3.6	0.003	3.2 x 10^{-3}	3.6	0.003
P4	production	release	1.2	9.1 x 10^{-4}	1.4 x 10^{-3}	1.2	0.001
P5	production	-	9.5	7.2 x 10^{-3}	7.7 x 10^{-3}	9.5	0.008
PP1	production and processing	-	1 134	0.864	0.864	1 134	0.932
PP2	production and processing	release	43.4	0.033	0.034	63.0	0.052
PP3	production and processing	release	20	0.015	0.016	21	0.017
PP4	production and processing	release	3.2	2.4 x 10^{-3}	2.9 x 10^{-3}	4.4	0.004
PP5	processing	release, monitoring data	5.1	3.9 x 10^{-3}	4.4 x 10^{-3}	6	0.005
PP6	production and processing	release	0.17	6.4 x 10^{-4}	1.1 x 10^{-3}	1.0	8.3 x 10^{-4}
PP7	production and processing	-	432	0.329	0.330	432	0.355

PP8	production and processing	release	8.7	0.025	0.026	42	0.034
PP9	production and processing	release	4.4	3.4 x 10^{-3}	3.8 x 10^{-3}	4.5	0.004
PP10	production and processing	-	436	0.332	0.333	436	0.359
PP11	production and processing	release	424	0.323	0.323	424	0.349
	other processing		2.6	0.013	0.014	89	0.015

3.1.6.1.1 Calculation of PEC_{local} for production, or production and processing

Generic scenario gives a maximum

$PEClocal_{air_ann} = 3.55$ mg m^{-3}

for production and processing using T = 1 x 10^6 t/a (prod + proc), f_{prod} = 0.001 (A 1.2, MC 1c), f_{proc} = 0.0001 (A 3.3. MC 1c), $T_{emission}$ = 300 d/a (prod + proc).

Using site-specific information results in a highest

$PEClocal_{air_ann} = 0.864$ mg m^{-3}

for the same life cycle step.

For production only, a maximum $PEClocal_{air_ann}$ of 0.008 mg m^{-3} was obtained using site-specific information.

3.1.6.1.2 Calculation of PEC_{local} for other processing

Using generic emission factors a $PEClocal_{air_ann}$ of 0.014 mg m^{-3} was obtained for the processing of ethylbenzene not including styrene (Table 3.13). Input data used were $T_{ethylbenzene}$ = 26 400 t/a, f = 0.0001 (A 3.3, MC 1c), $T_{emission}$ = 300 d/a, f_{ms} = 0.2 (B 3.2).

3.1.6.1.3 Calculation of PEC_{local} for disposal

No calculation was performed (see 3.1.2.3).

3.1.6.2 Measured data

There are a number of measurements of ethylbenzene in the atmosphere dating from 1984 - 1995. Concentrations vary greatly depending on the location and conditions. Over the Pacific Ocean ethylbenzene was measured in the range of 5 to 10 ng/m^3. In remote or rural areas (e.g. the Black Forest) measurements showed concentrations in the range of 0.01 to 3 µg/m^3. In major cities in B, CH, D, NL, USA monitoring data are mainly between 1 µg/m^3 and 100 µg/m^3, with a maximum value of about 10 mg/m^3 measured in Zurich/Switzerland. Most values are between 1 µg/m^3 and 10 µg/m^3. Measurements at industrial sites or places with heavy traffic result in values between 3 µg/m^3 and 200 µg/m^3. For details please refer to Appendix II.

There are very few monitoring data provided specifically by industry for this risk assessment. At one production site ethylbenzene was measured in the range of 0 – 136 ng/m^3 (131 measurements). One sporadic measurement at a downwind location of a processing site was given as 6.6 ng/m^3 (no further information).

Ethylbenzene can be released during combustion processes, e.g. power stations and waste incinerators. Only very few measurements exist.

Jay and Sieglitz (1995) analysed the emission of a municipal German incineration plant. They identified about 250 individual compounds representing ca. 42 % of the total organic carbon

(TOC) in the emissions. Ethylbenzene concentration was determined to be about 3 µg/m^3. However, the authors mention a probable error of 50 %.
Measurements at another municipal German incineration plant identified an ethylbenzene concentration of < 5 µg/m^3 (Dohmann et al., 1999).
Concentrations of ethylbenzene in the exhaust of a Japanese waste incineration plant are cited as 38 µg/m^3 and 85 µg/m^3 (BUA, 1997). The same reference gives the quantity of ethylbenzene released by incineration plants in Germany as about 0.06 % of the traffic-related releases.

3.1.7 PEClocal for unintentional uses

As described in Chapter 3.1.7 significant emissions of ethylbenzene could occur from unintentional use of ethylbenzene. Main known source are the "mixed xylenes" which are predominantly used as petrol additive. Petrol-related emissions are only considered for the background concentration.
However, a generic calculation to obtain a PEClocal is performed for formulation and technical use of mixed xylenes as solvent (see Chapter 3.1.2.4.2). The fraction of main source was derived from the appropriate B-Tables, based on the estimated quantity of 7.1 x 10^4 t/a ethylbenzene in about 3.5 x 10^5 t/a mixed xylene solvents.
The following table shows the results. Background concentrations were used as given in Table 3.17.
Soil scenario was only calculated for life cycle step "technical use" since formulation is supposed to take place at site with industrial wwtp. Input to soil is assumed to be by deposition ($DEPtotal_{ann}$ = 2.65 mg m^{-2} d^{-1}) and sludge application (Csludge = 1.24 x 10^4 mg kg^{-1}).

Table 3.14 PEClocal for unintentional use of "mixed xylenes" as solvent for water, air and soil

		PEC local $_{water}$ [µg/l]	**PEC local $_{air_ann}$ [mg/m^3]**	**PEC local $_{soil}$ [mg/kg]**
formulation	IC3/UC48	766	0.109	-
technical use	IC14/UC48	639	2.43	7.67

These PEClocal are higher (soil, water) or in the upper range (atmosphere) of PEClocal derived in Chapters 3.1.4, 3.1.5 and 3.1.6 in case default values had to be used there. Most PEClocal for sites where specific data were available are clearly below the above values. However, one has to keep in mind that all values of the above table are derived by using default values and that these have to be considered as worst-case assumptions.

3.1.8 Secondary poisoning

The log Kow of 3.13 indicates a potential for bioaccumulation. According to the TGD a BCF of 91 can be estimated from this value. However, experimental data (see 3.1.3.3) indicate that bioaccumulation of ethylbenzene is lower than predicted. Therefore, an assessment of secondary poisoning is not necessary.

3.1.9 Calculation of $PEC_{regional}$ and $PEC_{continental}$

For the calculation of the background concentration intentional and unintentional releases of ethylbenzene are taken into account. Intentional releases result from the identified and significant life cycle stages of ethylbenzene: production and processing as chemical intermediate. Unintentional releases occur by means of production and use of petrol as well as formulation and use of mixed xylene solvents.
The assumption is made that all producers and processors possess a waste water treatment plant.
Petrol-related emissions are only considered for the air compartment. All effluents from the formulation of mixed xylene solvents are thought to be treated in a wwtp, however, a connection rate of 80 % is believed to be realistic for the life cycle step of use of these solvents.
Table 3.15 and Table 3.16 summarise the total regional and continental releases.

Table 3.15 **Summary of regional releases of ethylbenzene**

		release to wwtp [t/a]	**release to surface water [t/a]**	**direct release to air [t/a]**	**release to soil [t/a]**
intentional	production, processing	31	0	254	105
unintentional	petrol-related			2 116	
	formulation and use of mixed solvent	135	28	6 426	8
total regional		166	28	8 796	113

Table 3.16 **Summary of continental releases of ethylbenzene**

		release to wwtp [t/a]	**release to surface water [t/a]**	**direct release to air [t/a]**	**release to soil [t/a]**
intentional	production, processing	280	0	2 285	941
unintentional	petrol-related			19 041	
	formulation and use of mixed solvent	1 214	256	57 829	70
total continental		1 494	256	79 155	1 011

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Regional and continental background concentrations of ethylbenzene in different compartments were calculated using the values of Table 3.15 and Table 3.16 and SimpleBox 2.0a. Results are shown in Table 3.17 (see Appendix III).

Table 3.17 **Continental and regional background concentrations**

	unit	**regional**	**continental**
PEC surface water	mg l^{-1}	6.40 x 10^{-5}	7.20 x 10^{-6}
PEC air	mg m^{-3}	4.62 x 10^{-4}	1.43 x 10^{-4}
PEC agr soil	mg kg_{wwt}^{-1}	7.40 x 10^{-5}	9.50 x 10^{-6}
PEC porewater agr soil	mg l^{-1}	9.56 x 10^{-6}	1.23 x 10^{-6}
PEC nat soil	mg kg_{wwt}^{-1}	1.23 x 10^{-5}	3.81 x 10^{-6}
PEC sediment	mg kg_{wwt}^{-1}	5.62 x 10^{-4}	7.03 x 10^{-5}

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT ASSESSMENT)

3.2.1 Aquatic compartment (incl. sediment)

3.2.1.1 Toxicity test results

For ethylbenzene many ecotoxicity tests are reported. Due to the volatility of the substance only studies using flow-through or closed systems with analytical measurement of ethylbenzene concentrations are suitable for effect assessment purposes. Other available acute studies using static testing methods without supporting analysis were therefore not used in this assessment.

The most relevant results of acute toxicity tests with aquatic organisms are summarized in Table 3.18 and are described in more detail below.

Table 3.18 Most relevant results of acute toxicity tests with aquatic organisms

Test organism/ source	**duration**	**test design/ remarks**	**analytical data**	**effect concentration [mg/l]**	**effect**
Vertebrates, short-term toxicity					
Menidia menidia (Boeri, 1987a)	96 h	flow-through saltwater	y	96 h-LC_{50} = 5.1	mortality
Pimephales promelas (Geiger et al., 1986)	96 h	flow–through freshwater	y	96 h-LC_{50} = 12.1	mortality

Test organism/ source	**dura-tion**	**test design/ remarks**	**analyti-cal data**	**effect concentration [mg/l]**	**effect**
Oncorhynchus mykiss (Galassi et al., 1988)	96 h	semi-static, closed bottles OECD 203 freshwater	y	96 h-LC_{50} = 4.2	mortality
Morone saxatilis (Benville and Korn, 1977)	96 h	static, open system more than 99% of test substance was lost within 24 h saltwater	y	96 h-LC_{50} = 4.3 [a)]	mortality
Poecilia reticulata (Galassi et al., 1988)	96 h	semi-static, closed bottles OECD 203 freshwater	y	96 h-LC_{50} = 9.6	mortality
Invertebrates, short-term and long-term toxicity					
Mysidopsis bahia (Boeri, 1988)	96 h	flow-through saltwater	y	96 h-LC_{50} = 2.6	mortality
Daphnia magna (Vigano, 1993)	24 h 48 h	static, EPA method F closed system freshwater	y	24 h-LC_{50} = 2.3-2.9 48 h-LC_{50} = 1.8-2.4	immobili-zation
Ceriodaphnia dubia (Niederlehner et al., 1998)	7 d	semi-static, EPA Whole Effluent Testing Program method, modified to minimize volatiliza-tion freshwater	y	7 d-LC_{50} = 3.6 7 d-IC_{50} = 3.3 (repro) 7 d-LOEC = 1.7 (repro) 7 d-NOEC = 1.0 (repro) 48 h-LC_{50} = 3.2	mortality / reproduction
Daphnia magna (Abernethy et al., 1986; Bobra et al., 1983)	48 h	static, closed system freshwater	n	48 h-LC_{50} = 2.1 [b)]	mortality
Artemia salina (Abernethy et al., 1986)	24 h	static, closed system saltwater	n	24 h-LC_{50} = 15.4 [b)]	mortality
Daphnia magna (MacLean and Doe, 1989)	48 h	static, closed system freshwater	y	48 h-EC_{50} = 2.9	immobili-zation
Artemia salina (MacLean and Doe, 1989)	48 h	static, closed system saltwater	y	48 h-EC_{50} = 9.2	immobili-zation
Crago franciscorum (Benville and Korn, 1977)	24 h 96 h	static, open system more than 99% of test substance was lost within 24 h saltwater	y	24 h-EC_{50} = 2.2 [a)] 96 h-EC_{50} = 0.49 [a)]	y
Daphnia magna (Galassi et al., 1988)	24 h	OECD 202 effect: immobiliza-tion freshwater	y	24 h-IC_{50} = 2.2	immobili-zation
Plants					

Test organism/ source	**dura-tion**	**test design/ remarks**	**analyti-cal data**	**effect concentration [mg/l]**	**effect**
Selenastrum capricornutum (Boeri, 1987b)	96 h	TSCA 797.1050 freshwater	y	72 h-E_RC_{50} = 5.9 72 h-NOEC = 3.4 96 h-E_RC_{50} = 5.0 96 h-E_RC_{10} = 3.4	growth rate
Skeletonema costatum (Boeri, 1987c)	96 h	TSCA 792.1050, static saltwater	y	72 h-E_RC_{50} = 7.7[b)] 72 h-NOEC = 4.5 [b)]	growth rate
Selenastrum capricornutum (Galassi et al., 1988)	72 h	growth inhibition test freshwater	y	72 h-E_RC_{50} = 4.6	growth rate

[a)] test result is not valid [b)] exponential growth in the control only over 72 h

3.2.1.1.1 Fish

The short-term toxicity of ethylbenzene to the fathead minnow (*Pimephales promelas*) was examined by Geiger et al. (1986) using a flow-through water test supported by chemical analysis. Fish were exposed at about 26°C for 96 h in dechlorinated laboratory water or unfiltered lake water (pH 7.4, oxygen content 7.0 mg/l, water hardness 45.6 mg/l $CaCO_3$). Different concentrations of test solutions were applied by diluting (factor 0.5) a stock solution of the test substance. The toxicant concentrations causing 50% mortality of the fish with corresponding 95% confidence intervals were calculated using the corrected average of the analysed tank concentrations. For ethylbenzene a 96 h-LC_{50} of 12.1 mg/l (95% confidence limits 11.5-12.7 mg/l) was found.

Galassi et al. (1988)) studied the acute toxicity of ethylbenzene in fish with *Oncorhynchus mykiss* (rainbow trout) and *Poecilia reticulata* (guppy) as test organisms. Toxicity tests were carried out according to the OECD Guideline 203 “Fish acute toxicity tests” (1981). Due to the volatility of the test substance tests were performed in closed bottles. At 48 hours from the beginning of the test, test solutions were renewed. Based on mean measured concentrations a 96 h-LC_{50} of 4.2 mg/l for *Oncorhynchus mykiss* and a 96 h-LC_{50} of 9.6 mg/l for *Poecilia reticulata* was obtained from this study.

Benville and Korn (1977) investigated the toxicity of ethylbenzene to the striped bass *Morone saxatilis*. Fish were exposed in a static system for 96 h with five test concentrations (nominal concentration range from 1.0 to 20 mg/l). A saturated solution of ethylbenzene was prepared and diluted to the desired concentration with natural seawater (salinity 25‰, temperature 16°C). The study was designed as a single dose test where the concentration was decreasing over time. Based on analytical results it was observed that more than 99% of the initial concentration in the test system was lost within 24 hours. As no attempt was made to supplement the toxicant and it is not known how the mean exposure concentration over the whole test duration was calculated, the test result is regarded as not valid.

A further acute toxicity bioassay was conducted by Boeri (1987a) using the Atlantic silverside *Menidia menidia* as test organism. The test was performed under flow-through conditions with five test concentrations ranging nominally from 21 to 100 mg/l and a dilution water control. Chemical analysis of ethylbenzene in the test vessels was conducted on samples collected at 0, 48 and 96 hours after the start of the test. Suitable precautions were taken to pre-

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vent losses of test substance by volatilization in the test samples. As dilution water natural seawater (salinity 20-21‰, temperature 21-22°C, dissolved oxygen 6.9-9.2 mg/l, pH 7.7-8.2) was used. Twenty fish per treatment divided in two groups (average length 12 mm, average weight 0.008 g, age not stated) were exposed. Referring to mean, measured concentrations a 96 h-LC_{50} of 5.1 mg/l (95% confidence limits 4.4-5.7 mg/l) was found.

3.2.1.1.2 Aquatic invertebrates

The 96 hr acute toxicity of ethylbenzene to the mysid *Mysidopsis bahia* was determined by Boeri (1988). The test was conducted under flow-through conditions with five test concentrations of ethylbenzene ranging nominally from 1.0 to 9.1 mg/l and a dilution water control. As dilution water filtered natural seawater (salinity 20‰, pH 7.8-8.0, $CaCO_3$ 117 mg/l, temperature 25 +/- 1°C) was used. Twenty mysids were randomly distributed among two replicates of each concentration level. Test vessels consisted of loosely covered glass aquaria that contained 9 l of media and mysids were fed live *Artemia salina* during the test. The number of live mysids and the occurrence of abnormal behavior were determined daily. Aeration was not employed during the test and the photoperiod was adjusted to 14 h light and 10 h dark. Based on mean measured concentrations a 96 h-LC_{50} of 2.6 mg/l (95% confidence limits 2.0-3.3 mg/l) was derived.

Vigano (1993) investigated the ecotoxic effect of ethylbenzene on juvenile *Daphnia magna* fed on different food rations. Animals were tested in closed bottles that were completely filled with test solutions. Concentration of dissolved oxygen, measured at the end of the tests (48 h), was always higher than 5 mg/l (approx. 60% saturation). The test medium had a hardness of 150 mg/l as $CaCO_3$, alkalinity 120 mg/l as $CaCO_3$, Ca/Mg = 4, Na/K = 1, water temperature 20°C. Cultures were maintained under a photoperiod of 16 h light and 8 h dark. Toxic effects were recorded as percentage of immobility at 24 and 48 h of exposure. Test results were based on measured concentrations. The toxicity test results at 48 h of exposure revealed only minor effects of feeding levels. A 24 h-EC_{50} of 2.3-2.9 mg/l and a 48 h-EC_{50} of 1.8-2.4 mg/l respectively was obtained from this study.

The response of the daphnid *Ceriodaphnia dubia* to ethylbenzene was determined by Niederlehner et al. (1998) using the standard short-term and chronic test method developed for U.S. EPA's Whole Effluent Testing Program (U.S. EPA 1994) The test design was modified to minimize volatilization of test chemicals. Organisms were tested in completely filled and tightly closed bottles. Tests were conducted in artificial moderately hard water (pH 7.6, hardness 68.3, alkalinity 88.0, dissolved oxygen above 7.0 ppm). Light was provided at a photoperiod of 16 h light and 8 h dark. Solutions were renewed daily. Survival, behaviour, and reproduction were observed at the time of daily renewal. Acute toxicological results were based on the geometric mean of two measured concentrations for each treatment level measured at the beginning of the test. Chronic toxicological results were based on the geometric mean of four measured concentrations of each treatment level measured during the duration of the experiment. 84.4% of initial test concentration was present at the end of the study. The toxicity studies in *Ceriodaphnia dubia* found with a 48 h-LC_{50} of 3.2 mg/l, a 7 d-LC_{50} of 3.6 mg/l and a 7 d-IC_{50} of 3.3 mg/l (reproduction) almost identical values for the acute and the repeated exposure toxicity. As long-term NOEC a 7 d-NOEC of 1.0 mg/l (reproduction) was derived.

A further acute toxicity test was conducted by Abernethy et al. (1986) with two planktonic crustaceans (freshwater *Daphnia magna* and saltwater *Artemia salina*) as test organisms. A

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saturated aqueous solution of the test substance was prepared and diluted to provide at least five exposure concentrations. As dilution water distilled water and saltwater (salinity 30‰) respectively was used. To minimize volatilization loss of the toxicant from water, test chambers were filled completely and sealed. Bioassays were conducted in the dark at approx. 23°C for *Daphnia* and at approx. 20°C for *Artemia* tests. Toxicant solutions were not aerated during the exposure period, the lowest oxygen concentration measured after 48 h was 5 mg/l in *Daphnia* tests. As the effective concentrations were not measured, results of this study should be used with care. Referring to nominal concentrations a 48 h-LC_{50} of 2.1 mg/l for *Daphnia magna* and a 24 h-LC_{50} of 15.4 mg/l for *Artemia salina* was obtained.

An acute immobilzation study with *Daphnia magna* in reconstituted freshwater and *Artemia salina* in synthetic seawater (salinity 30‰) was performed by Maclean and Doe (1989). As test substance the water soluble fraction of ethylbenzene was used. Test chambers were capped immediately with no air space. Tests with *Artemia salina* were conducted in the dark at a temperature of approx. 20°C, tests with *Daphnia magna* were carried out with a photoperiod of 16 h light and 8 h dark at temperature of approx. 20°C. Initially and at test termination selected test solutions were analysed using fluorescence spectroscopy. Test solutions were also measured for temperature, dissolved oxygen and pH. For *Daphnia magna* a 48 h-EC_{50} of 2.9 mg/l and for *Artemia salina* a 48 h-EC_{50} of 9.2 mg/l was derived.

Galassi et al. (1988) studied the acute toxicity of ethylbenzene in *Daphnia magna*. Toxicity tests were carried out according to the OECD Guideline 202 "Immobilization". The determination of 24-h IC_{50} (immobilization concentration for 50% of the organisms) on Daphnia was performed in closed bottles, completely filled with the test solution. A 48 h-IC_{50} of 2.2 mg/l based on the mean values of the analytically detected concentrations was reported.

Benville and Korn (1977) studied the toxicity of ethylbenzene to the bay shrimp *Crago franciscorum*. Mature shrimp were exposed for 96 h in a static system with five test concentrations (nominal concentration range from 1.0 to 20 mg/l). A saturated solution of ethylbenzene was prepared and diluted to the desired concentration with natural seawater (salinity 25‰, temperature 16°C). The study was designed as a single dose test where the concentration was decreasing over time. Based on analytical results it was observed that more than 99% of the initial concentration in the test system was lost within 24 hours. As no attempt was made to supplement the toxicant and it is not known how the mean exposure concentration over the whole test duration was calculated, the test result is regarded as not valid.

3.2.1.1.3 Algae

The acute toxicity of ethylbenzene to the freshwater algae *Selenenastrum capricornutum* and to the diatom *Skeletonema costatum* was determined by Boeri (1987b; 1987c). Tests were conducted with five test concentrations of ethylbenzene ranging nominally from 15 to 100 mg/l and a dilution water control. As test water deionized water (pH 4.2, $CaCO_3$ < 5.0 mg/l, temperature approx. 24°C) and natural seawater (pH 8.0, salinity 30‰, temperature approx. 20°C) respectively was used. To minimize the loss of test substance by volatilization test vessels consisted of 40 ml glass vials filled to capacity and were sealed with Teflon caps. Test vessels were replicated 16 times, allowing three vials to be sacrificed for algal counts at 24, 48, 72 and 96 hours after the start of the test and four vials to be taken for chemical analysis at 0, 48 and 96 hours after the start of the test. Analytical determination of test material was performed on media from one replicate test vial at the initiation and termination of the test. All calculations were performed using mean, measured concentrations. A 96 h-E_RC_{50} of 5.0 mg/l

(95% confidence limits 4.6-5.5 mg/l) and a 96 h-E_RC_{10} of 3.4 mg/l was derived for *Selenastrum capricornutum*. Since the algae in the study with *Skeletonema costatum* were only during the first 72 hours in the exponential growth phase, the effects assessment for *Skeletonema costatum* is based on this period leading to a 72 h-E_RC_{50} of 7.7 mg/l (95% confidence limits 6.79-8.79 mg/l) and a 72 h-NOEC of 4.5 mg/l.

Galassi et al. (1988) studied the acute toxicity of ethylbenzene in *Selenastrum capricornutum*. Toxicity tests were carried out according to the OECD Guideline 201 "Alga, Growth Inhibition Test". For the determination of 72 h-EC_{50} (concentration resulting in 50% growth inhibition) on *Selenastrum* the test vessels were modified in order to maintain constant concentration and to allow sampling of the culture medium without opening the vessel. Algal growth was evaluated by a Coulter Counter. A 72 h-E_RC_{50} of 4.6 mg/l based on the mean values of the analytically detected concentrations was reported.

3.2.1.1.4 Microorganisms

Several data sets are reported for microorganisms including studies with mixed or single-species inoculum of aerobic and anaerobic bacteria. Additionally, results from protozoa are available. From the data reported, the most relevant are described in more detail below.
Blum and Speece (1991) investigated the toxicity of several chemicals to aerobic heterotrophs and *Nitrosomonas*. Assays were carried out in sealed serum bottles under similar experimental conditions:
Seed bacteria for the aerobic heterotroph culture were obtained from the mixed liquor of an activated sludge wastewater treatment plant. Diffused aeration provided complete mixing and aeration in the reactor. The reactor was fed a complex carbon source (COD 3800 mg/l). The hydraulic and the solids retention time was 5 days. The nitrifier populations were estimated to be only 1 % of the total bacteria population. Inhibition of oxygen uptake was used as criterion for the toxic inhibition of the aerobic heterotrophs. Data were collected at 15, 27, 38 and 49 hours after the start of the test.
The seed bacteria for the nitrifying enrichment culture was obtained from the mixed liquor of an activated sludge plant treating meat-packing, rendering, and hide-curing wastewater. Diffused aeration provided complete mixing and aeration in the reactor. The culture was fed two times per day approximately 1000 mg/l ammonia-nitrogen. The hydraulic and the solids retention time was 25 days. As the criterion for the toxic inhibition of *Nitrosomonas* the inhibition of ammonia consumption was used.

Table 3.19 Toxicity in aerobic heterotrophs *and Nitrosomonas (*Blume and Speece, 1991*)*

Species	exposure period	Temp. [°C]	effect concentration	Initial atmosphere	effect
Aerobic heterotrophs	15 h	25 or 35	15 h-IC_{50} = 130 mg/l	pH =7 N2 : O2 = 1 : 1	Inhibition of oxygen consumption
Nitrosomonas	24 h	25	24 h-IC_{50} = 96 mg/l	pH =7 N2 : O2 = 1.6 : 1	Inhibition of ammonia consumption

In a respiration inhibition test according to OECD Guideline 209 (test was modified to minimize the loss of volatile organic compunds) Volskay and Grady (1990) observed after 30 minutes of incubation a 30%-decrease in oxygen consumption. The concentration of ethyl-

benzene was at the limit of solubility in the medium (approximately 150 mg/l). The activated sludge source used for the experiment was maintained in continuous flow reactor (solids retention time 2 days, temperature 10°C) receiving a complex synthetic feed designed to mimic the soluble content of domestic wastewater. Because the culture was grown primarily for other purposes, the feed also contained different aromatics. In a respiration inhibition kinetic analysis (RIKA) with butyric acid as substrate the same authors recorded after 3 hours of exposure 100% inhibition for the same initial concentration.
Bringmann and Kühn (1977) studied the effects of ethylbenzene on bacteria in a cell multiplication inhibition study. For *Pseudomonas putida* a toxicity threshold of 12 mg/l (exposure period 16 hours) was obtained. As other more reliable test results are available for ethylbenzene this result is not used for the assessment.
Table 3.20 summarizes the test results for different protozoa which were obtained from cell multiplication inhibition studies by Bringmann and Kühn (1981). As the test vessels were sealed only with metal caps and the effective concentrations of ethylbenzene were not measured, the reported toxicity threshold values should be used with care.

Table 3.20 Toxicity in protozoa (Bringmann and Kühn ,1981)

Species	**exposure period**	**pH**	**Temp. [°C]**	**effect concentration**
Chilomonas paramaecium (saprozoic flagellates)	48 h	6.9	20	48 h-TGK (EC_5)> 56
Uronema parduzci (holozoic, bacterial-eating ciliates)	20 h	6.9	25	20 h-TGK (EC_5)> 110
Entosiphon sulcatum (holozoic, bacterial-eating flagellates)	72 h	6.9	25	72 h-TGK (EC_5)= 140

In an oxygen consumption test with mixed bacterial populations Krebs (1991) determined a 24 h-EC_{10} of 21 mg/l and a 24 h-EC_{50} of 53 mg/l. The oxygen consumption test is a method, where the intoxication of the biochemical degradation of organic substances by heterotrophic bacteria occurring in surface waters is measured via a reduced oxygen depletion in sealed incubation bottles. As this method is designed to assess the harmful effects of waste waters on the self purification capacity of surface waters, which are not representative for an activated sludge system, this test result is regarded as not suitable for the determination of a $PNEC_{micro\text{-}organism}$.

3.2.1.2 Calculation of Predicted No Effect Concentration (PNEC)

3.2.1.2.1 Determination of PNECaqua

For ethylbenzene short-term results are available for organisms representing three trophic levels including freshwater and saltwater species. The relevant LC_{50}-/EC_{50}-values are in the narrow range of 1.8 mg/l (daphnids) to 4.6 mg/l (algae) showing a similar sensitivity of the three trophic levels. The most sensitive organism appears to be the aquatic invertebrate *Daphnia magna* with a 48 h-EC_{50} of 1.8 mg/l.

As two long-term NOECs from two trophic levels are available (aquatic invertebrates and algae) according to TGD an assessment factor of 50 should be used. The most sensitive species in long-term studies is the saltwater invertebrate *Ceriodaphnia dubia* with a 7 d-NOEC of 1.0 mg/l.

However, it was pointed out by Member States that ethylbenzene is a neutral organic substance well-known to act by baseline toxicity. Consequently the TC NES agreed to use an assessment factor of 10 in consistency with the approach taken for other neutral organic chemicals. Applying an assessment factor of 10 to the long-term result of aquatic invertebrates leads to

$$\mathbf{PNEC_{aqua} = 1.0\ mg/l\ /\ 10 = 100\ \mu g/l}$$

3.2.1.2.2 Determination of $PNEC_{microorganisms}$

According to the different endpoints and sensitivities of the used test systems, the TGD recommends the following assessment factors (AF):

Activated sludge (respiration inhibition)	15 h-EC_{50}	= 130 mg/l	AF = 100	⇒ PNEC = 1.3 mg/l
Nitrosomonas	24 h-EC_{50}	= 96 mg/l	AF = 10	⇒ PNEC = 9.6 mg/l

The most sensitive microorganism to ethylbenzene was *Nitrosomonas* representing the nitrification step in a sewage treatment plant (STP).
Although the PNEC derived from the activated sludge test is lower than the PNEC derived from the lowest effect value for *Nitrosomonas*, it is proposed to use the latter for the risk assessment according to TGD. Therefore,

$$\mathbf{PNEC_{microorganisms} = 9.6\ mg/l}$$

3.2.1.3 Toxicity test results for sediment organisms

As no experimental results with benthic organisms are available the $PNEC_{sed}$ can be provisionally calculated using the equilibrium partitioning method. According to TGD this method uses the $PNEC_{aqua}$ for aquatic organisms (= 100 µg/l) and the Ksusp_water of 11.7 m^3/m^3 as inputs and leads to

$$PNEC_{sed} = \frac{K_{susp_water}}{RHO_{susp}} \cdot PNEC_{water} \cdot 1000$$

$$\mathbf{PNEC_{sed} = 1017\ \mu g/kg_{\ wwt}}$$

3.2.2 Terrestrial compartment

3.2.2.1 Toxicity test results

3.2.2.1.1 Earthworm

In an earthworm contact test with four earthworm species (*Eisenia fetida*, *Allolobophora tuberculada, Eudrilus eugeniae* and *erionyx excavatus*) as test organisms Callahan et al. (1994) found a LC_{50} of 4.93 µg kg^{-1} body weight after two days of exposure. As the report does not specify which species was used for ethylbenzene, this test result is not assignable.

A further study of 48 hours duration using filter paper media was conducted in an indoor laboratory with laboratory obtained *Eisenia fetida* (earthworm). In this study Neuhauser et al. (1985) found a 48 h-LC_{50} of 47 µg cm^{-2} contact area (95% confidence level 42-53 µg cm^{-2}).

3.2.2.2 Calculation of Predicted No Effect Concentration ($PNEC_{soil}$)

The only available test results for the terrestrial compartment originate from contact tests with soil dwelling organisms. A $PNEC_{soil}$ cannot be deduced from these data.
If there are no data on effects to terrestrial organisms available, for an indicative risk assessment, the $PNEC_{soil}$ can be provisionally calculated using the equilibrium partitioning method. Applying this method and using K_{soil_water} of 13.2 m^3/m^3 leads to

$$PNEC_{soil} = \frac{K_{soil_water}}{RHO_{soil}} \cdot PNEC_{water} \cdot 1000$$

$PNEC_{soil, calculated}$ = 776 µg/kg (wet weight)

3.2.3 Atmosphere

3.2.3.1 Biotic effects

<u>Terrestrial plants</u>
On exposing leaves of the runner bean (*Phaseolus multiflorus*) and parsnip (*Pastinaca sativa*) to ethylbenzene at a range of vapour concentrations for 1 h it was found that the concentrations causing no damage and those completely killing the leaves were close. The author subsequently determined an EC_{50} of 27 and 48 g/m^3 respectively (Ivens, 1952).

Determination of $PNEC_{air}$

The only available experimental data on terrestrial plants are obtained from fumigation studies with *Phaseolus multiflorus* and *Pasticana sativa*.

Data base is considered to be not sufficient for the derivation of the $PNEC_{air}$ for ethylbenzene.

3.2.3.2 Tropospheric ozone formation

The formation of tropospheric ozone involves complicated chemical reactions between NO_x and VOC driven by the solar radiation. In order for these reactions to occur in substantial quantities, meteorological conditions must prevail that prevents dispersion of NO_x and hydrocarbons. After a night time accumulation NO_x reacts with sunlight to produce NO and highly reactive atomic oxygen. The atomic oxygen may react with many compounds in the air, i.e. O_2 to produce O_3 or VOC to produce free radicals. The time scale of ozone production is such that ozone concentrations may build up over several days under suitable weather conditions, and that this pollutant and its precursors can be transported over considerable distances (European Commission DG XI, 1998).
There is as yet no consensus on the quantitative yield of these reactions, making modelling of these processes difficult. In addition to the VOC speciation and concentrations, VOC/NO_x ratio, solar radiation and meteorological conditions vary from city to city within the EU. Since the environmental conditions differ considerably, a certain concentration of VOC may lead to very different ozone concentrations within the EU. For example European Commission DGXI (European Commission DG XI, 1998) used a simplified EMEP model calculations and showed how a change in the VOC concentration may affect the ozone formation to a small extent in some parts of Europe (NO_x limited region), while in other parts of Europe a change in the VOC concentration will lead to a considerable change in the ozone formation (high NO_x regions). Thus there is no simple relationship between the VOC and NO_x concentrations and the resulting tropospheric ozone creation. The ozone concentrations may at some places of Europe even be higher at the same VOC concentration and at lower NO_x concentrations than may be the case at other places. Likewise the time trends of the tropospheric ozone concentration for Europe in general cannot not be forecasted by predicting the future concentrations of VOC and NO_x.
Nevertheless, the member countries in UNECE have agreed to use a Photochemical Ozone Creation Potential (POCP) factor system where the individual VOC's potential to create ozone is given as a relative equivalence factor expressed as g ethylene / g VOC (gas) (Hauschild and Wenzel, 1998). Here their relative importance has been evaluated against ethylene, which is given a value of 100. Two sets of factors exist corresponding to a low and high NO_x situation.
Hauschild and Wenzel (1998) proposed POCP equivalence factors for ethylbenzene of 0.5 g C_2H_4/g ethylbenzene gas in a low NO_x situation and 0.6 g C_2H_4/g ethylbenzene gas in a high NO_x situation.
To evaluate the relative importance of ethylbenzene for the creation of troposheric ozone using the POCP factor system the VOC composition within the region of concern has to be known. For a simple evaluation of the relative importance of the isolated commercial product ethylbenzene for the creation of ozone the VOC composition from industrial sources as well as the VOC composition from other sources e.g. traffic emissions have to be known. For a more in depth evaluation also the solar radiation and the NO_x concentrations have to be taken into account. These will of course vary considerably in Europe, between regions and between

individual sites within the region as will also the VOC composition which depends on composition of the regional / local industrial sector and the traffic.
In the following an attempt to evaluate the relative contribution of non-isolated ethylbenzene (traffic emissions) and isolated ethylbenzene to the ozone creation potential has been performed.

3.2.3.2.1 Creation of tropospheric ozone due to non-isolated ethylbenzene in car exhaust based on one monitoring event

In Table 3.21 the mean road site concentrations of individual NMVOCs (non-methane volatile organic compounds) at a site in Copenhagen during 5 d in December 1997 is shown. Using the POCP equivalence factors it is possible to estimate the relative contribution of non-isolated ethylbenzene to the potential overall tropospheric ozone creation for such a NMVOC composition. It has to be emphasised that NMVOC composition from this site in Copenhagen is only used as an example, and that it is unlikely in this specific case that considerable ozone concentrations will build up within the region of Copenhagen as a consequence of these ethylbenzene concentrations due to low solar radiation and the prevailing wind conditions.

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Table 3.21 **Monitoring results of different NMVOCs at Jagtvej, Copenhagen December 1-5 1997 (Christensen (1999), and the relative contribution to potential ozone creation. Table from the EU Toluene RAR, Final Report March 2001**

Substance	Mean ppbv	Range ppbv	S.D. ppbv	Median ppbv	POCP g C2H4/g gas[5] low NOx	High NOx	Relative O3 creation[6] low NOx	high NOx
Pentane	2.4	0.4-5.7	1.2	2.5	0.3	0.4	2.12E-03	2.83E-03
trans-2-Pentene	0.2	0.01-0.5	0.1	0.2	0.4	0.9	2.29E-04	5.16E-04
2-Methyl-2-butene	0.4	0.02-0.9	0.2	0.3	0.5	0.8	5.73E-04	9.17E-04
cis-2-Pentene	0.1	0.01-0.3	0.1	0.1	0.4[7]	0.9	1.15E-04	2.58E-04
2,2-Dimethylbutane	0.9	0.04-2.3	0.5	0.9	0.3	0.3	9.51E-04	9.51E-04
Cyclohexane	0.5	0.04-1.1	0.3	0.5	0.25	0.25	4.30E-04	4.30E-04
2,3-Dimethylbutane	0.4	0.03-1.0	0.2	0.4	0.4	0.4	5.64E-04	5.64E-04
2-Methylpentane	2	0.2-5.2	1.1	2.1	0.5	0.5	3.52E-03	3.52E-03
3-Methylpentane	1.1	0.1-2.7	0.6	1	0.4	0.4	1.55E-03	1.55E-03
n-Hexane	0.8	0.1-2.3	0.5	0.8	0.5	0.4	1.41E-03	1.13E-03
Isoprene	0.2	0.01-0.6	0.1	0.2	0.6	0.8	3.34E-04	4.46E-04
2-Methyl-1-Pentene	0.04	0.01-0.1	0.02	0.02	0.5[8]	0.9	6.88E-05	1.24E-04
cis-2-Hexene	0.03	0.01-0.1	0.01	0.02	0.5	0.9	5.16E-05	9.29E-05
2,4-Dimethylpentane	0.2	0.01-0.7	0.1	0.2	0.4[9]	0.4	3.28E-04	3.28E-04
Methyl-cyclohexane	0.3	0.02-0.6	0.1	0.3	0.5	0.6	6.02E-04	7.22E-04
2- and 3-Methylhexane	1.4	0.1-3.7	0.8	1.3	0.5	0.5	2.87E-03	2.87E-03
n-heptane	0.7	0.1-1.9	0.4	0.6	0.5	0.5	1.43E-03	1.43E-03
Benzene	3.4	0.2-8.0	1.7	3.3	0.4	0.2	4.34E-03	2.17E-03
2- and 3-Methylheptane	0.4	0.01-1.0	0.2	0.3	0.5	0.5	9.34E-04	9.34E-04
Toluene	10.2	0.8-21.5	5.6	8.9	0.47	0.6	1.81E-02	2.31E-02
Ethylbenzene	2	0.1-4.9	1.1	1.9	0.5	0.6	4.34E-03	5.21E-03
o-Xylene	2.7	0.1-6.2	1.4	2.6	0.2	0.7	2.34E-03	8.20E-03
m- and p-Xylene	5.5	0.3-12.7	2.9	5.5	0.5	0.95	1.19E-02	2.27E-02
Relative contribution of non-isolated ethylbenzene: %							7.34	6.41

The result of this calculation shows that if the VOC composition is as measured in the Copenhagen study non-isolated ethylbenzene potentially would exhibit approx. between 6.4 and 7.4 % of the total VOC contribution to ozone creation.

3.2.3.2.2 Creation of tropospheric ozone due to ethylbenzene based on estimated emissions

As described the creation of tropospheric ozone is dependent on the occurrence of VOC, NO_x, solar radiation and thus OH-radicals in a complicated relationship. The VOC composition will

[5] POCP equivalence factors from Hauschild & Wenzel (1998) except for cyclohexane from EU RAR

[6] Calculated at Standard Temperature and Pressure (STP)

[7] Data for *trans*-2-Pentene used

[8] Average data for alkanes with double bonds used

[9] Average data for alkanes without double bonds used

be highly variable and depend on the industrial sources, traffic emissions and natural sources. The contribution from isolated commercial ethylbenzene will depend on the composition of local and regional industry. Therefore, average calculations are likely to underestimate the magnitude of the problem within certain regions with high exposure potential.
The total NMVOC emitted in EU15 is shown in the table below.

Table 3.22 Emission of NMVOC in EU15 (EEA - European Environment Agency, 2001)[10]

NMVOC in EU15 (Kilotons)										
1980	1985	1990	1991	1992	1993	1994	1995	1996	1997	1998
16435	16248	16097	15521	14690	14323	14203	13736	13078	12715	12478

The mean total emission of NMVOC in 1998 is approx. 12 500 kt/a. This value is in the following table related to the known emissions of ethylbenzene (cf. 3.1.9).

Table 3.23 Fraction of ethylbenzene emissions to gas phase of total NMVOC emissions in EU15

	Emission [t/a]	**% of 12 500 kt/a**
Isolated (production and processing)	2559	0.02
Non-isolated (traffic and mixed solvent)	85412	0.68
Total emissions for EU	87971	0.70

The proportion of isolated ethylbenzene relative to total NMVOC is approx. 0.02 %. If non-isolated emissions are included in this estimate, the portion of ethylbenzene rises to about 0.7 % of the total NMVOC emissions.
The POCP equivalence factor for the total NMVOC is not known because the composition of individual NMVOC species is not available. Ethylbenzene may have a slightly higher photochemical ozone creation potential than the average NMVOC and thus contribute slightly more to the ozone creation than indicated by the proportion of isolated ethylbenzene relative to total NMVOC.
It has to be emphasised that the local and regional NMVOC composition may have a higher concentration of ethylbenzene than indicated by the average calculations due to differences in local NMVOC sources.
To conclude isolated ethylbenzene contributes in the order of 0.02 % to the total NMVOC emission. Thus isolated ethylbenzene in general only contributes to a small extent to the total SMOG problem, however, for a single substance among hundreds of different VOCs the contribution may be significant.

3.2.4 Secondary poisoning

As the available bioaccumulation studies indicate that ethylbenzene has only a low bioaccumulation potential, it is not necessary to perform a risk assessment for secondary poisoning.

[10] More up-to-date value not available at that source.

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3.3 RISK CHARACTERISATION [11]

3.3.1 Aquatic compartment (incl. sediment)

3.3.1.1 Surface water

Using the derived PNECaqua of 100 µg/l, the following PEC/PNEC ratios are obtained for surface water. Please refer to Chapter 3.3.4 for sites located at the sea.

Table 3.24 PEC/PNEC ratios for aquatic compartment (surface water)

		site-specific information	**$PEClocal_{water}$ [µg/l]**	**$PEClocal_{water}/PNEC_{aqua}$**
P1	production	effluent concentration, emission days	1.31	0.013
P2	production	emission factor, river flow rate, emission days	0.06	6×10^{-4}
PP1	production and processing	effluent concentration, dilution	0.07	7×10^{-4}
PP2	production and processing	release for production, effluent concentration	14.1	0.14
PP3	production and processing	effluent discharge rate, municipal wwtp, emission days	5.0	0.05
PP4	production and processing	effluent concentration, dilution, emission days	10.1	0.10
PP6	production and processing	effluent concentration, dilution, emission days	0.09	9×10^{-4}
PP8	production and processing	river flow rate, emission days, effluent concentration	0.19	2×10^{-3}
PP9	production and processing	effluent concentration	0.19	2×10^{-3}

[11] Conclusion (i) There is a need for further information and/or testing.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

PP10	production and processing	WWTP influent concentration, dilution	0.09	9×10^{-4}
PP11	production and processing	effluent concentration, elimination –in WWTP, effluent discharge rate	5.81	0.06
	other processing (generic)	-	16.7	0.17

PEC/PNEC < 1 could be derived for all known sites at which production, or production and processing of ethylbenzene take place. A PEC/PNEC < 1 was also derived for the generically calculated life cycle step “other processing”.

Conclusions to the risk assessment for the aquatic compartment (surface water):

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to all sites where production, or production and processing take place.

3.3.1.2 Sediment

No experimental results with benthic organisms are available, hence only a provisional $PNEC_{sed}$ was calculated using the equilibrium partitioning method. No risk characterisation was performed. $PEC/PNEC_{sed}$ is covered by $PEC/PNEC_{aqua}$.

3.3.1.3 Waste water treatment plants

Using the derived $PNEC_{microorgansims}$ of 9.6 mg/l, the following PEC/PNEC ratios are obtained. Sites with non-biological or without any WWTP are not listed in this table.

Table 3.25 PEC/PNEC ratios for microorganisms

		site-specific information	**Clocal $_{eff}$ (= PEC $_{stp}$) [mg/l]**	**$PEC_{stp}/PNEC_{microorganisms}$**
P1	production	effluent concentration, emission days	0.05	5×10^{-3}
P2	production	emission factor, river flow rate, emission days	0	0

P5	production and processing	emission days, effluent concentration	1 x 10^{-3}	1 x 10^{-4}
PP1	production and processing	effluent concentration, dilution	2 x 10^{-4}	2 x 10^{-5}
PP3	production and processing	effluent discharge rate, municipal wwtp, emission days	0.050	5 x 10^{-3}
PP4	production and processing	effluent concentration, dilution, emission days	1.0	0.10
PP6	production and processing	effluent concentration, dilution, emission days	0.01	1 x 10^{-3}
PP7	production and processing	effluent discharge rate, effluent concentration	0.062	6 x 10^{-3}
PP8	production and processing	river flow rate, emission days, effluent concentration	0.02	2 x 10^{-3}
PP9	production and processing	effluent concentration	0.005	5 x 10^{-4}
PP10	production and processing	WWTP influent concentration, dilution	5 x 10^{-4}	5 x 10^{-5}
PP11	production and processing	effluent concentration, elimination in WWTP, effluent discharge rate	0.23	0.02
	other processing (generic)	-	0.665	0.07

All PEC/PNEC ratios are below 1, and so it is unlikely that ethylbenzene poses a risk to microorganisms in the WWTP.

Conclusions to the risk assessment for the waste water treatment plants:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to all sites where production, or production and processing take place.

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3.3.2 Terrestrial compartment

Releases of ethylbenzene into the terrestrial compartment are to be expected as a result of deposition and sludge application. C_{sludge} used was calculated from production and processing at PP3 the only site known to discharge effluents to a municipal WWTP. A $PEClocal_{soil}$ of 0.066 mg/kg was obtained for site PP3. Comparison with the calculated $PNEC_{soil}$ of 776 µg/kg results in a PEC/PNEC of 0.08.
For comparison, the $PEClocal_{soil}$ at site PP1 was calculated. Input takes place only via deposition (maximum deposition rate at that site). The derived $PEClocal_{soil}$ of 0.039 mg/kg is below $PEClocal_{soil}$ (PP3). There is no indication of a risk.
A further calculation for site PP1 using the deposition rate of $DEP_{total\ ann}$ (PP1) = 0.932 mg/(m^2 d) and the sludge concentration calculated for PP3 of C_{sludge} of 107 mg/kg_{dw}, resulted in a $PEClocal_{soil}$ of 0.104 mg/kg which again leads to PEC/PNEC < 1.

Conclusions to the risk assessment for the terrestrial compartment:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to production and processing of ethylbenzene.

3.3.3 Atmosphere

On account of the atmospheric half-life ($t_{1/2}$ = approx. 2.3 days), abiotic effects on the atmosphere, such as ozone depletion in the stratosphere, are not to be expected in the case of ethylbenzene.
Direct releases into the atmosphere occur during production and processing of pure ethylbenzene (about 2 540 t/a for Europe). Indirect releases come from stripping processes in waste water treatment plants. According to the SimpleTreat calculations 46.7 % of the releases to WWTP are accounted to air (about 311 t/a). Thus, WWTP are a not negligible source of ethylbenzene.
The calculated $Clocal_{air_ann}$ ranging from < 0.001 mg/m^3 to 0.9 mg/m^3. The 90th percentile of all sites for the production and/or processing of pure ethylbenzene is 0.330 mg/m^3 and the mean $Clocal_{air_ann}$ is 0.116 mg/m^3.
As derived in Chapter 3.2.3.1, the effect data are very scanty and insufficient for the derivation of a distinct PNEC. However, they allow the statement that ethylbenzene seems not to be of concern for plants with regard to exposure via the atmosphere except at very high concentrations (g/m^3). Therefore, only an indicative risk characterization for the atmospheric compartment is conducted using selected measured or calculated environmental concentrations. As highest ethylbenzene concentrations in air the PEC_{local} (100 m distance from point source) of 0.9 mg/m^3 is chosen. This value is a factor of 30 000 below the concentration range at which effects on plants were observed. In view of these ratios it should be concluded that in the present immission situation no harmful effects on outdoor vegetation resulting from exposure to ethylbenzene in air are to be expected.
Result
(ii) There is at present no need for further information and/or testing.

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It is known that ethylbenzene contributes to tropospheric VOC and contributes to the tropospheric formation of ozone. The photochemical formation of ozone and other compounds depends on emission of all VOCs and other compounds in a complex interaction with other factors.

Changes in VOC emissions lead to changes in ozone formation. The efficiency of VOC emission reductions in reducing ground level ozone concentrations may vary from place to place and depends on the occurrence of NO_x, the solar radiation and the prevailing wind conditions. Thus the effects on ozone creation of emissions arising from the production and use of the isolated commercial product ethylbenzene may differ substantially between different regions in the EU.

The industrial use of the commercial product ethylbenzene contributes significantly to the overall emission of ethylbenzene, however, emission of ethylbenzene in exhaust gases expelled from motor vehicles seem to be the largest single source.

Based on a rough estimation utilising available information, the current risk assessment indicates that emission of ethylbenzene from the use and production of the commercial product ethylbenzene may be in the order of 0.02 % of total NMVOC emissions. Locally and regionally this proportion may vary substantially due to differences between regions in the VOC emission pattern from industrial sectors using ethylbenzene. Even a simple evaluation of the photochemical ozone creation potential of the emission of isolated ethylbenzene is difficult to perform, when the emission pattern of individual NMVOCs is not available.

Effects of ozone exposure are documented on plants, animals and humans. Reporting on monitoring results are most frequently done in relation to exceedance of thresholds for information or warning of the human population, but this reporting may also give indication on the magnitude of environmental effects, because effect concentrations seem to be in the same order of magnitude for both vegetation and humans. The threshold values set by the European Union to protect human health and the vegetation are frequently exceeded (cf. e.g. De Leeuw et al, 1996)

In 1995 90% of the EU population (both urban and rural) experienced an exceedance of the current EU threshold for health protection (110 240 µg/m3, 8h average) for at least one day during the summer 1995. Over 80% experienced exposure above the threshold for more than 25 days. The highest concentrations (≥240 µg/m3) were recorded in Italy and Greece (De Leeuw et al., 1996; WHO, 1999).

In 1999 the threshold for information of the public in EU (180 $\mu g/m^3$, 1h average) were not exceeded in 4 member states while up to 70 % of the monitoring stations in other member states did exceed this threshold (Sluyter and Camu, 1999). On average 27 % of all monitoring stations in EU did exceed the threshold. The number of days that the threshold was exceeded ranged from 2 days in Luxembourg to 68 days in Italy (out of 153 days in the reporting season).

The severity of exceedance of the EU threshold for health protection (110 $\mu g/m^3$, 8 h average) has been estimated by WHO (WHO, 1999). The 1995 summer ozone incidence is estimated to have caused 1500-3700 deaths (0.1-0.2 % of all deaths) and further 300-1000 extra emergency hospital admissions due to respiratory diseases. "It is likely that the total number of health impacts is higher than the estimated impact of the days with high levels only. This is suggested by epidemiological studies where the effects can be seen also below the 110 $\mu g/m^3$ level." (WHO, 1999).

If these figures are used to estimate the impact of emissions from the production and use of the commercial product ethylbenzene through formation of ozone then this emission may

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have probably caused 1 death in the summer of 1995 if a linear relationship exists between the emission of ethylbenzene, the emission of NMVOCs and the creation of ozone. Similarly, the vegetation and wildlife may be severely affected by ozone incidences and ethylbenzene is likely to contribute to these effects.
However, no simple relationship has been established between the proportion of ethylbenzene to total NMVOC emitted - and thus also between emissions arising from the use of the commercial product ethylbenzene - and the creation of tropospheric ozone.

Result
(iii) There is a need for limiting the risk; risk reduction measures which are being applied shall be taken into account.

Conclusions to the risk assessment for the atmosphere:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (ii) applies to biotic effects of ethylbenzene in the atmosphere.

Conclusion (iii) applies to the contribution of the commercial product ethylbenzene to the formation of ozone. In the context of the consideration of which risk reduction measures that would the most appropriate, it is recommended that under the relevant Air Quality Directives a specific in-depth evaluation be performed. Such an evaluation should focus on the contribution of isolated as well as non-isolated ethylbenzene to the complex issue of ozone and smog formation and the resulting impact on air quality.

3.3.4 Marine Assessment including PBT Assessment

3.3.4.1 PBT Assessment

The following table shows the PBT/vPvB criteria as defined in the TGD and the values relevant for ethylbenzene. The description of the relevant tests can be found in Chapter 3.1.3 (**P** and **B**) and in Chapter 3.2 (**T**).

Table 3.26 Data for ethylbenzene and PBT/vPvB criteria according to TGD

Criterion	**PBT-criteria**	**vPvB-criteria**	**Ethylbenzene**
P	Half-life > 60 d in marine water or > 40 d in freshwater or half-life > 180 d in marine sediment or > 120 d in freshwater sediment	Half-life > 60 d in marine- or freshwater or half-life > 180 d in marine or freshwater sediment	readily biodegradable in surface water (half-life: 15d)
B	BCF > 2000	BCF > 5000	BCF < 100
T	Chronic NOEC < 0.01 mg/l or CMR or endocrine disrupting effects	Not applicable	7d NOEC (daphnid): 1.0 mg/l

Ethylbenzene has to be considered as readily biodegradable. There are no tests on degradation of ethylbenzene in soil or sediment available.
A BCF of 92 was derived from log Kow of 3.13 according to TGD. Measured BCF in fish showed BCF ≤ 20, however the validity of the tests is limited.
The lowest long-term effect value of 1.0 mg/l was found for *Ceriodaphnia dubia*.

It can be concluded that ethylbenzene does not meet the PBT criteria.

3.3.4.2 Marine Assessment

There are no indications that ethylbenzene persists or accumulates in the environment. However, it is known that there are 5 sites located at the sea. PEClocal for these sites are estimated using the defaults described in the TGD for the marine assessment. If available, site-specific data are used.
There is information that two of the 5 marine sites operate a WWTP. All other sites discharge effluents directly to the sea. A standard dilution factor of 100 was used. In a first approach the marine regional background concentration was derived from the fresh water value by division by 10:

$$PEC_{regional}\text{ (seawater)} = 6.4 \times 10^{-3}\ \mu g\ l^{-1}.$$

The value was added to the $Clocal_{water}$ to obtain the respective $PEClocal_{water}$ (see Table 3.9) for the marine sites.
The $PNEC_{marine}$ was derived by dividing the $PNEC_{aqua}$ by 10:

$$PNEC_{marine} = \frac{PNEC_{aqua}}{10} = \frac{100\ \mu g\ l^{-1}}{10} = \underline{10\ \mu g\ l^{-1}}$$

Using this $PNEC_{marine}$, the following PEC/PNEC ratios for marine environment can be derived.

Table 3.27 PEC/PNEC ratios for marine sites

		site-specific information	**$PEClocal_{seawater}$ [µg/l]**	**$PEClocal_{seawater}/PNEC_{marine}$**
P3	production	waste water discharge rate, waste water concentration, emission days, discharge to sea	0.08 1	8.1×10^{-3}
P4	production	waste water discharge rate, waste water concentration, emission days, discharge to sea	0.02 5	2.5×10^{-3}
P5	production and processing	emission days, effluent concentration	0.01 6	1.6×10^{-3}
PP5	processing	effluent discharge rate, emission days, discharge to sea	1.07	0.107
PP7	production and processing	effluent concentration, effluent discharge rate	0.63	0.063

The PEC/PNEC < 1 for all marine sites.

Conclusions to the risk assessment for the marine environement:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to all identified marine sites. Ethylbenzene does not meet the PBT or vPvB criteria.

3.3.5 Unintentional uses

As described in Chapter 3.1.2.4 significant emissions of ethylbenzene could occur from unintentional use of ethylbenzene. Main known sources are the "mixed xylenes" which are predominantly used as petrol additive. Emissions are only considered for the background concentration.
A generic calculation to obtain PEClocal was performed for formulation and technical use of mixed xylenes as solvent (see Chapter 3.1.7). If a risk characterisation was to be performed a possible risk could be identified for surface water and soil.

There are only scarce data regarding the unintentional uses. Since unintentional uses are generally not covered by Council Regulation (EEC) 793/93 the Rapporteur does not have the possibility to improve the data base. Available information has been included in the risk assessment since it is considered valuable information. However, no quantitative risk characterisation has been performed.

There are indications that there may be a risk to surface water and soil arising from formulation and technical use of mixed xylenes as solvent containing ethylbenzene. It is recommended this to be further evaluated.

3.3.6 Secondary poisoning

No risk characterisation was performed since experimental data indicate that bioaccumulation of ethylbenzene is low.

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4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

Ethyl benzene is naturally present in crude oil. Commercially it is mainly produced by alkylating benzene with ethane. Ethyl benzene is a raw material for the production of styrene (99.5 %). Styrene is further processed to polystyrene which is used in large volumes in the automobile industry, in the building industry and for packaging. A small percentage of ethyl benzene (0.5 %) is used as a chemical intermediate, e.g. in the manufacture of acetophenone, cellulose acetate, diethyl benzene, propylene oxide.

There are nine producers in the EU; one company is a trader only. Ethyl benzene is produced in a volume of approx. 5 million tons per year in Europe. The actual production volume in 2006 was 3.857.500 t/a (production capacity 2006, 4.832.400 t/a).

For details see chapter 2.

Some petroleum refineries isolate a reformate stream during the distillation of crude oil into petroleum products. It may be further processed to isolate a mixed xylene stream. The “mixed xylenes” (also called xylene-range aromatic solvent) is a commercial product (CAS: 1330-20-7) contain generally about 80 % o-, m-, and p-xylenes and 15 to 20 % ethyl benzene (range of 10 – 45 %) (CEFIC, 2004). The majority of the mixed xylenes produced is blended into gasoline (ECETOC, 1986). Ethyl benzene serves as a “anti-knock” agent (octane enhancer) (Fishbein, 1985). The final concentration of ethyl benzene in gasoline is approximately 2 % (by weight) (CEFIC, 2004). Ethyl benzene can be discharged during handling and combustion of gasoline (automobiles and aeroplanes). These petrol related emissions, e.g. from crude oil production, gasoline distribution, at service stations, are estimated in a risk assessment report for “Gasoline” supported by CONCAWE (CONCAWE Draft Report, 2004). In this report the highest time weighted average concentration (TWA) of ethylene benzene is 5.9 mg/m^3 (railcar operators - top loading, without vapour recovery). On account of the low concentration of ethyl benzene in gasoline and that ethyl benzene stemming from mixed xylenes (unintentional uses) the use of ethyl benzene in gasoline is not part of this risk assessment report.

Ethyl benzene is also known in paints, lacquers and inks. The European Council of Paint, Printing Inks and Artist's Colours (CEPE, 2000) had launched a survey among its members. A questionnaire was sent to all major paint manufactures plus most of the medium-sized companies as well as a number of small companies asking for information on the use of ethyl benzene in paints and solvents. It became apparent that > 99 % of ethyl benzene used in paints is stemming from “mixed xylene” solvent. The intentional use of ethyl benzene in paints seems not to be of relevance anymore. That might also be due to higher flammability and higher price of ethyl benzene compared to the “mixed xylene” solvent. Ethyl benzene can be sepa-

rated; however, this technology is economically not favourable. In addition industry has emphasised their endeavour to develop solvent free systems and water based paints.

4.1.1.2 Occupational exposure

Industrial activities using ethyl benzene present opportunities for occupational exposure. Exposure ranges depend on the particular operation and the risk reduction measures in use.

The following occupational exposure limits (OEL) and short term exposure levels (STEL) apply in the EU and USA (Ariel, 2008):

Country	**OEL**		**STEL**	
Norway (2007)	**20 mg/m^3**	**5 ml/m^3**	**-**	**-**
France (2008)	**88.4 mg/m^3**	**20 ml/m^3**	**442 mg/m^3**	**100 ml/m^3**
Sweden (2007)	**200 mg/m^3**	**50 ml/m^3**	**450 mg/m^3**	**100 ml/m^3**
Iceland (2001)	**200 mg/m^3**	**50 ml/m^3**	**884 mg/m^3**	**200 ml/m^3**
The Netherlands (2007)	**215 mg/m^3**	**50 ml/m^3**	**430 mg/m^3**	**100 ml/m^3**
Denmark (2008)	**217 mg/m^3**	**50 ml/m^3**	**-**	**-**
Finland (2007)	**220 mg/m^3**	**50 ml/m^3**	**442 mg/m^3**	**100 ml/m^3**
Swiss (2007)	**435 mg/m^3**	**100 ml/m^3**	**435 mg/m^3**	**100 ml/m^3**
Ireland (2007), Greece (2001), USA:OSHA (2005), ACGIH (2008)	**435 mg/m^3**	**100 ml/m^3**	**545 mg/m^3**	**125 ml/m^3**
Germany (2008), Austria (2007)	**440 mg/m^3**	**100 ml/m^3**	**880 mg/m^3**	**200 ml/m^3**
United Kingdom (2007)	**441 mg/m^3**	**100 ml/m^3**	**552 mg/m^3**	**125 ml/m^3**
Spain (2008)	**441 mg/m^3**	**100 ml/m^3**	**884 mg/m^3**	**200 ml/m^3**
Belgium (2007)	**442 mg/m^3**	**100 ml/m^3**	**551 mg/m^3**	**125 ml/m^3**
Italy (2008), Luxembourg (2002), Portugal (2001)	**442 mg/m^3**	**100 ml/m^3**	**884 mg/m^3**	**200 ml/m^3**

The following scenario is regarded to be relevant for occupational exposure:

- Production and further processing in the chemical industry (4.1.1.2.1)
- Use of paints, lacquer, inks containing ethyl benzene (4.1.1.2.2)

Ethyl benzene itself is not use as solvent by the formulation of products (e.g. paints, lacquers, inks, and cleaner). The existence of ethyl benzene in these products results from the usage of "mixed xylenes" as solvent. The exposure against "mixed xylenes" is not discussed in the

ethyl benzene risk assessment. The use of paints is described nevertheless because of the wide dispersion use of paints and a content of estimate 20 % ethyl benzene.

The exposure assessment generally aims at assessing exposure levels representing the reasonable worst case situation. The reasonable worst case is regarded as the level of exposure which is exceeded in a small percentage of cases over the whole spectrum of likely circumstances of use for a specific scenario.

The assessment of inhalation exposure is mainly based on measured exposure levels from which – if possible – 90th percentiles are derived as representing reasonable worst case situations. If available, only data measured later than 1990 are used in exposure assessment. Scenarios are clustered as far as possible to make the description transparent. If quantitative exposure data are not available, model estimates are used.

Beside inhalation exposure, dermal exposure is assessed for each scenario. Two terms can be used to describe dermal exposure:

Potential dermal exposure is an estimate of the amount of a substance landing on the outside of work wear and on the exposed skin.

Actual dermal exposure is an estimate of the amount of a substance actually reaching the skin.

Within the framework of existing substances there is an agreement between the EU member states, to assess - as a rule - dermal exposure as exposure to hands and parts of the forearms. In this, the main difference between both terms – potential and actual - is the protection of hands and forearms by work wear and – more important – the protection by gloves. Within this exposure assessment, the exposure reducing effect achievable by gloves is only considered if information is provided indicating that, for a certain scenario, gloves are a widely accepted protective measure and that the gloves are fundamentally suitable for protection against the substance under consideration. As a measure for the latter, tests according to DIN EN 374 are taken as a criterion. For most down stream uses it is commonly known that gloves are not generally worn. In these cases, dermal exposure is assessed as actual dermal exposure for the unprotected worker. Since quantitative information on dermal exposure is often not available, the EASE model is usually used for assessing dermal exposure.

4.1.1.2.1 Production and further processing in the chemical industry (scenario 1)

Ethyl benzene is produced by a catalyzed reaction of ethylene and benzene at approx. 40 bar and temperatures around 250 °C. All manufacturers use ethyl benzene as the raw material for the production of styrene monomer. Therefore the ethyl benzene production plant and the styrene monomer plant are often situated at the same site and directly connected to each other. In some cases ethyl benzene is shipped in bulk to a styrene monomer plant. All ethyl benzene plants are completely closed systems, working under controlled conditions. The process is highly automated and runs continuously. Another production method is the fractionation of mixed xylene streams which is, however, employed to a much lesser extent. These streams occur in petroleum refineries during distillation of cruide oil into petroleum products and contain ~ 80 % o-, m-, p-xylenes (“mixed xylene stream) and ~ 15-20 % ethyl benzene.

Ethyl benzene is mainly a raw material for the production of styrene (99.5 %). Styrene is further processed in the chemical industry to polystyrene which is used in large volumes in the automobile industry, in the building industry and for packaging. A small percentage of ethyl

benzene (0.5 %) is used as a chemical intermediate, e.g. in the manufacture of acetophenone, cellulose acetate, diethyl benzene, propylene oxide.

The following information based on a questionnaire that has been sent to all ethylene benzene producers in Europe and compiled by CEFIC – Styrene Steering Committee (CEFIC, 2007).

Occupational exposure might take place under the following activities or conditions:

• Normal operating conditions: The companies indicate that exposure to ethyl benzene during normal operating conditions might occur during 10 – 90 % of the shift working hours.
• Sampling: Most companies describe that samples are taken via a closed loop system. Samples are taken several times during a shift. The duration of the sampling time is 1 – 5 min. Exposure to ethyl benzene may occur in case of bottle overfilling or liquid spills.
• Ship loading and unloading: Exposure can occur when hose is disconnected. At that moment half face masks with organic vapour cartridge are worn. Potential exposure for each individual is approximately 9-10 days/year, 20 minutes/day. The location is in open air with good natural ventilation.
• Maintenance: It is estimated that maintenance takes approx. 10 % of the total operating time. During maintenance, workers are potentially exposed to ethyl benzene, up to 80 % of their working time. In most cases the maintenance work is done in the open air which warrants good natural ventilation. Some maintenance operations include opening of the process equipment. In one case a complete turn around maintenance scheme is followed once every 3.5 to 5 years.
• Cleaning of filters: Some filters in the production line are changed two to four times a year. Changing a filter may take up to 30 minutes.
• Cleaning of tanks during maintenance works: This takes place once every two years.
• One company indicates that cleaning of a settling trap occurs once every two weeks during2 hours.

In all cases the exposure route to ethyl benzene is through inhalation and skin contact.

The reported number of workers per site range from 20 to 380. It is not quite clear from the provided information whether all these people are regularly exposed to ethyl benzene. The workers are predominantly of male gender and have an age between 20 and 62 years.

Information was asked in the questionnaire about measures taken to prevent occupational exposure to ethyl benzene by engineering controls and the use of personal protection equipment. One company did not give any information about measures taken for the prevention of exposure to ethyl benzene.

A variety of engineering controls were mentioned:

• All processes are completely closed
• Vents of the process are connected to a flare system or a site off-gas system
• Sample collection is always done in closed loop systems
• Where loading or unloading operations are performed, these are always connected to remote vapour venting or vapour collection systems.
• Fugitive emissions are monitored.
• Repair and maintenance work can only be done after obtaining dedicated work permits.
• Certification systems are used for all equipment removed from the plant for remote maintenance within a workshop. Equipment is fully decontaminated and labelled prior to transfer.

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• Liquid spills, vents and drain streams are being collected and processed in a waste water treatment plant of different types.

The type of personal protection equipment (PPE) in use is described in different levels of detail by the individual companies. Following are compilations of the different types of PPE, as they have been described:

In most plants operators wear as standard outfit safety helmets, flame retardant coveralls and safety shoes. More particular types of personal protection are described as follows: Eye protection: In most cases safety goggles are worn as a standard measure. In cases where splashes might occur, face shields are prescribed. Skin protection: Safety gloves are worn; in a few cases specifically the use of Viton or nitril rubber gloves is recommended. Chemical resistant disposable overalls are used during high risk tasks. Respiratory protection: Under normal operating conditions no breathing protection is prescribed. However, in specific (low) exposure situations breathing masks with a Type A filter are worn. In high risk situations (e.g. maintenance, equipment opening, etc), breathing protection with air respirators or self-contained breathing apparatus may be used.

Inhalation exposure

Measured data

Tab. 4.1: Summary of exposure data at workplaces during production

Job category / activities	Years of measurement	Number of samples	Measurement data [mg/m³]	50th percentile [mg/m³]	90th percentile [mg/m³]	95th percentile [mg/m³]
8-h TWA						
Production (total)	1999 – 2001	254 (p)	-	0.05	1.3	6.9
Maintenance	1999 – 2001	26 (p)	-	0.11	19	56
Filling/storage	1999 – 2001	25 (p)	-	0.19	1.4	1.5
Laboratory	1999 – 2001	50 (p)	-	0.06	1.4	1.5
Production	2001	25 (p)	0.01 – 6.7	0.04	0.25	1
Production	2000	200	0.005 – 5	0.2	-	-
Production	1995	7	<0.01 – 0.18	-	-	-
Filling	1995	4	0.01 – 0.07	-	-	-

-: no data available
p: personal air sampling

Due to the measurement method and the sampling strategy applied, the measurement results are regarded as valid.

The CEFIC – Styrene Steering Committee questionnaire reported in total 46150 individual exposure measurements. The number of individual measurements differs substantially from one company to another.

Tab. 4.2: Summary of exposure data (period of measurements 2002 – 2007)

Function	Activities	No of data points	Type of measurement	min [mg/m^3]	max [mg/m^3]
Process operators	**Normal operation**	**480**	**TWA**	**0**	**94.2**
		17	**STEL**	**<0.09**	**20.7**
	Change of filters	**4**	**STEL**	**0.9**	**96.8**
	Equipment openings	**47**	**TWA**	**0.02**	**1.0**
	Turn around activities	**442**	**TWA**	**0.02**	**51.0**
	Stop of styrene plant	**201**	**TWA**	**<0.4**	**15.8**
	Not specified	**28048**	**TWA**	**0.03**	**19.8**
	Not specified	**54**	**Not specified**	**<0.004**	**0.48**
Maintenance personnel	**Normal operation**	**47**	**TWA**	**0.02**	**0.31**
	Equipment openings	**8**	**TWA**	**0.02**	**0.04**
	Change of filters	**4**	**STEL**	**0.18**	**257**
	Not specified	**4177**	**TWA**	**<0.02**	**22.9**
	Not specified	**13**	**Not specified**	**<0.2**	**1.0**
Contractors	**Equipment openings**	**150**	**TWA**	**0.02**	**33**
	Turn around activities	**436**	**TWA**	**0.02**	**91.5**
	Stop of Styrene plant	**107**	**TWA**	**<0.4**	**110**
Laboratory	**Not specified**	**5541**	**TWA**	**0.009**	**99**
Shipping operators	**Normal operation**	**43**	**TWA**	**<0.09**	**0.66**
	Not specified	**6331**	**TWA**	**<0.02**	**3.4**

TWA – 8-h time weighted average
STEL – Short term exposure level

A distinction is made between the descriptions of different groups of personnel as well as different activities. Some companies have reported summary data only, including median and 90th percentiles scores, others have given a long list of individual measurements. It is therefore not possible to give a total calculation of average, median and percentile scores.

The following information is available about the methods used to monitor occupational exposure:

• Air samples are taken with an active sampling pump. The air samples are fed through an adsorption medium, such as active carbon or similar. The adsorbed ethyl benzene is analysed by means of GC-MS and calculated back to an average value over the sampling period.
• Passive sampling: operators wear an active carbon badge on which ethyl benzene is absorbed. The badge is analysed by means of GC.
• One company reported a limited number of exposure data, obtained by means of determination of mandelic acid in urine samples. These data are not included in the report.
• In cases where procedures are indicated, officially recognised procedures, such as NIOSH 1501 or the equivalent German or Italian methods are reported.

The measurements are divided into two groups, i.e. long-term time weighted average results and short-term measurements:

• Long-term measurements are normally done to measure the average exposure in relation to the legal maximum allowable concentration over a working period of 8 hours. The sampling time in the reported long-term measurements is not reported, except for two companies where the sampling times ranged from 65 - 774 minutes.
• Short-term measurements give an indication about the peak values that may be measured during relatively short periods of time, usually 15 – 30 minutes. Only in two cases sampling periods of 15 and 30 minutes were reported.

• A number of tasks such as draining of pump seals and changing filters usual took a time between 10 and 60 minutes. These data points are considered to be a STEL measurement.

A limited number of companies provided statistical data, such as median or average values and percentile scores of their submitted data points. These statistical data will be reported here. They will not be included in Table 4.2, because of the limited number of cases where they have been reported.

Four companies have reported a median value of their measurements. This median value is calculated on a total of 359 data points in 12 groups of data. For these groups the lowest reported median value is 0.04 mg/m^3 (in a group of 39 data points), the highest value is 0.44 mg/m^3 (in a group of 20 data points).

For 11 groups of data points with in total 1267 data an average value has been reported. The lowest reported average value was 0.009 mg/m^3 (in a group of 8 data points), the highest reported average value was 1.8 mg/m^3 (in a group of 125 data points).

90th percentile scores have been reported for 7 groups of data with in total 229 data points. The lowest reported percentile score was 0.44 mg/m^3 (in a group of 30 data points), the highest reported percentile score was 8.8 mg/m^3 (in a group of 55 data points).

The highest single data point reported was 257 mg/m^3 (STEL), measured during changing a filter. This is a task which normally lasts 15 to 30 minutes.

The majority of exposure data are below 0.09 mg/m^3 with a range of <0.02 – 110 mg/m^3.

Summary of the exposure level

Inhalation exposure has to be assessed for the production and further processing of ethyl benzene in fields with high levels of protection (large-scale chemical industry).

For the assessment of health risks of daily inhalation exposure to ethyl benzene during the production and further processing an 8-h time weighed average concentration (8-h TWA) of 1.3 mg/m^3 (90th percentile of measurement data) should be taken to represent a reasonable worst case situation. Higher short term exposure levels occur (257 mg/m^3, change of filters).

It is to be assumed that the substance is processed daily. Consequently, the duration and the frequency of exposure to ethyl benzene are assumed to be daily and for the entire length of shift.

<u>Dermal exposure</u>

According to information from industry the plants for production of ethyl benzene and for the production of styrene or other chemical intermediates using ethyl benzene as a source material consist of fully closed components such as reactors, pipes, pumps, etc..

Dermal exposure could occur during activities like coupling and decoupling of transfer lines, sampling, cleaning, maintenance and repair work.

Modelled data

For the unprotected worker, according to the EASE model, potential dermal exposure is assessed as follows (default assumptions for coupling and decoupling of transfer lines, according to Technical Guidance Document - TGD, 2003):

Input parameters: Non dispersive use, direct handling, incidental
Level of exposure: 0 – 0.1 mg/cm^2/day.

Considering an exposed area of 420 cm^2 (equivalent to one hand) the model yields an exposure level of 0 - 42 mg/person/day.

For assessing actual dermal exposure levels, it has to be considered that the substance is manufactured and further processed primarily in closed systems and that the use of PPE (here gloves and eye protection) is highly accepted in the large-scale chemical industry. The extent of protection by PPE (here gloves) depends inter alia on the suitability of the recommended material with regard to the permeation properties of substance.

According to the information provided by the industry suitable gloves are used. Since no measurement results are available, a protection efficiency of 90 % (according to TGD, 2003) is taken as a default value leading to an exposure level of 4.2 mg/person/day. The value is regarded to represent the reasonable worst case situation.

Summary of the exposure level

For assessing the health risks from daily dermal exposure in the area of production and further processing, an exposure level of 4.2 mg/person/day should be taken. This exposure assessment is based on the assumption, that gloves are suitable for the protection against ethyl benzene.

Exposure to the eyes is largely avoided by using eye protection.

4.1.1.2.2 Use of paints, lacquers, inks containing ethyl benzene (scenario 2)

According a survey performed by the European Council of Paint, Printing Inks and Artist's Colours > 99 % of ethyl benzene used in paints stemming from "mixed xylene" solvent. This "mixed xylenes" are mainly used as solvent in spray paints, primers, paint removers, thinners, wood stains, varnishes and other finishes, and cleaners for automotive and household uses (OECD, 2002).

Lacquers and paints are applied by spraying, dipping, brushing, rolling or covering by pouring in different industrial and skilled trade sectors, e.g. treatment and processing of metal and wood, mechanical engineering, electronic industry, vehicle production and repair as well as building trade. The European Council of Paint, Printing Inks and Artist's Colours (CEPE, 2004) gave more details on the uses of paints containing ethyl benzene. The majority of these paints are used as industrial paint in the following sectors: automotive, vehicle refinishes, marine, can coating, wood furniture, electrical insulating, and printing inks. Spraying, curtain coating, dipping, coil coating is the main application techniques used in industry. Spray application of paints and coatings often results in a significant potential inhalation and dermal exposure. Spraying can be performed manually or automatically (spray cabins). In addition to inhalation exposure caused by the evaporation of the substance, droplet aerosols may be a source of exposure.

The concentration range of ethyl benzene in paints and lacquers is wide. According to the available information typical concentrations of ethyl benzene in paints, lacquers, inks, thinner or hardener is < 1 % - < 25 %.

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In view of occupational exposure, the application of paints (e.g. by spraying, brushing, and rolling) are relevant. The concentration of ethyl benzene in end products is assumed to be 20 %.

Inhalation exposure

Measured data

Tab. 4.3: Measurements listed were performed by the German Worker's Compensation Funds (BGAA, 2000)

Job category / activities	Years of measure-ment	Number of samples	Technical measures	50th-percentile [mg/m³]	90th-percentile [mg/m³]	95th-percentile [mg/m³]
8h time-weighted average						
Surface coating (brush/ roller application)	1991-1995	649 113	Without LEV with LEV	3 3	34 19	55 114
Surface coating (spraying, metal-working)	1991-1995	196 819	Without LEV with LEV	7 3	43 19	64 32
Surface coating (spraying, woodworking)	1991-1995	22 690	Without LEV with LEV	2 3	13 11	14 16
Surface coating, mechanical	1991-1995	200 285	Without LEV with LEV	2 3	24 19	44 35
Glueing, floor laying	1991-1995	256 65	Without LEV with LEV	5 < 0.5	29 12	38 23
Surface cleaning	1991-1995	146 147	Without LEV with LEV	2 2	30 12	51 25

LEV: local exhaust ventilation

Measurement method: A defined volume is sucked through an activated charcoal tube using a sampling pump. After desorption with diethyl ether, quantitative analysis is carried out by means of gaschromatography using a flame ionisation detector. Due to the measurement method and the sampling strategy applied, the measurement results are regarded as valid.

In the literature measurement data on personal solvent exposure from six studies for Dutch commercial painters were described (Burstyn, 2002). Most of the measurements were collected during the application of solvent-based paints. Data were available for painting done in houses and shipyards, during spray-painting and manual painting with brush, a roller, or a brush and a roller. Altogether 259 measurements of ethyl benzene exposure were collected between 1990 and 1999.

Tab. 4.4: Ethyl benzene exposure levels during the use of different paints

Paint type	number of samples	Measurement data [mg/m³]	Geometric mean [mg/m³]	GSD [mg/m³]

all	259	0.002 – 86	0.3	11.94
water-based (acrylate)	48	0.002 – 1.73	0.05	8.67
high-solid and two-component	44	0.021 – 42.32	1.02	6.05
other solvent-based	108	0.023 - 86	1.04	6.36

GSD: geometric standard deviation

On account that the measured data are from 1990 – 1999 and the industry has emphasised their endeavour to develop solvent free systems and waterbased paints the 50th percentile values are regarded to represent the exposure situation for the given scenario.

On the basis of the presented exposure data, 7 mg/m^3 obtained at real workplaces is regarded to represent the reasonable worst case situation of daily exposure during painting. The exposure situation “spray painting” is not treated separately from other painting techniques, because the measurement results are in the same order of magnitude.

It is assumed, that activities relevant for exposure performed daily during the entire length of the shift.

Conclusions

For the assessment of health risks from daily inhalation exposure to ethyl benzene during the use of paints (spraying, brushing, rolling) an 8-h TWA of 7 mg/m^3 (workplace measurements) should be taken. Higher short-term exposure levels occur.

Dermal exposure

For the use of paints, lacquers, inks containing ethyl benzene in small and medium sized enterprises and the skilled-trade area it cannot be excluded that gloves are not regularly worn and that immediate dermal contacts occur during activities like spraying, brushing, rolling of paints.

For the spray application of paints in the industrial and skilled-trade area it is to be assumed that protective gloves are not regularly worn (Voullaire, 1995). Taking into consideration that personal protective equipment is not generally worn during painting works, the estimation of dermal exposure levels is performed for the unprotected worker.

Analogous data

In the Technical Guidance Document (TGD, 2003) dermal exposure estimates for specific exposure scenarios (here: spray painting of large areas and brushing and rolling of liquids) are described. These estimates are taken to assess the daily dermal exposure during painting works.

Application of paint by airless spraying to relatively large areas

Dermal exposure during spray painting is due to the deposition of spray mist, back bouncing, contact with contaminated spray gun and possibly also with freshly painted surfaces. The estimates are based on an experimental study in 3 off-shore facilities where containers were painted (Lansink, 1998) and on studies by HSE and IOM on airless spray application of

antifouling paint (HSE, 1999). On the basis of the 90th percentile of the extrapolated results of Lansink *et al*. (1998) and the 95th percentile of the HSE data a reasonable worst case (RWC) estimate of 10.000 mg on an exposed area of 840 cm² was derived. For the typical case an exposure value of 2.500 mg is described. In consideration of ethyl benzene content of 20 % dermal exposure through direct skin contact during spraying of the formulations is estimated to 2000 mg/person/day (RWC) and 500 mg/person/day (typical value).

Application of liquids by brushing and rolling

Exposure due to contaminated tools and splashing of (small) droplets, as well as direct contact with freshly painted surfaces based on experimental study of consumers applying wood preservatives (Roff, 1997) and field study of consumers applying anti-fouling paints by roller and brush on their boats (Guiver, 1997; Guiver and Foster, 1999). The extrapolated result of Roff for workers without gloves was used as a basis for the RWC estimate (10.000 mg on an exposed area of 840 cm²). This value compared reasonably with the values measured by Guiver (1997) for amateurs painting boats without gloves or with previously used gloves. The median value presented by Roff (1997) for spirit-based fluids and no gloves was used as the basis for the typical value (1.700 mg). In consideration of ethyl benzene content of 20 % dermal exposure through direct skin contact during brushing and rolling of the formulations is estimated to 2000 mg/person/day (RWC) and 340 mg/person/day (typical value).

Conclusions

For assessing the risk of daily dermal exposure during painting works (spraying, brushing and rolling) an exposure level of 2000 mg/person/day should be taken (analogous data, according to TGD, 2003). The typical value is between 340 - 500 mg/person/day.

It cannot presuppose that eye protection is regularly worn. For assessing the risks, hand eye contacts as well as possible splashes to the eye should be considered.

4.1.1.2.3 Summary of occupational exposure

Ethyl benzene is primarily used for the production of styrene (99.5%). A small percentage (0.5 %) is used in the production of other chemicals. It is concluded that the intentional use of ethyl benzene in the solvent sector is only marginal and is not further considered in the risk assessment (details, see chapter 2.2.2.3). Some petroleum refineries isolate a reformate stream during the distillation of crude oil into petroleum products. The reformate stream may be further processed to isolate a mixed xylene stream which contents ethyl benzene.

The existence of ethyl benzene in paints, lacquers, inks, and cleaner results from the usage of "mixed xylenes" as solvent. The exposure against "mixed xylenes" is not discussed in the ethyl benzene risk assessment. The use of paints is described nevertheless because of the wide dispersion use of paints and a content of 20 % ethyl benzene.

For occupational exposure there are two scenarios:

- Production and further processing in the chemical industry
- Use of paints, lacquers, inks containing ethyl benzene

The relevant inhalation and dermal exposure levels are given in table 4.5.

For the large scale chemical industry, it is assumed that the production and further processing of ethyl benzene is mainly performed in closed systems. Exposure occurs if the systems are breached for certain activities, e.g. coupling-decoupling of transfer lines, maintenance, cleaning. As concerning dermal exposure the industry provided information that suitable gloves are used regularly. This is considered assuming a protection efficiency of 90 %. For the other scenario dermal exposure is assessed for the unprotected worker.

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Table 4.5: Summary of exposure data (reasonable worst case) of ethyl benzene which are relevant for occupational risk assessment

				Inhalation		**Dermal**	
				Reasonable worst case		**Reasonable worst case**	
Scenario	**Activity**	**Frequence Days/year**	**Duration Hours/day**	**Unit mg/m^3**	**Method**	**Unit mg/person/day**	**Method**
Production							
1) Production and further processing	Coupling-decoupling transfer lines, shipping, maintenance	daily	shift	1.3	Workplace measurement (90th percentile)	4.2	EASE (suitable gloves)
Uses							
2) Use of paints, lacquers, inks (containing 20 % ethyl benzene)	spray painting, brushing, rolling	daily	shift (assumed)	7	Workplace measurement	2000	Analogous data (TGD)

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4.1.1.3 Consumer exposure

In the Swedish (103 consumer products listed) and the Swiss product register (401 consumer products listed), ethylbenzene is listed as a solvent in two major categories of products: a) 170 paints and lacquers (n = 350), especially for metal e. g. cars, vehicles and heaters, b) resin filling materials, adhesives (n = 21). Other categories are concerning cleaners, primers, lubricants, metal care, and solvents. The concentration range is wide within the categories mentioned. The Swiss product register mentions most of the products in a range up to < 100%; in the BgVV data base of poison control centres a maximum content of 28% is given. Although the latter data base is not complete, it can be assumed that as a worst case approach the maximum content of ethylbenzene would be around 30% in paints and adhesives, which applies also for solvents according to the Swiss product register. The concentration in cleaning product has been indicated in the Swiss product register evaluation not to exceed 1%.

Table 4.1.1.3-1 lists the major uses of ethylbenzene mentioned in the BgVV data base, which is very similar to the Swiss and Swedish product register information, together with it's contents and the assumed duration of use and contact as well as the use-frequencies, in accordance to the scenario and model characterisation of the CONSEXPO models.

Table 4.1.1.3-1: Categories of consumer products in which ethylbenzene has been identified as a constituent, and the respective input variables for model estimations taking the CONSEXPO-software

CONSEXPO results ethylbenzene exposure												
	Contact scenario				**Exposure scenario**							
Product category	*CONSEXPO model*	*Frequency of exposu-re (1/year)*	*Duration of use (h)*	*Duration of expo-sure (h)*	*CONSEXPO scenario*	*Area of release (m²)*	*Room volume (m³)*	*Air excha-nge rate (m³/h)*	*Mass of Product used per event (g)*	*Weight fraction[12]*	*Emission rate (mg/min)*	*Mol weight matrix (g(mol)*
Painting materials												
Paints for cars	painting	2	1	2	spray, well		60	12		0.015	50	
Paints, general	painting	6	2	8	painting	2	30	6	200	0.28		120
Sealing for floors	painting	1	2	5	painting	12	30	6	1200	0.06		120
Primer	painting	4	2	5	painting	5	30	6	500	0.025		120
Thinner	user defined	52	0.5	5	evaporation from mixture	0.0005	30	6	100	0.25		120
Repair equipment												
Adhesives for porcelain, stone	user defined	12	0.5	2	source and ventilation		30	6		0.2	4	120
Hardener	user defined	4	2	4	source and ventilation	1	30	6		0.02	100	
Enamel, bathroom equipment repair	user defined	4	1	4	painting	0.2	20	4	50	0.01		120
Other												
Car-care/cleaner (inside the car)	cleaning car/ motor bike	52	0.5	2	evaporation from mixture	1	5	2.5[13]	(100)	0.06		30

[12] Percent of substance used as ingredient in product
[13] The air exchange rate eas set to 2.5 m³/h, because it is impossible to clean a car without open doors.

Nail hardener	painting	365	0.5	24	painting	0.0025	30	6	0.05	0.05		30

Inhalation exposure

All exposures concerning adults were related to males with body weight of 57 kg (= 5th percentile of BW), and to females having a body weight of 45 kg [also 5th percentile].

In the adults (females and males), the breathing volume during the use of chemical products is assumed to account for 34 m^3/d[14)] for light activity and, for the time after use, it was set to 20 m^3/d.

All other data taken for estimations are given in Table 1.

From Product data information, the following scenarios have be characterised. The uses were categorised to 1) paints, 2) repairing materials and 3) other materials. A subcategory "paints, general" has been characterised to summarize all paints and lacquers for different purposes (for metal, heaters etc.) that can be painted.

1. Scenario: Painting (= external exposure for inhalation)
   For evaluation of this scenario the examples a) car painting by spraying, b) painting (general), c) floor sealings, d) primers, and e) thinners have been chosen as scenarios.

   a) Car painting
   Parts of a car (e.g. exhaust-pipe or rims) may be painted in a closed environment e.g. a garage, by taking a spray. The duration of this event will last for half an hour, the frequency of use is twice per year.

   The amount of paint is assumed to account for 200 g, the maximum content of ethylbenzene is 1.5%, the room volume is 60 m^3, the duration of exposure is 2 hours.

   b) Painting in the home, e.g. furniture, doors, heaters etc.
   For this scenario it is assumed that an area of 2 m^2 is painted with an amount of 500 g of a paint containing ~ 30% of ethylbenzene. The duration is 2 hours, the contact time is 24 hours and frequency of use 6 per year.

   c) Floor sealing
   It is assumed that 1000 g of a floor sealer have been used for 12 m^2.

   d) Primers
   Use data of primers are very similar to paints, data are depicted in Table 1.

   e) Thinners
   For thinners, exposure can occur by evaporation of ethylbenzene from open cans. If thinners are put together with e.g. paints, the exposure to ethylbenzene from the thinner would be covered by that from the resp. paint which is already considered.

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[14] Unfavourable assumption for limited exposures

2. Repair

Ethylbenzene is mentioned as an ingredient in some materials used to repair stony articles, particularly e.g. porcelain, enamel, and as hardeners. As examples for these uses, the exposure to ethylbenzene c) from an adhesive for porcelain, where a small area is brought to contact to another and the solvent is evaporated from the fissure, b) as a hardener of plastic material, and c) as a solvent in enamel repair has been considered.

3. Other uses

a) Car cleaners

There are products mentioned in the BgVV product data base containing ethylbenzene as an ingredient in car cleaners containing up to 6% of ethylbenzene. The frequency may account for weekly use.

b) As a further scenario occurring every day the use of nail hardeners may lead to exposure from inhalation and to dermal exposure. Nail hardeners may be used every day, the amount is 50 mg, and the area of contact is 25 cm² (all nails of fingers and toes).

The input variables are listed in Table 1, due to the selected model and scenarios from the CONSEXPO Program. For the scenario "source-and-ventilation" the emission rates were estimated as follows. It is assumed that the substance is evaporated from the volume contacting air. The layer thickness (TL) of this volume (V) is assumed to be 0.01 cm. It is also assumed that the total content of ethylbenzene is evaporated from this volume within the given time (TI) interval (min). If the density of the product is 1, than the volume, expressed in ml, is similar to weight in g. The equation to calculate the emission is than T * V * WF/TI (g/min).

Exposures were calculated for users and non-users (bystanders). For users, the event concentrations were estimated by taking the duration of contact as given in Table 1. For bystanders (who are represented (i) by other persons than the user and (ii) the user after use), the contact time was set to 24 h as a worst case for exposure of a whole day.

Table 4.1.1.3-2: Estimates of exposure

CONSEXPO results ethylbenzene exposure	**CONSEXPO results /all values in mg/m³**		
	Room air conc. per event (user)[15]	Room air conc. per event (bystander)[16]	Room air conc. per year (user and bystander)
Painting materials			
Paints for cars	0.02	not applicable	0.00009
Paints, general	2600	523.00	2.80
Sealing for floors	1200	82.00	1.13
Primer	420	108.00	0.24

[15] Contact time in CONSEXPO set to event duration (table 1) as indicated, personal volume of user 5m³

[16] Contact time in CONSEXPO set to 24 hours, personal volume of non-user = room volume

Thinner	5.7	0.96	0.028
Repair equipment			
Adhesives for porcelain, stone	17	0.83	0.027
Hardener	1340	not applicable	2.4
Enamel, bathroom equipment repair	0.036	0.009	0.00002
Other			
Car-care/cleaner (inside the car)	551	not applicable	6.5
Nail hardener	0.11	0.017	0.017

Results of inhalation exposure estimations

Acute exposure:

The results of model estimations of exposure to ethylbenzene from various uses and scenarios are shown in Table 3. For acute exposures, the highest amounts that can be reached are listed (max). From this table it can be taken that paints containing high amounts of ethylbenzene may lead to high exposures from inhalation. The highest values were obtained from not specified paints in which weight fractions of up to 30% were identified which amounts to ~ 320 mg/kg per day for males and 480 mg/kg per day for females. Other high but considerably lower exposures than for paint exposures were obtained for hardeners (70-80 mg/kg per day) and primers (40-60 mg/kg per day) for users, and 30-40 mg/kg per day for bystanders from floor sealer use.

Chronic exposure

Chronic exposure, as a sum of all the listed scenarios as a worst case concept, would lead to an average daily exposure of ~5 to ~6 mg/kg/day. Total exposure per year would then reveal ~ 1500 mg/kg.

Dermal exposure

Theoretically, from all of the scenarios listed above, dermal exposure via air is possible. For all scenarios, the exposed area is that of the face and the hands and forearms. The total area is 3160 cm^2. An exposure estimate taking CONSEXPO dermal exposure modelling reveals that dermal exposure via air can be neglected taking the paint scenario leading to the highest air concentrations.

Dermal exposure can be expected for nail hardeners. Due to the very small contact area (finger- and toe-nails with very poor absorption) CONSEXPO reveals very low exposure result (< 0.0001 MG) mg/kg. Dermal exposure from nail hardeners can therefore be neglected.

Conclusion

It can be concluded that exposure to consumers from use of ethylbenzene occurs via the inhalation route. For acute effects, highest exposures (~ 480 mg/kg/d) occur due to high concen-

trations of ethylbenzene in paints. For chronic effects the sum of all exposures amounts to 5-6 mg/kg/d. Due to lower body weight, exposure to females is greater than in males.

Table 4.1.1.3-3: Estimated exposures for female and male adults from inhalation of ethylbenzene

	Acute exposures				Exposure at the day of use				Exposure during whole year		
Scenario	**user**, during use		**user** after use		**user**		**bystander**		**user and bystander**		
	males	females	males	females	males	females	males	females	males	females	
Materials to painted											
Paints for cars	0.0005	0.0006	0.0	0.0	0.0005	0.0006	0.0	0.0	0.0	0.0	mg/kg/day
Paints, lacquers not specified	258	327	61	154	319	482	183	232	0.98	1.2	mg/kg/day
Sealing for floors	74	94	11	22	85	116	28	36	0.39	0.50	mg/kg/day
Primer	26	33	15	29	41	62	37	48	0.08	0.106	mg/kg/day
Thinner	0.35	0.44	0.13	0.25	0.48	0.70	0.33	0.42	0.009	0.012	mg/kg/day
Repair material											
Adhesives for porcelain, stone	0.42	0.53	0.13	0.19	0.55	0.73	0.29	0.36	0.009	0.012	mg/kg/day
Hardener	66	84	0.0	0.0	66	84	0.0	0.0	0.84	1.0	mg/kg/day
Enamel, bathroom equipment repair	0.0018	0.0023	0.0013	0.0023	0.003	0.0046	0.0032	0.004	0.0	0.0	mg/kg/day
other											
Car-care/cleaner (inside the car)	13	17	0.0	0.0	13	17	0.0	0.0	2.2	2.8	mg/kg/day
Nail hardener	0.03	0.04	0.0	0.0076	0.03	0.05	0.0060	0.0076	0.006	0.0076	mg/kg/day

Value to be taken for risk characterisation	258	327	61	154	319	**482**	183	232	4.6	**5.8**	mg/kg/day
	max	max	max	max	max	max	max	max	sum	sum	

4.1.1.4 Indirect exposure via the environment

According to Appendix VII of Chapter 2 of the TGD, the indirect exposure to humans via the environment through food, drinking water and air is estimated for a local and a regional approach. For the local concentrations the generic scenario for production and processing at site PP2 is used, representing the local worst case. This scenario is compared to an average intake due to exposure via the regional background concentration. In the Appendix IV the input data and results of the calculations are presented. The following table shows the input parameters selected.

Table 4.4 Input parameter for calculation of indirect exposure ("Production and processing at PP2")

Parameter	Value
$PEClocal_{water_ann}$	0.0116 mg l^{-1}
$PEClocal_{air_ann}$	0.034 mg m^{-3}
$PEClocal_{grassland}$	0.00261 mg kg^{-1}
$PEClocal_{agri_soil_porewater}$	2.80×10^{-4} mg l^{-1}
$PEClocal_{grassland_porewater}$	3.34×10^{-4} mg l^{-1}
$PEClocal_{agri_soil_groundwater}$	2.80×10^{-4} mg l^{-1}
$PECregional_{water}$	6.40×10^{-5} mg l^{-1}
$PECregional_{air}$	4.62×10^{-4} mg m^{-3}
$PECregional_{agri_soil}$	7.40×10^{-5} mg kg^{-1}
$PECregional_{agri_soil_porewater}$	9.56×10^{-6} mg l^{-1}

The resulting total daily doses are:

$DOSE_{tot_local}$ = 9.247 $\mu g \cdot kg_{b.w.}^{-1} \cdot d^{-1}$

$DOSE_{tot_regional}$ = 0.111 $\mu g \cdot kg_{b.w.}^{-1} \cdot d^{-1}$

The calculated doses comprise the following routes

Table 4.5 Routes of exposure (percentage of total dose) for regional and local model

route	**regional model, percentage of total dose (%)**	**point source model; percentage of total dose (%)**
drinking water	0.83	1.79
air	89.41	78.79
stem	0.40	0.35
root	0.68	0.24
meat	<0.01	<0.01
milk	<0.01	<0.01
fish	8.67	18.82

The most significant route of exposure in the regional as well as in the local model is that via air.
However, it has to be noted, that the applied model calculations are of preliminary nature and have to be revised as soon as further information become available.

4.1.1.5 (Combined exposure)

4.1.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

Not all study descriptions (especially study reports from industry) were available to the rapporter. It is indicated in the following text, where the full study reports were missing. In these cases, the available summaries of the studies were used in chapter 4.1.2.

4.1.2.1. Toxicokinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

In vivo studies

Inhalation

Six male Harlan-Wistar rats were exposed to ^{14}C-ring radiolabelled (5mCi/mmole) ethylbenzene vapour at a chamber concentration of about 1 mg/l (corresponding to 230 ppm ethylbenzene) for 6 hr **(Chin et al., 1980b)** in two different experiments (3 animals per experiment). Urine, cage washings and feces were collected at 6, 24 and 48 hr. Exhaled CO_2 and exhaled

radioactivity were also assessed. Approximately 44 % of the total amount of radioactivity inhaled was absorbed. Ethylbenzene was shown to be efficiently distributed throughout the body in rats following inhalation exposure to radiolabelled ethylbenzene. Mean total radioactivity remaining in the tissues after 42 hrs was 0.2 %. Amounts >0.001 % were in liver, gastrointestinal tract, adipose tissue, lungs and kidneys. Traces (amounts <0.001%) were in thyroid gland, adrenal glands, blood cells, plasma, brain, bone marrow, spleen and carcass. Approximately 90 % of the absorbed radioactivity was excreted within 24 hrs after start of the exposure. The major route of excretion was via the kidneys: 42 hrs following inhalation exposure, 82.6 % of the absorbed radioactivity was determined in the urine. Exhalation via the lungs was also a major route of excretion: 8.2 % of the absorbed radioactivity was determined as exhaled gases, 0.03 % of the absorbed radioactivity was excreted as CO_2 within 42 hrs following inhalation exposure. Feces contained only minor amounts (0.7 % of the absorbed radioactivity).

Engström (1984b) investigated the metabolism of ethylbenzene in male Wistar rats (6 animals/dose group) after 6 hr exposures to 0, 300, or 600 ppm (corresponding to 0, 1305 and 2610 mg/m^3) ethylbenzene by analyzing urines collected up to 48 hrs after start of the exposures. Analysis of minor metabolites was carried out as described by Engström (1984a). During the 48 hr sampling period, 83 % and 59 % of the absorbed dose were excreted via urine at 300 and 600 ppm, respectively, indicating that a higher exposure level did not lead to an increased excretion of urinary metabolites. Fourteen different metabolites from ethylbenzene were identified. The main metabolites were mandelic acid (23 % and 28 % of all metabolites at 300 and 600 ppm, respectively), benzoic acid (24 % (300 ppm) and 27% (600 ppm)), 1-phenylethanol (23 % (300 ppm) and 25 % (600 ppm)), phenylglyoxylic acid (10 % (300 ppm) and 11% (600 ppm)), phenylacetic acid (7 %), omega-hydroxyacetophenone (5 %), 1-phenyl-1,2-ethandiol (1%), acetophenone (0.2 % (300 ppm) and 0.1 % (600 ppm)), phenylglyoxal (1.4 % (300 ppm) and 1.2 % (600 ppm)), and traces of 2-phenylethanol. While side-chain oxidation was the major route of metabolism, some ring oxidation also occurred but only to a minor amount as indicated by small amounts of 4-hydroxy-acetophenone (0.5 % (300 ppm) and 0.7 % (600 ppm)) and traces of m-hydroxy acetophenone and 2-ethylphenol found in the urine samples. Neither 3-ethylphenol nor 2-hydroxyacetophenone was found. Both glucuronide and sulfate conjugation of alcoholic and phenolic metabolites occurs in rodents but with glutathione conjugation apparently playing a minor role in the rat.

Charest-Tardif et al. (2006) investigated the inhalation pharmacokinetics of ethylbenzene in male and female B6C3F1 mice following single and repeated exposures. In the single dose study, mice received 75, 200, 500 or 1000 ppm (corresponding to 326, 870, 2175 and 4350 mg/m^3) ethylbenzene for 4 hrs. For comparison of kinetics following single and multiple exposures mice were exposed to 75 or 750 ppm ethylbenzene (concentrations of the NTP carcinogenicity study) for 6 hrs/day for 1 or 7 consecutive days. C_{max} in blood, AUC (blood conc. x time) and the rate constant for elimination of ethylbenzene were calculated.
It was found that the kinetics at 75 ppm was comparable for acute and repeated exposures, whereas the rate of metabolism was enhanced at 750 ppm during repeated exposures. Furthermore, ethylbenzene kinetics was saturable at concentrations exceeding 500 ppm (and therefore at 750 ppm used in the NTP bioassay) but was in the linear range at the lower concentration of the NTP bioassay. C_{max} and AUC were disproportionately higher at 750 ppm than at 75 ppm in mice of both sexes.

A disproportional increase of ethylbenzene concentrations in blood was also observed in rats **(Faber et al., 2006)**: ethylbenzene concentrations in blood in adult rats and their offspring

increased at a rate that was greater than proportional to dose. Ethylbenzene levels in maternal blood, collected on PND 22, after a 6-hour exposure to 25, 100, or 500 ppm (corresponding to 109, 435 and 2175 mg/m^3) were 0.11, 0.56, and 11 mg/l, respectively. The mean concentrations in the blood of pups (males/females), culled from the dams sampled above, in the 25-, 100-, and 500-ppm groups were 0.021/0.025, 0.26/0.24, and 11.4/12.7 mg/ml, respectively.

Marked interspecies difference among rats and guinea pigs in the disposition of inhaled ethylbenzene could be observed after repeated inhalation exposure **(Cappaert et al., 2002)**. The concentrations of ethylbenzene in the blood of female Wag/Rij rats and female albino guinea pigs exposed to ethylbenzene concentrations of 500 ppm (2175 mg/m^3) (8 hrs/d for 3 consecutive days) reached 23 and 3 µg/ml, respectively, at the end of the first day. Ethylbenzene concentrations in blood after the last of three daily exposures had diminished to 6 µg/ml in rats and to <2 µg/ml in guinea pigs.

Species differences after inhalation exposure were also described by **Chin et al.,1980a**: three Harlan-Wistar rats (not data on sex is given) were exposed by inhalation to ethylbenzene vapors of a concentration of approximately 1 mg/l (corresponding to 230 ppm). One female beagle dog was exposed for 3.5 hr by inhalation (total amount of ethylbenzene applied: 50 µl). Quantitative differences in the percentages of metabolites (the structures of the metabolites were not elucidated) excreted in the urine were also reported by **Chin et al. (1980a)**. Although similarities in the types of metabolites recovered following inhalation exposure were reported, quantitative differences, albeit minor ones, were noted in the ratio of metabolites present in the urine.

The temporal changes of ethylbenzene metabolism and enzyme activities after inhalation exposure were also investigated by **Pedersen and Schatz (1998)** (only abstract available). When Sprague-Dawley rats (information on sex is not given) were exposed for 1 or 3 days (6 hrs/day) at 300 ppm (1305 mg/m^3), a 32 % decrease in blood levels of ethylbenzene was measured between 1 and 3 days of exposure. This decrease in blood concentrations was accompanied by increases in hepatic microsomal CYP450 enzyme expression at the protein- and activity level. For example, CYP2B6-dependent benzoylresorufin dealkylase (BROD) activity increased from 123 % to 241 % between 1 and 3 days of exposure in relation to baseline; protein levels were similarly increased. CYP2E1 associated N-nitrosodimethylamine demethylase (NDMA) activity increased from 115 % to 129 % of controls. CYP1A1-associated ethoxyresorufin dealkylase activity (EROD) changed from 89 % to 138 % compared to controls. In addition, the *in vitro* rates of ethylbenzene microsomal metabolism to 1-phenylethanol increased by 19 % (substrate concentration of 0.5 mM) in comparison to baseline in tissues from animals exposed for 3 days to ethylbenzene. While exposures to ethylbenzene increased baseline levels of several CYP450 enzymes, the level of CYP2C11 protein after 3 days of exposure was only 82 % of control values. The preliminary conclusion of the authors was that while ethylbenzene induced its own metabolism, CYP2B1 was unlikely to play a major role in ethylbenzene metabolism.

In order to investigate the influence of long term repeated exposure to ethylbenzene on its own metabolism and on accumulation in fat, adult male Wistar rats were exposed to 0, 50, 300 or 600 ppm (corresponding to 0, 218, 1305 and 2610 mg/m^3) ethylbenzene (6 hrs/d, 5 d/week, for 2, 5, 9 and 16 weeks). Urine was collected on the first day of exposure and thereafter on the monday and thursday of the second, fifth and ninth week. Urine was analyzed for the following metabolites: 1-phenylethanol, omega-hydroxyacetophenone and mandelic-, phenylglyoxylic-, hippuric-, and phenaceturic acids. Perirenal fat samples were obtained on the fridays of experimental weeks 2, 5, 9 and 16. Results demonstrated that the level of expo-

sure, but not the duration of exposure can have a marked effect the pattern of the metabolites in the urine **(Engström et al., 1985)**. The concentration of total metabolites in urine showed a dose-related increase, but this was less than expected from linear extrapolation. Excretion of 1-phenylethanol and omega-hydroxyacetophenone were enhanced with increasing dose, whereas mandelic-, hippuric- and phenylglyoxylic acid were diminished. The concentration of ethylbenzene in the perirenal fat increased with an increase in the dose, but not linearly (50 ppm: 0.08-0.24 umol/g fat, 300 ppm: 1.6-2.0 umol g fat, 600 ppm: 2-3 umol/g fat). Significant interindividual variation in both urinary metabolite levels and ethylbenzene concentrations in fat could be observed between animals.

In order to investigate the influence of exposure to ethyl acetate on blood concentrations of ethylbenzene, adult female Sprague-Dawley rats were exposed by inhalation for 2 hrs to either 120, 240, 350 or 650 ppm (corresponding to 522, 1044, 1523 and 2828 mg/m^3) ethylbenzene alone or in combination with 1000 or 4000 ppm ethyl acetate. Blood was collected immediately after exposure and analyzed for ethyl benzene concentrations. The results demonstrated that co-exposure of rats with ethyl acetate lowered the blood concentrations of ethyl benzene by percentages between 0.4 and 26.8 %. Reduction of ethylbenzene blood concentrations was statistically significant at 650 ppm ethylbenzene **(Freundt et al., 1989)**.

Elovaara et al. (1984) studied the metabolic interaction of simultaneously inhaled m-xylene and ethylbenzene in the male Wistar rat. Animals (3 per combination of solvents) were exposed for 6 hrs a day for 5 days at the following combinations of concentrations of m-xylene (XYL) and ethylbenzene (EB) [concentrations are given in ppm, corresponding concentrations in mg/m^3 are given in brackets]: (1) 0 XYL + 0 EB; (2) 75 XYL + 25 EB (109 mg/m^3); (3) 300 XYL + 100 EB (435 mg/m^3) and (4) 600 XYL + 200 EB (870 mg/m^3). Additionally, four groups of six rats received m-xylene (300 or 600 ppm) or ethylbenzene (100 or 200 ppm (435 and 870 mg/m^3)) alone for a single 6 hrs exposure. Urine was collected during the exposure and during the 18 hr interval between exposures. Perirenal fat samples were analyzed for m-xylene and ethylbenzene. Urine was analyzed for the metabolites of m-xylene and ethylbenzene (hippuric acid, mandelic acid, phenylglyoxylic acid, 1-phenyl ethanol and phenaceturic acid). Thioethers were also measured in the urine. m-Xylene and ethylbenzene were present in the fat after 5 days exposure in the same molar ratio 3:1 as in the inspired air. Solvent concentration in the fat increased with dose level. Excretion of thioethers in the urine increased linearly up to 12-fold. 1-Phenyl ethanol appeared in the urine earlier than metabolites produced from later steps in the biotransformation. The metabolite pattern of m-xylene showed no difference between mixed and pure equimolar exposures whereas that of ethylbenzene metabolites was variable. The metabolism of ethylbenzene in the mixture was twice that following sole exposure. The authors noted that this finding was unexpected and it was based on a single data point.
Combined exposure to ethylbenzene and m-xylene resulted in a dose- and exposure-related increase in metabolism which at the highest combined dose level of 600 ppm m-xylene + 200 ppm ethylbenzene was greater than expected from the individual components.

The urinary excretion of mandelic acid was investigated in female Sprague-Dawley rats exposed for 15 days (6hrs/day) to either ethylbenzene alone (250 and 1000 ppm (1088 and 4350 mg/m^3)) or to the following combinations of ethylbenzene (EB) and methylethylketone (MEK): (1) 250 ppm EB (1088 mg/m^3) + 1000 ppm MEK; (2) 250 ppm EB + 3000 ppm MEK; (3) 1000 ppm EB (4350 mg/m^3) + 1000 ppm MEK; (4) 1000 ppm EB + 3000 ppm MEK **(Saillenfait et al., 2006)**. Urine was collected for 16 hrs after the first and the last exposure. The excretion of mandelic acid was 2- to 5-fold higher after 15 exposures than after one exposure to 1000 ppm EB, alone or in combination. After repeated treatments, the urinary

level of mandelic acid was significantly greater after co-exposure to 1000 ppm EB and 3000 ppm MEK when compared with 1000 ppm EB alone. Mandelic acid tended to decrease with increasing concentration of MEK, after one exposure to EB at either concentration or after repeated exposure to 250 ppm EB.

Tardif et al. (1996) studied the interaction of ethylbenzene, toluene and m-xylene on the blood concentrations of each single component by exposing male Sprague-Dawley rats for 4 hrs to 100 and 200 ppm (435 and 870 mg/m^3) of each single component or to binary and ternary mixtures with total concentrations of 300 ppm. Blood concentrations were measured 5, 30, 60, 90 and 120 min following the end of exposure. Details are only given for the data obtained with ethylbenzene: after exposure to 100 ppm ethylbenzene, the blood concentrations (estimated from figures) were 1.9 mg/l and 0.3 mg/l in samples taken 5 and 120 min after the end of exposure and 7.8 mg/l and 2.0 mg/l for an exposure to 200 ppm, respectively. The area under the blood concentration curves (AUC: mmol x min/l) were 1.3 (± 0.07) after an exposure to 100 ppm and 5.58 (± 0.98) for 200 ppm. Thus, increasing the exposure from 100 to 200 ppm led to a more than proportional increase in blood concentration and AUC indicating that metabolism is approaching saturation at 200 ppm. Exposure to binary and ternary mixtures resulted in significantly higher concentrations of ethylbenzene as a result of metabolic interaction between the solvents.

(Römer et al., 1988) investigated the influence of ethanol on blood concentrations of inhaled ethylbenzene. Adult female Sprague-Dawley rats (3 animals per group) were exposed by inhalation to 180 ppm (7833 mg/m^3) ethylbenzene for 2 hrs. One group received 20 mmol ethanol i.p. before exposure, whereas the other group received physiological saline i.p. Animals preatreated with ethanol exhibited 2.4-fold higher levels of ethylbenzene in blood in comparison to animals pretreated with physiological saline.

Dermal

Percutaneous absorption studies were conducted with ring-labelled [^{14}C]-ethylbenzene in male albino hairless mice (4.1 mg ethylbenzene on a surface of 0.8 cm² for 4 h, occlusive) **(Susten et al., 1990)**. Absorbed ethylbenzene determined from the measured radioactivity in urine, feces, carcass, skin application site and expired air was 3.4 % of the nominal dose. The absorption rate was calculated to 37 µg cm^{-2} min^{-1}. The total percentage recovered (including also wipe of skin area) was 95.2 %.The main route of excretion was via the urine and feces (measured together 65.6 % of absorbed dose). For most mice, the excretion rate was fastest during the first 15 minutes.

In a study to determine the dermal absorption of ethylbenzene (and other VOCs), either pure substance (2 ml) or aqueous solutions (2 ml) of different concentrations (1/3 saturated, 2/3 saturated and saturated) were applied to the dorsal skin (3.1 cm^2, occlusive exposure cell, for 24 h) of male Fischer F344 rats **(Morgan et al., 1991).** The rats (215 - 300 g) were fitted with a jugular indwelling catheter and blood samples were taken during the exposure (after 0; 0.5; 1; 2; 4; 8; 12 and 24 hrs) and analyzed by gas chromatography. The number of rats tested was not reported. After exposure, the volume of test solution remaining in the exposure chamber was measured. A peak level of 5.6 µg ethylbenzene per ml blood was measured one hr after application of the pure substance. The blood level decreased slightly over the 24 hr exposure period to approximately 3 µg/ml. No ethylbenzene could be detected in the blood at any time when ethylbenzene was applied in aqueous solution.

The authors calculated the volume of test substance solutions absorbed during the 24-hr exposure period by subtracting the amount that remained at the end of exposure from the 2 ml applied originally (amounts absorbed were: 0.24 ml (pure substance), 0.20 ml (saturated solution), 0.18 ml (2/3 saturated solution), 0.17 ml (1/3 saturated solution). The absorption of 0.24 ml undiluted ethylbenzene would lead to an absorption rate of 47 µg cm^{-2} min^{-1}. When exposure cells contained only distilled water, 0.18 ml water was absorbed over the 24 hrs. Aqueous solutions remaining in the exposure chambers contained less than 1 % of the initial test concentration. The authors state that evaporation of the test material during exposure was considered unlikely because sealed caps (using teflon coated silicone liners) were used on the exposure cells. The initial concentration of ethylbenzene in the aqueous test solutions was 134, 84 and 47 µg/ml for saturated, 2/3 and 1/3 saturated solutions respectively. In summary, undiluted ethylbenzene was absorbed through the skin of rats giving a peak blood level 1 hr after application. Following exposure to aqueous solutions of ethylbenzene the test substance was not detected in the blood.

Oral

Smith et al. (1954a) administered a single oral dose of ethylbenzene by gavage to rabbits (433 mg/kg bw). Urine was collected (time period not specified). Levels of glucuronic acid and ethereal sulphate were measured in the urine. 32 % of the dose administered was recovered as glucuronides. There was no excretion of etheral sulphate.

In a subsequent publication **(Smith et al, 1954b)** the stereochemical aspects of ethylbenzene metabolism was investigated in 6 rabbits given 15 g/kg bw of ethylbenzene orally. Urine was collected for 36 hrs and after derivatisation, the pure methyl ((-) methylphenylcarbinyl-tri-O-acetylglucosid)uronate was isolated as well as the (+) isomer.

Sollenberg et al. (1985) investigated the excretion of mandelic acid and phenylgloyxylic acid after administration of single oral doses of ethylbenzene (350 mg/kg bw in corn oil) to four male Sprague-Dawley rats. Urine was collected as voided (no information about the duration of sampling is given), extracted with diethyl ether and analyzed by isotachophoresis and HPLC. It could be demonstrated, that the excretion of mandelic acid and phenylglyoxylic acid in rats begins with the first urine voiding after dosing and that peak concentrations in urine are reached between 15 and 19 hrs after dosing. Undetectable levels are achieved after 48 hrs.

Climie et al. (1983) investigated metabolism and excretion after single oral administration of ring-labelled ^{14}C-ethylbenzene (30 mg/kg bw; 44.5 µCi) to 6 female Wistar rats. Urine and feces were collected daily for 48 hrs. Total radioactivity in excreta was determined by liquid scintillation, metabolites were identified by thin layer chromatography and mass spectroscopy. A rapid excretion took place mainly by urine (80.3 % and 82.4 % of the applied radioactivity after 24 and 48 hrs, respectively) and to a much lesser extent by feces (1.1 % and 1.5 % of the applied radioactivity after 24 and 48 hrs, respectively). As major metabolites mandelic acid (23 % of the dose), hippuric acid (34 %) and the glucuronide of 1-phenylethanol (8 %) were identified. A further metabolite representing 8.5 % of the dose could not be identified.

Drummond et al. (1989) investigated the stereochemistry of mandelic acid formation after oral administration of ethylbenzene (100 mg/kg bw) to male Wistar rats. Urine was collected at 24 hr-intervals for 96 hrs and urine was analyzed by mass spectrometry, HPLC and NMR. Only the R-enantiomer of mandelic acid was excreted after ethylbenzene exposure.

Bakke and Scheline (1970) studied the hydroxylation of ethylbenzene following a single oral administration of ethylbenzene in propylene glycol (100 mg/kg bw) to 5 male albino rats. The animals were fed a diet containing neomycin in order to reduce the levels of normally occurring simple urinary phenols. After administration, urine was collected over a period of 48 hrs and analyzed by gas- and thin-layer chromatography after enzymatic hydrolysis and extraction. A major metabolite was 1-phenylethanol from side-chain oxidation besides traces of 2-phenylethanol, but quantitative data were not given. 1-Phenyl-1,2-ethandiol was not detected. Ring hydroxylation was a minor pathway (0.3% of 4-ethylphenol was found).

In Chinchilla rabbits, which received approximately 300 mg/kg bw ethylbenzene by gavage, the main metabolites were identified as hippuric acid (24 – 36 % of the dose), methyl-phenylcarbinyl glucosiduronic acid (in amounts comparable to the amounts of hippuric acid) and phenaceturic acid (10 – 20 % of the dose). Mandelic acid was a minor metabolite (2%) **(El Masry et al., 1956)**.

Other routes: i.p. administration

In a study in male rabbits (no information on strain was given) the animals received a single i.p. injection of 1.0 g ethylbenzene per rabbit (corresponding to approximately 230 – 260 mg/kg bw). Two series of experiments were performed using five animals per experiment. Urine was collected over a 48 hr period and treated with ß-glucuronidase before isolation of metabolites (**Kiese and Lenk, 1974).** Analysis of the urine showed that 2 – 10 % of the applied dose was excreted as 1-phenylethanol. Minor metabolites included omega-hydroxyacetophenone (0.04 - 0.11 % of the applied dose), 4-hydroxyacetophenone (0.13 %) and 3-hydroxyacetophenone (0.03 %).

McMahon and Sullivan (1966) investigated the stereochemistry of urinary methylcarbinol formation after i.p. administration of ethylbenzene (1.1 g/kg bw) to untreated rats and to rats pretreated by daily i.p. applications of phenobarbital (40 mg/kg bw) for four days (no data on strain and sex is given). Non-induced rats converted ethylbenzene to methylphenylcarbinol composed of 90.3 % of the D-(+) isomer. In Phenobarbital-treated rats, a reduction in stereo-specifity was observed (77.4 % of the D-(+)-isomer).

Sullivan et al. (1976) investigated the stereospecifity of the conversion af ethylbenzene to mandelic acid. After administration of ethylbenzene (100 mg/kg bw) to male albino rats, optical activity of urinary mandelic acid was determined. It could be demonstrated that mandelic acid formed in vivo was laevo mandelic acid of high optical purity. The metabolic steps leading to mandelic acid were elucidated (in this study, several compounds were investigated, whereby the dosing frequency was dependent on the toxicity of the respective substance. Concerning ethylbenzene, no further details were given whether single administration or repeated administration for three days was used).

Ex vitro studies

Elovaara et al. (1984) investigated the effects of simultaneous inhalation of m-xylene and ethylbenzene (6 hrs/day, for 5 days) on the microsomal drug metabolizing enzymes of the liver and the kidneys. Organs were obtained after the 5th 6 hr-period from animals exposed to the following combinations of concentrations of m-Xylene (XYL) and ethylbenzene (EB) [given in ppm, mg/m^3 in brackets]: (1) 0 XYL + 0 EB, (2) 75 XYL + 25 EB (109 mg/m^3), (3) 300 XYL + 100 EB (435 mg/m^3) and (4) 600 XYL + 200 EB (870 mg/m^3). CYP450 protein, EROD-activities and UDP-glucuronosyltransferase activity were determined in the micro-

somal preparations. The CYP450 protein content was increased in liver microsomes, but not in kidney microsomes. EROD activity was highest in the 600 + 200 ppm dose, however, a clear increase could only be observed in kidney microsomes, but not in liver microsomes. UDP-glucuronosyltransferase activity was enhanced only by treatment with the highest dose, and more clearly in the liver than in the kidneys.

Elovaara et al. (1985) exposed male Wistar rats (6 hrs/day, 5 days/week) to 0, 50, 300 or 600 ppm (corresponding to 0, 218, 1305 and 2610 mg/m^3) ethylbenzene. Animals were killed after 2, 5, 9, or 16 weeks of exposure. After exposure to 600 ppm, total liver microsomal protein, but not CYP450 total protein was slightly increased. 7-Ethoxycoumarin O-deethylase (ECOD)-activity was increased 1.8-fold and UDP-glucuronosyltransferase activity was increased 2.3-fold. The increase in liver cytosolic D-glucuronolactone dehydrogenase paralleled the up to 2-fold glucuronidation activity. In the kidneys, ECOD-activity was increased 3.5 fold and UDP-glucuronosyltransferase activity was increased up to 1.8-fold. Ethylbenzene did not deplete hepatic glutathione, whereas kidney glutathione was slightly (up to 1.3-fold) increased. Urine excretion of thioethers was increased with dose and was 8-fold the control levels at 600 ppm.

Toftgard and Nilsen (1982) investigated the effects of ethylbenzene on *in vitro* enzymatic activities in rat liver, kidney and lung. Male Sprague-Dawley rats were exposed by inhalation to 2000 ppm (8700 mg/m^3) ethylbenzene (6 hrs/d, 3 d). On the morning of the 4th day, lung, livers and kidneys were taken for preparation of microsomes. Tissues from animals exposed to air only were taken for controls. In liver, incubations with 4-[4-^{14}C]androstene-3,17-dione, benzo[a]pyrene (B[a]P), 7-ethoxyresorufin and n-hexane were performed. The metabolism of 7-ethoxyresorufin was increased 3 times compared to controls. Formation of 2- and 3-hexanol from n-hexane was increased 10-fold compared to controls and the formation of 16-hydroxylated metabolites from 4-[4-^{14}C]androstene-3,17-dione was also increased; the formation of the 4,5-dihydrodiol from (B[a]P) was also 10-fold increased. In kidney microsomes, dealkylation of 7-ethoxyresorufin and formation of 1-hexanol were increased two- and 2.5-fold. In lung microsomes, on the other hand, dealkylation of 7-ethoxyresorufin and hydroxylation of n-hexane, were reduced compared to controls.

Stott et al. (1999; 2003) investigated mixed function oxidase (MFO) activities in kidneys, livers and lungs of Fischer F344 rats and B6C3F1 mice of both sexes after inhalation exposure. Animals were exposed to 0, 75 or 750 ppm (corresponding to 0, 326 and 3262 mg/m^3) ethylbenzene for 6 hrs per day for either one week (6 rats and 30 mice per sex and exposure level) or to 0 and 750 ppm ethylbenzene for four weeks (8 rats and 40 mice per sex and per exposure level). Microsomes from rat kidneys, mouse liver and mouse lung (lungs were pooled) were prepared and the following enzyme activities were determined: ethoxyresorufin O-deethylase (EROD)-activity (index for CYP1A1), methoxyresorufin O-demethylase (MROD)- activity (index for CYP1A2; investigated only in the four week study), pentoxyresorufin O-depentylase (PROD) activity (index for CYP2B1/2), ethoxycoumarin O-dealkylase (ECOD) activity (index for several different CYP enzymes), p-nitrophenol hydroxylase (p-NPH) activity (index for CYP2E1) and UDP glucuronosyl transferase (UGT) activity. Changes in renal enzyme activities after ethylbenzene exposure in rats of both sexes were small: p-NPH activity in males, PROD activity in females and UGT activity in both sexes of rats exposed to 750 ppm for one week were increased 89 %, 71 % and 29-30 % of control levels. p-NPH activity in females was increased slightly. PROD and ECOD activities in both sexes of rats were below the detection limits. There were no changes in enzyme activity levels in rats exposed to 75 ppm for one week. After 4-week exposures, most enzyme activities were

similar or somewhat lower than control values with the only statistically identified changes being a decrease in the activities of MROD and PROD activities in exposed females.
In mouse liver, PROD and ECOD activities were slightly decreased in both sexes after one week exposure to 75 ppm. EROD activities were elevated in both sexes after one week of high dose exposure. In males, PROD and ECOD activities were also elevated whereas p-NPH activity was not elevated in males. Treatment-related alterations of ECOD- and p-NPH-activities in females as well as alterations of UGT activities in both sexes were not observed after one week exposure.
After four week exposures to 750 ppm, liver PROD activity remained increased in males and females (81 % and 130 % relative to controls, respectively), p-NPH activities were also increased. In females, EROD and UGT activities were also increased.
In the mouse lung, several enzyme activities (EROD, PROD and ECOD) were decreased in a dose-dependent manner relative to controls after one week of exposure, but no significant net changes in pulmonary p-NPH activity were observed in treated animals. After four weeks of exposure, p-NPH and UGT activities were increased in males. In females, the activities of EROD, MROD and PROD were statistically decreased.

McMahon and Sullivan (1966) investigated *in vitro* the stereochemistry of formation of methylcarbinol from ethylbenzene in liver microsomes from either untreated rats or rats pretreated by daily i.p. applications of phenobarbital (40 mg/kg bw) for four days (no data on strain and sex is given). In microsomes from non-induced rats, ethylbenzene was converted to methylphenylcarbinol composed of 80.9 % of the D-(+) isomer. In phenobarbital-treated rats, a reduction in stereospecifity was observed (66.7 % of the D-(+) isomer was detected).

The effect of ethylbenzene on hepatic and pulmonary enzymes was investigated in liver and lung microsomes prepared from tissues of male Sprague-Dawley rats 24 hrs after i.p. injection of 5 mmol/kg (approx. 530 mg/kg) ethylbenzene in corn oil **(Pyykkö et al., 1987)**. In the lungs, the concentration of CYP450 protein was decreased by about 60% of control, whereas it was increased by about 50 % of control in hepatic microsomes (percentages were derived from figures).
CYP450-related aryl hydrocarbon hydroxylase-, 7-ethoxycoumarin O-deethylase (ECOD)- and 7-ethoxyresorufin O-deethylase (EROD) activities in liver microsomes were statistically significantly increased compared to controls. In lung microsomes, aryl hydrocarbon hydroxylase- and ECOD-activities were statistically significantly lower compared to controls, whereas EROD activity was higher compared to control values (statistically not significant).

The induction of cytochrome P450 isoenzymes was studied in liver microsomes obtained from male Sprague-Dawley rats following i.p. administration of ethylbenzene in corn oil over 4 days with daily doses of 5 mmol/kg (~530 mg/kg). Various CYP450 substrates were investigated and the activity was significantly increased for aminopyrine N-demethylation, aniline hydroxylation, 7-ethoxycoumarin O-dealkylation and testosterone hydroxylation at the 7α- and 16ß-positions. Testosterone hydroxylations were also increased at 2ß-, 6ß-, and 15α-position and slightly decreased at 2α- and 16α-position. Immunoblotting of eleven forms of CYP450 enzymes demonstrated that CYP450 isoenzymes CYP2B1, CYP2B2, CYP2C6, CYP2E1 and CYP3A2 were increased, while that of CYP2C11 was decreased. The content of total CYP450 measured photometrically was not changed. The *ex vivo* results were supplemented by *in vitro* experiments studying testosterone hydroxylation by rat microsomal preparations: ethylbenzene at concentrations of 0.25 and 25 mM inhibited testosterone hydroxylation at all hydroxylation sites investigated. As derived from the figures given, inhibition was most pronounced on 15α-position (15α-hydroxylation is catalyzed by CYP2A2), 16ß-position

(16ß-hydroxylation is catalysed by CYP2B1), 16α-position and 6ß-position (catalysed by CYP3A2) **(Imaoka and Funae, 1991)**.

Sequeira et al. (1992) investigated sex differences of a variety of catalytic activities as well as CYP450 protein levels in liver microsomes prepared from male and female Holtzman rats treated with ethylbenzene (10 mmol/kg bw, approx. 1080 mg/kg bw, intraperitoneally injected for 3 days). Benzphetamine N-demethylation, 7-ethoxycoumarin O-deethylation and p-nitroanisole O-demethylation were induced in both sexes after treatment with ethylbenzene. In females, the increase was always higher compared to males. Aminopyrine N-demethylase, aniline p-hydroxylation and dimethylnitrosamine N-demethylation were induced exclusively in the female rat. Concomitantly, CYP450 protein content was doubled in female rats and did not change in male rats after ethylbenzene treatment. The largest effect of treatment was the formation of aromatic hydroxylated metabolites of toluene and an increase in the rate of o-cresol formation by 4- and 9-fold in female and male rats, respectively. Western immunoblotting indicated that ethylbenzene treatment induced CYP2B1/2B2 to a greater extent in male rats while induction of CYP2E1 occurred only in females. Ethylbenzene exposure did not affect significantly the level of CYP1A1.

In a further experiment **Sequeira et al. (1994)** investigated the temporal changes of CYP450 expression in liver microsomes from male Holtzman rats treated either with a single i.p. injection of ethylbenzene (10 mmol/kg bw) or in rats receiving a similar treatment for 3 consecutive days. CYP450 isozymes were determined by benzphetamine N-demethylation, by O-demethylation of p-nitroanisole, aminopyrine N-demethylation, aniline p-hydroxylation and dimethylnitrosamine N-demethylation and by immunoblotting. Furthermore, toluene metabolism (formation of benzyl alcohol, o-cresol and p-cresol) were also investigated. Two general patterns of induction became evident: 1.) CYP2B-dependent activities (benzphetamine N-demethylation, O-demethylation of p-nitroanisole) were induced both after 1 and 3 days of exposure, while 2.) CYP2E1-dependent activities (aniline p-hydroxylation and dimethylnitrosamine N-demethylation) were induced only after the single injection but normalised after 3 days of treatment. Changes in enzymatic activities were consistent with changes in isoenzyme levels as determined by immonoblotting. These results demonstrated that CYP2E1 levels may be increased after acute exposures but not after prolonged administration. Furthermore, CYP2C11 protein levels were reduced in microsomes from livers of ethylbenzene-treated animals.

Yuan et al. (1994) studied CYP3A-dependent activities in liver microsomes from male Holtzman rats treated with a single i.p. injection of ethylbenzene in corn oil (10 mmol/kg bw) and rats treated only with corn oil. Microsomes and mRNA were prepared from animals sacrificed at various times after injection. The effects on CYP3A-dependent 2ß-testosterone hydroxylation, immunoreactive protein levels and CYP3A1- and CYP3A2 mRNA levels were determined. The enzyme levels (based on 2ß-testosterone hydroxylation and CYP3A protein) were maximally induced 24 h after treatment and diminished thereafter. Despite the increases in CYP3A protein and 2ß-testosterone hydroxylation, CYP3A2 mRNA levels were not affected and CYP3A1 mRNA levels were not dectable in either control liver microsomes or microsomes from ethylbenzene-treated animals. The authors conclude that CYP3A enzymes can be enhanced by either translational activation or protein stabilization.

In a follow-up study **Yuan et al. (1997)** investigated the time course of changes in CYP450 expression and toluene metabolism following acute exposure to ethylbenzene. Male Holtzman rats were given a single i.p. injection of ethylbenzene in corn oil or corn oil alone and

sacrificed 1, 2, 5, 10, 15, 24 and 48 hrs after the injection. The effect of ethylbenzene on toluene metabolism was investigated *in vitro* by comparing toluene metabolism in microsomal preparations from ethylbenzene treated and control (corn oil treated) animals. Besides total CYP450 protein, various CYP450 enzymes were investigated by measurement of microsomal activities (testosterone 16ß-hydroxylation and 2α-hydroxylation as indices of CYP2B1/2- and CYP2C11-dependent activities; N,N-dimethylnitrosamine-demethylation and formation of p-aminophenol from aniline as markers for CYP2E1-dependent activities; 7-ethoxyresorufin O-deethylase (EROD) activity as an index for CYP1A1 activity), by determination of CYP450 specific proteins by Western Blotting (CYP1A1, CYP2B1/2, CYP2C11, CYP2E1) and mRNA determinations (CYP1A1, CYP2B1, CYP2B2, CYP2C11 and CYP2E1).

CYP2B1/2-dependent testosterone 16ß-hydroxylation and CYP2B1/2 immunoreactive protein were elevated 30-fold after ethylbenzene administration, reaching maxima by 24 hrs and remaining elevated 48 hr after exposure. Changes of CYP2B1 and CYP2B2 mRNAs preceded those of the proteins. Similar results were observed for CYP1A1. CYP2E1 mRNA levels were elevated after ethylbenzene administration. However, the elevation in CYP2E1 dependent activities and CYP2E1 immunoreactive protein preceded the changes in mRNA. CYP2C11 on the other hand was rapidly suppressed (within the first 2 – 10 hrs) after ethylbenzene administration.

Toluene was almost entirely metabolized to benzylalcohol in microsomes from untreated animals. In liver microsomes from ethylbenzene-treated rats, significant quantities of benzyl alcohol, o-cresol, and p-cresol were produced. 5 – 10 hrs after treatment, there was a 40 % reduction in benzyl alcohol production (which is consistent with the decrease in CYP2C11). 24 hrs after ethylbenzene treatment, rates of benzyl alcohol formation returned to control levels whereas there was a 7-fold increase in o-cresol and a greater than 50-fold increase in p-cresol production (consistent with the induction of CYP2B1/2, CYP2E1 and CYP1A1).

In vitro studies

Dermal

The *in vitro* penetration rate of ethylbenzene (1 ml) through the excised abdominal skin from SD-JCL rats (no information on sex was provided) was determined by using a glass diffusion cell, where 0.9 % saline containing penicillin and streptomycin was used as receptor fluid **(Tsuruta, 1982)**. The application skin area was 2.55 cm², the application time 3 to 6 hrs. A penetration rate of 0.11 µg cm^{-2} min^{-1} was calculated. The time to reach steady state diffusion (lag time) was 2 hrs.

McDougal et al. (2000) investigated the *in vitro* skin penetration of JP-8 fuel containing approximately 1,200 µg ethylbenzene/ml fuel (0.15 % w/w). Back skin from male F344/CrlBr rats was used in static diffusion cells, physiological saline containing 6 % Volpo 20 (polyethylene glycol-20 oleyl ether) served as receptor medium, the receptor solution was sampled at half-hour intervals for 4 hrs and analyzed by headspace GC/MS. An ethylbenzene flux of 0.377 µg cm^{-2} hr^{-1} (0.0004 µg cm^{-2} hr^{-1}) and a permeability coefficient of 3.1×10^{-4} cm hr^{-1} during a 4-hr period was derived from excised rat skin which had received 2 ml of JP-8 fuel containing approximately 1,200 µg ethylbenzene/ml fuel (0.15 % w/w).

The effect of pre-exposure to jet fuel on the absorption of JP-8 jet fuel hydrocarbons (such as ethylbenzene) was investigated by **Muhammad et al. (2005)**. Skin (500 µm thickness) from pre-exposed or non-exposed female weanling Yorkshire pigs was investigated in flow-through diffusion cells (dosing surface area: 0.64 cm^2). A total of 20 µl of a dosing mixture of

different hydrocarbons diluted in ethanol-water as solvent was applied onto the skin. Cells were perfused and perfusate samples were taken for up to 300 min and analyzed by gas chromatography. The steady state flux, permeability, and diffusivity values of ethylbenzene in pig skin treated with JP-8 *ex vivo* were 1.04 µg cm^{-2} hr^{-1}, 0.06 x 10^{-3} cm hr^{-1}, and 715 x 10^{-6} cm^2 hr^{-1}. Skin from preatreated animals yielded steady state flux, permeability, and diffusivity values of ethylbenzene after additional *ex vivo* treatment of 3.32 µg cm^{-2} hr^{-1}, 0.19 x 10^{-3} cm hr^{-1}, and 1065 x 10^{-6} cm^2 hr^{-1}, indicating that pre-exposure to jet fuel enhances the subsequent in vitro percutaneous absorption of ethylbenzene.

Other in vitro studies

A study with hepatic and adrenal microsomes from adult male guinea pigs of the English Smooth Hair variety indicated that ethylbenzene has a far greater affinity for adrenal than hepatic CYP450 **(Greiner et al., 1976)**.

In vitro isotope studies have shown that formation of 1-phenylethanol in rat liver 15 000 g supernate (no information on strain and sex was provided) occurs by direct oxygenation of ethylbenzene by front side displacement **(McMahon et al., 1969)**.

After incubation of ethylbenzene with liver microsomes from adult Chinook salmon, identified 1-phenylethanol and benzyl alcohol were identified as metabolites **(Kennish et al., 1988)**.

Kaubisch et al. (1972) investigated the ring hydroxylation of ethylbenzene in liver microsomes from methylcholanthrene preatreated (40 mg/kg for 2 days, sacrifice at day 3) male Sprague-Dawley rats. Both 4-hydroxyethylbenzene and 2-hydroxyethylbenzene could be identified.

Nakajima and Sato (1979) investigated the effect of fasting on the *in vitro* metabolism of ethylbenzene in liver homogenates from Wistar rats of both sexes following fasting for up to 3 days. There was no significant effect on microsomal protein or CYP450 content but hepatic metabolism of ethylbenzene was approximately doubled in the fasted rats as measured by ethylbenzene disappearance. A sex difference was noted in *in vitro* metabolism in fed and one day fasted rats, however this difference became less as the duration of the fasting increased.

Midorikawa et al. (2004) investigated the metabolism of ethylbenzene in liver microsomes from male Sprague-Dawley rats which have been pretreated by oral administration of phenobarbital (60 mg/kg bw) daily for three days before preparation of microsomes. After incubations (for 30 min) and purification, metabolites were identified by HPLC and GC/MS. The major metabolism of ethylbenzene was by side chain oxidation producing the metabolites 1-phenylethanol (3 % of ethylbenzene) and acetophenone. The ring hydroxylated metabolites 2-ethylphenol (0.0048 %) and 4-ethylphenol (0.014 %) were also measured but only in very low amounts.

Beliveau and Krishnan (2000) determined the concentration dependency of the blood:air partition co-efficient by the vial equilibrium method using either rat blood or a mixture of n-octanol and water. The blood:air partition co-efficient decreased significantly with increasing ethylbenzene concentration with no significant change for n-octanol-water:air partitioning. The authors comment that the concentration-dependent nature of the blood:air partition co-

efficient does not need to be considered for PBPK modelling of rat inhalation exposure up to several thousand ppm.

In a study reported by **Saghir et al. (2007)** (the original study is not available to the rapporteur, the description was taken from the industry version of the RAR), ethylbenzene was incubated at concentrations ranging from 0.22 to 7mM with liver and lung microsomes of mouse, rat and humans to measure formation of 1-phenylethanol, acetophenone, 2,5-ethylquinone and 3,4-ethylquinone. Molar conversion varied broadly depending on the tissue and species with none of the metabolites being formed in incubations with human lung microsomes. The side chain hydroxylated metabolites (1-phenylethanol and acetophenone) were found at much higher levels than the ring hydroxylated metabolites, (i.e. 2,5-ethylquinone and 3,4-ethylquinone) but with large variations between the species. For example, molar conversion to 1-phenylethanol (the major metabolite) ranged from 1 % in rat lung at 7mM ethylbenzene to 58 % in mouse lung at 0.22 mM ethylbenzene. Similarly, the highest level of ring hydroxylated metabolites was found in mouse lung with no evidence of ring hydroxylation being measured in human lung. Overall the pulmonary metabolism of ethylbenzene ranked according to species was mouse > rat > human. The authors hypothesize that the more efficient metabolism of ethylbenzene to ring oxidized metabolites by mouse lung may be the underlying reason for the lung specific toxicity seen in the mouse.

4.1.2.1.2 Studies in humans

<u>In vivo studies</u>

Inhalation – Controlled exposure

Bardodej and Bardodejova (1970) exposed human volunteers (no information on number and sex of volunteers is given) to 23 - 85 ppm ethylbenzene (100 – 370 mg/m^3) in the inspired air during 8 hrs. Due to the lack of experimental detail and lack of analytical sensitivity for determination of urinary metabolites, the value of the results is limited. However, it was demonstrated that in man only trace amounts of ethylbenzene were excreted in expired air following cessation of exposure. 64 % of the inspired vapor was retained in the respiratory tract. The following percentages of the retained amount were eliminated as urinary metabolites: 64 % mandelic acid, 25 % phenylglyoxylic acid and 5 % methyl phenyl carbinol. Acetophenone, phenylethylene glycol and omega-hydroxyacetophenone were not demonstrated at a limit of detection of 2 %. Hippuric acid and mercapturic acids were not increased in the urine (level of detection 2 %).

A lower pulmonary retention rate in human volunteers was reported by **Gromiec and Piotrowski (1984)**. After the exposure of six healthy men (aged between 27 and 32 years) for 8 hrs to ethylbenzene vapours of 18, 34, 80, 150 and 200 mg/m^3 (corresponding to 4, 8, 18, 35 and46 ppm), the pulmonary retention rate was 49 % of the inhaled dose. The main metabolite in the urine was mandelic acid (55 % of retained ethylbenzene; 22.6 % were excreted during the 8 hr exposure, up to 43.6 % were excreted during 14 hrs after exposure). Mandelic acid excretion was biphasic with biological half-life values of 3.1 and 24.5 hrs.

Drummond et al. (1989) exposed 2 healthy male volunteers (25 and 39 years) to ethylbenzene vapours (435 mg/m^3, corresponding to 100 ppm) in an exposure chamber for 4 hrs. Urine was collected before exposure (one sample per volunteer), at 1 hr intervals during exposure and for 48 hrs after exposure. By analyzing the stereochemistry of the excreted mandelic acid, only the R-form was found.

Engström and Bjurström (1978) investigated the total uptake, alveolar concentrations and fat content of m- and p-xylene and ethylbenzene in 12 male volunteers after exposure to industrial xylene which consisted of 40.4 % ethylbenzene. Six subjects were exposed to 200 ppm industrial xylene during 30 min of rest and 90 min of exercise at a work load of 50 W. Six subjects were exposed to 100 ppm for 30 min at rest and during three 30 min periods of exercise at work loads of 50, 100 and 150 W. Needle biopsies of subcutaneous adipose tissue was taken 0.5, 2, 4 and about 22 hrs after the end of the exposures. The estimated solvent retention in adipose tissue was 5 %. Since there was no indication of differences in turnover rates of chemicals within the mixture, it is likely that the retention of ethylbenzene in adipose tissue was approximately 2 % of the total uptake. The mean concentrations of ethylbenzene (and xylene) in both groups of subjects were comparable at the time points 4 hr and 22 hr after the end of the exposure. Therefore the authors concluded that elimination of ethylbenzene (and xylene) from adipose tissue is slow.

Tardif et al. (1997) have investigated the excretion of phenyl glyoxylic acid and mandelic acid after controlled exposure of human volunteers in a dynamic exposure chamber. Exposure duration was 7 hrs. Urine was collected after the first 3 hrs of exposure, at the end of exposure and during 17 hrs after exposure. Concerning results, reference is given to **Tardif et al. (1991)**, but in **Tardif et al. (1991)**, no further results are presented.

Inhalation - Occupational exposure

Angerer and Lehnert (1979) investigated ethylbenzene concentrations in blood and urinary excretion of phenolic compounds in four female workers exposed for a workday to vapours of a solvent consisting of 25.25 % ethylbenzene, 6.72 % o-xylene, 52.63 % m-xylene, 15.24 % p-xylene and a small amount (0.16 %) of toluene. Blood analysis was performed at the end of the working day, urine was collected for a 24 hr period following exposure. Average concentrations of ethylbenzene were 34 and 41 ppm (corresponding to approximately 150 and 180 mg/m^3), average concentrations of xylene (all isomers) were 66 and 81 ppm. At the end of the shift, blood ethylbenzene concentrations were between 0.5 and 0.8 mg/l. Urine analysis demonstrated, that about 1.1 - 1.4 % of the retained ethylbenzene was metabolized to 2-ethylphenol while no 2,4-dimethylphenol, as a metabolite of m-xylene, could be detected. This finding seems to indicate that under simultaneous exposure of ethylbenzene and xylene a competitive mechanism takes place and ring oxidation of xylene is reduced by ethylbenzene.

Ethylbenzene concentrations in whole blood collected from 30 workers at the end of their work shifts (the maximum ethylbenzene concentration in the workplace was 5 ppm (corresponding to 22 mg/m^3)) correlated significantly with the average concentrations of occupational exposure to ethylbenzene. No significant correlations could be observed between solvent concentrations in the breathing zone air and the metabolites phenylglyoxylic acid and mandelic acid in shift-end urine samples. Corrections for urine density in terms of creatinine concentration or specific gravity did not improve the correlations **(Kawai et al. 1992),** although it had been demonstrated by others **(Ogata and Tagutchi, 1988)** that variation coefficients for urinary metabolites of hydrocarbons such as ethylbenzene were lower when corrected for creatinine. However, in an earlier study, the same authors found a statistically significant correlation between urinary excretion of mandelic acid and ethylbenzene exposure in 121 male workers exposed to mixed solvents (including an ethylbenzene time-weighted average [TWA] of 0.9 ppm (corresponding to 4 mg/m^3)) in a metal-coating factory, whereas no correlation was observed between ethylbenzene exposure and phenylglyoxylic acid urinary excretion **(Kawai et al., 1991).**

Concentrations of ethylbenzene metabolites in before-shift and after-shift urine were significantly higher in workers exposed from 85 – 921 ppm (corresponding to 370 – 4006 mg/m^3) ethylbenzene in a styrene plant than in control workers exposed to 33.4 – 66.8 ppm (corresponding to 145 – 290 mg/m^3) **(Holz et al., 1995).**

In a study conducted in Italy, blood concentrations of ethylbenzene in non-smoking policemen working as traffic wardens showed no significant differences between before and after work shift values or from blood ethylbenzene concentrations in policemen working indoors (**Fustinoni et al. 1995**). Indoor and outdoor mean air concentrations (measured by personal air samplers) were 21 and 37 mg/m^3 (corresponding to 5 and 8.5 ppm), respectively. Before and after shift blood ethylbenzene concentrations were 140 and 163 ng/l in indoor workers, respectively, and were 158 and 184 ng/l in outdoor workers, respectively.

Combined dermal and inhalation exposure

An initial concentration of ethylbenzene in blood of 2.6 µg/mL and a half-life of 27.5 hours were estimated in a 44-year-old man who died after a massive inhalation and dermal exposure of ≥10 hours to gasoline (**Matsumoto et al. 1992**). This absorption value may have been slightly overestimated, however, because possible contributions from dermal exposure were not addressed.

Dermal

To determine dermal absorption of ethylbenzene vapors, three experiments were carried out with one male volunteer who was exposed for 2 hrs at concentrations of 650 to 1300 mg/m^3 (corresponding to 150 - 300 ppm) **(Gromiec and Piotrowski, 1984)**. The exposed skin accounted for about 90-95 % of the total skin area. Clean breathing air was provided by means of a gas-tight respirator. Urine was collected before, during and after exposure and analysed for mandelic acid. The levels of urinary mandelic acid during and after exposure were within physiological limits indicating that the skin is not a relevant route of entry of ethylbenzene vapours.

Studies in humans dermally exposed to liquid ethylbenzene, on the other hand, demonstrate absorption through the skin **(Dutkiewicz and Tyras, 1967)**. Absorption rates of 24–33 mg cm^{-2} hr^{-1} and 0.11–0.23 mg cm^{-2} hr^{-1} have been measured for male subjects exposed to liquid ethylbenzene (measured in seven individuals) and ethylbenzene from aqueous solutions (measured in 14 individuals), respectively. The average amounts of ethylbenzene absorbed after volunteers immersed one hand for up to 2 hours in an aqueous solution of 112 or 156 mg/l ethylbenzene were 39.2 and 70.7 mg ethylbenzene, respectively (the amount of ethylbenzene absorbed was calculated from the difference between the applied and the remaining ethylbenzene). The urinary excretion of mandelic acid in humans dermally exposed to ethylbenzene for 2 hours was only 4.6 % of the absorbed ethylbenzene. Urine was collected over a period of 24 hrs after the beginning of the experiment. No ethylbenzene was reported to be excreted in exhaled air. These results indicate, that skin absorption occurs after dermal exposure to neat liquid ethylbenzene or ethylbenzene diluted in water.

Oral

No studies concerning the toxicokinetics of ethylbenzene after oral exposures of humans are available.

In vitro studies

Sams et al. (2004) studied the initial hydroxylation of ethylbenzene to 1-phenylethanol by *in vitro* incubation with human liver microsomes from seven individual donors of both sexes, selective inhibitors and microsomes from recombinant insect cells infected with different human CYP450 enzymes. Production of 1-phenylethanol exhibited biphasic kinetics with a high affinity, low K_m component (K_m = 8 µM; V_{max} = 689 pmol/mg protein/min) which was inhibited 79 – 95 % by diethyldithiocarbamate and a low affinity, high K_m component (K_m = 391 µM; V_{max} = 3039 pmol/mg protein/min). The results suggested that CYP2E1 catalyzed the high-affinity component and CYP1A2 was involved in catalyzing the low-affinity component. As CYP2E1 was the major enzyme responsible for high-affinity side chain hydroxylation, the authors conclude that the variability of this enzyme should be incorporated into risk assessments.

4.1.2.1.3. Physiologically-based toxicokinetic modeling

Several models have been developed which simulate the kinetics of inhaled ethylbenene in animals and humans **(Dennison et al., 2003: Tardif et al., 1997 and Nong et al., 2007)**. Furthermore, a model of dermal absorption of ethylbenzene in humans has also been reported (Shatkin and Brown, 1991). Models for simulation of ethylbenzene kinetics after oral ingestions have not been reported.

Tardif et al. (1997) developed a PBPK model for simulating the kinetics of ethylbenzene, toluene and xylene following inhalation exposures of humans and rats to the individual chemicals or the ternary mixture. Tissue compartments were limited to lungs, liver, fat, richly perfused tissues and poorly perfused tissues. All metabolism was attributed to the liver. Partition coefficients were derived from equilibrium studies with isolated rat tissues, metabolism parameters were derived by fitting the model to observations of blood ethylbenzene kinetics as obtained from **Tardif et al. (1996)**. Physiological parameters and V_{max} were allometrically scaled across species as a function of body weight BW (scaling factor = $BW^{0.75}$). Metabolism parameters were optimized against observed blood kinetics of ethylbenzene in rats exposed for 4 hrs to binary combinations of 100 or 200 ppm (corresponding to 435 and 870 mg/m^3) of ethylbenzene and xylene or ethylbenzene and toluene. Predicted ethylbenzene blood concentrations were within 1 – 2 standard deviations of the observations. Furthermore, the model was validated by comparing predictions of the human model (allometrically scaled from the rat) to ethylbenzene concentrations observed in human subjects who were exposed to ethylbenzene (33 ppm / 144 mg/m^3) for 7 hrs/day on 4 different days. Predicted blood concentrations in subjects exposed to ethylbenzene were within 1-2 standard deviations of observations.

Dennison et al. (2003) developed a PBPK model for simulating the blood and elimination kinetics of components of gasoline which ethylbenzene is a component of. Tissue compartments were limited to lungs, liver, fat, richly perfused tissues and slowly perfused tissues. All metabolism was attributed to the liver. Partition coefficients for ethylbenzene were taken from the literature **(Tardif et al., 1997)**. Metabolism parameter values were derived by fitting the model to observations of blood ethylbenzene kinetics measured in rats during closed-chamber exposures to ethylbenzene. Optimization of the metabolism parameters for rats against observed close chamber air concentration kinetics achieved predicted elimination kinetics of

ethylbenzene that were similar to observations. By using a lumping approach, the model was used to investigate probable solvent interactions when combined exposure (e.g. in the case of gasoline exposure) takes place.

A physiologically based model of ethylbenzene inhalation in B6C3F1 mice has been developed by **Nong et al. (2007)** to help understand the pharmacokinetic basis why life time exposure to 750 ppm (3262 mg/m^3) ethylbenzene caused an increase in the incidence of hepatocellular ademonas (female mice) and lung adenomas (male mice), while exposures to lower doses, i.e. 250 ppm or 75 ppm, had no effect. Also, because tissue dose in animals can be used as the basis for deriving human-equivalent exposure concentrations using PBPK models. For simulating the inhalation kinetics of ethylbenzene in male and female mice, a five compartment model was developed, metabolism was described in liver and lungs, partition coefficients were determined. The model was validated by comparing a priori predictions with experimental data on venous blood concentrations of ethylbenzene determined during and following a single 6 hr inhalation exposure to 75 or 750 ppm (326 and 3262 mg/m^3). The blood and tissue kinetic data obtained in mice indicated a higher clearance than expected which could not be described by increasing the rate of hepatic metabolism in the PBPK model, but which was supported by pulmonary metabolism as a contributor for the enhanced ethylbenzene clearance in mice. Furthermore, the model supported an induction of hepatic clearance after repeated high inhalation exposures (750 ppm) to ethylbenzene. Concerning pulmonary metabolism it was discussed, that in addition to CYP2E1, the pulmonary enzyme CYP2F2 might also contribute to ethylbenzene metabolism as suggested earlier on the basis of molecular modeling (Lewis et al., 2002; 2003).

Shatkin and Brown (1991) described a model of dermal absorption of several nonpolar organic nonelectrolytes in dilute aqueous solution, one of which was ethylbenzene. The model included the compartments stratum corneum, viable epidermis and blood. Transfers of ethylbenzene in solution through the fully hydrated stratum corneum and viable epidermis were assumed to be diffusive, with passage through the stratum corneum being the rate-limiting step. Transfer from the viable epidermis to blood was assumed to be flow-limited. Sensitivity analysis revealed a relatively high influence of epidermal blood flow, epidermal thickness, and stratum corneum fat. Model predictions were compared to the estimates of dermal absorption of ethylbenzene in human **(Dutkiewicz and Tyras, 1967)**. The model predicted absorption percentages of 34 – 37 % of the bath ethylbenzene in 1 hr, compared to the observed mean absorption of 39 % (range: 0.33 – 0.54). The simulated kinetics of absorption were not reported or compared to observations.

4.1.2.1.4 Summary of toxicokinetics, metabolism and distribution

Ethylbenzene is absorbed from the lungs, gastrointestinal tract and through the skin. Inhalation studies in humans demonstrate that up to 64 % of the inhaled ethylbenzene vapour can be retained in the lungs **(Bardodej and Bardodejova, 1970)**, whereas for animals, slightly lower retention rates (44 %) have been reported **(Chin et al., 1980b)**. Therefore, for risk assessment purposes, inhalation absorption percentages of 65 % for humans and 45 % for animals should be taken into consideration.
No studies are available regarding the absorption of ethylbenzene after oral exposure of humans. Studies in animals indicate that ethylbenzene is quickly absorbed via this route. Up to 92 % of ethylbenzene metabolites could be detected in the urine of animals within 24 hrs after dosing **(El Masry et al., 1956)**. Therefore, as worst case, 100 % oral absorption in animals and humans is taken for risk characterisation. Studies in humans dermally exposed to liquid

ethylbenzene demonstrate rapid absorption through the skin, whereas no indications of absorption could be seen after exposure of humans to ethylbenzene vapors. Up to 45 % of ethylbenzene was absorbed in human volunteers, who had immersed one hand for up to 2 hrs in aqueous solutions of ethylbenzene **(Dutkiewicz and Tyras, 1967)**. In predictions based on a dermal PBPK model, human dermal absorption rates were between 34 and 37 % **(Shatkin and Brown, 1991)**. As worst case assumption, 50 % dermal absorption is taken for risk assessment purposes in humans. An even higher dermal absorption rate of up to 65.6 % was observed in animals **(Susten et al., 1990)**. Therefore, 70 % dermal absorption should be taken into account for animals.
Ethylbenzene is rapidly distributed through the body. After inhalation exposure of animals, highest amounts of radioactivity in tissues after 6 hr exposure to 230 ppm ethylbenzene were found in the carcass, liver and gastrointestinal tract and lower amounts detected in the adipose tissue **(Chin et al., 1980b)**. From humans exposed to a mixture of industrial xylene containing 40.4 % ethylbenzene it was derived, that the retention of ethylbenzene in adipose tissue was approximately 2 % of the total uptake **(Engström and Bjurström, 1978)**. There was, however, no evidence of ethylbenzene accumulation in fat-rich tissues.
Species differences have been shown concerning the metabolism of ethylbenzene. In humans exposed via inhalation, the major metabolites are mandelic acid (approximately 70 % of the absorbed dose) and phenylglyoxylic acid (approximately 59 % of the absorbed dose), which are excreted in the urine. Both metabolites are formed after side chain oxidation. In rats exposed by inhalation or orally, the major metabolites were identified as hippuric acid and benzoic acid (both metabolites amount to 38 % of the metabolites), 1-phenylethanol (25 % of the metabolites), mandelic acid (15 – 23 % of the metabolites) and phenylglyoxyloc acid (10 % of the metabolites). In rabbits, the most relevant metabolite is hippuric acid. Rabbits excrete higher levels of glucuronidated metabolites compared to rats and humans. Human and animal data demonstrate, that ring oxidation (and formation of metabolites resulting from ring oxidation) represents a minor pathway compared to side chain oxidation. In animals. ethylbenzene kinetics was saturable at concentrations above 500 ppm in mice **(Charest-Tardif et al., 2006)** and concentrations of 200 ppm in rats **(Tardif et al., 1996)**.
Ethylbenzene is rapidly metabolized and metabolites are eliminated rapidly from the body, primarily as urinary metabolites. Excretion is almost complete within 24 hrs after exposure with only about 0.2 % of absorbed dose remaining in the body within 42 hrs after inhalation exposure. In man, highest urinary concentrations of mandelic acid and phenylglyoxylic acid occurred 7 hrs after exposure, the biological half-life of both metabolites is 4 – 7 hrs **(Hagemann and Angerer, 1979)**. Comparable results were obtained for other metabolites, such as the ring oxidation product 2-ethylphenol and the phenolic metabolites m- and p-hydroxyacetophenone: within a period up to 8 hrs after exposure, half the quantity measured in 24 hr-urine could be determined **(Engström et al., 1984)**.
After inhalation exposure, exhalation is also an important pathway of excretion, whereas fecal excretion plays a minor role **(Chin et al., 1980)**.

4.1.2.1 Acute toxicity

Animal data:

Oral

A LD_{50} value of 5460 mg/kg (5090-5860 mg/kg) for male rats is given for ethylbenzene based on a 14-days observation period within a list of toxicological data for more than 300 com-

pounds (no data on purity). This LD_{50} value was determined in a test using 5 animals per dose group (number of dose groups not mentioned). Further information is not given **(Smyth et al., 1962)**.

In a test with young adult white rats of both sexes ethylbenzene was administered with a stomach tube to a total of 57 animals resulting in an oral LD_{50} of 3500 mg/kg (no data on purity of the test substance). Surviving rats were observed until recovery was ensured (usually about 2 weeks). At necropsy unspecified liver and kidney changes were found. Further information is not given **(Wolf et al., 1956)**.

In a preliminary test with fasted rats 7/10 animals died after oral administration of 2.5 ml of ethylbenzene 1:1 v/v in olive oil (no data on purity of the test substance). Surviving animals were observed for 3 weeks after dosing for evidence of abnormality in behaviour and activity. At necropsy hyperaemia and haemorrhage of the lungs were observed. It was stated that the pulmonary injury was undoubtedly the cause of death where gross haemorrhage was found. Respiratory failure might have been a contributory factor, but the principal cause of death was believed to be chemical pneumonitis with pulmonary oedema and haemorrhage. Also generalised hyperaemia and vasodilatation of the blood vessels of the gastrointestinal tract were detected. Liver and also spleen enlargement, but no thymus changes were observed **(Gerarde, 1959)**.

Inhalation

Four-hour exposure to substantially saturated vapour (produced at room temperature) killed 6/6 rats exposed to ethylbenzene, 2/6 rats were killed within 2 hrs and none of the rats died within 1 hr. A concentration of 8000 ppm of ethylbenzene for 4 hrs (35.2 mg/l) killed 6/6 rats, 4000 ppm for 4 hrs (17.6 mg/l) killed 3/6 animals, and 2000 ppm for 4 hrs (8.8 mg/l) resulted in no deaths. No more data are reported **(Union Carbide, 1949)**.

Six albino rats were exposed to a flowing stream of ethylbenzene vapour-laden air (no data on purity). The vapour-air mixture was generated by passing 2.5 l/min of dry air at room temperature through a fritted glass disc immersed to a depth of at least 1 inch in approximately 50 ml of ethylbenzene contained in a gas-washing bottle. No mortality was observed in a 1-hr inhalation period with concentrated vapours. Inhalation of metered vapour concentrations by rats was conducted with flowing streams of vapour prepared by various styles of proportioning pumps. A concentration of 2000 ppm (8.8 mg/l) resulted in 2/6 dead rats within 14 days after an inhalation period of 4 hrs, 4000 ppm (17.6 mg/l) resulted in 3/6 and 8000 ppm (35.3 mg/l) in 6/6 dead animals, therefore the 4 hour LC_{50} was 4000 ppm. Concentrations recorded are nominal and not analytically verified **(Smyth et al., 1962)**. The reports of Union Carbide (1949) and Smyth et al. (1962) most probably refer to the same experiment.

Dermal

The dermal LD_{50} for rabbits was higher than 2000 mg/kg:
An estimated LD_{50} of 17.8 ml/kg (15.5 g/kg) by skin penetration of ethylbenzene (no data on purity) resulted from a test on male rabbits. Ethylbenzene was applied to the clipped trunk of rabbits under "Vinylite" sheeting for 24 hrs with the following results: 3/5 rabbits died at a dose of 20 ml/kg, 2/5 at 15.8 ml/kg and 1/5 at 12.6 ml/kg. Male albino New Zealand strain

non-fasted rabbits weighing between 2 and 3 kg were used in this test, and the animals were observed for 13 days post exposure. No more data are reported **(Union Carbide, 1949)**.

Ethylbenzene (no data on purity) was applied to the entire trunk (the fur was removed by clipping) of male albino New Zealand rabbits for a 24-hours contact period under occlusion using 4 rabbits per dose group (number of groups not given). A dermal LD_{50} of 17.8 ml/kg (15.5 g/kg) resulted in this test. No further data are given **(Smyth et al., 1962)**. The reports of **Union Carbide (1949) and Smyth et al. (1962)** most probably refer to the same experiment.

Other routes

The intraperitoneal LD_{50} in male NMRI mice was determined prior to a micronucleus test at dose levels between 0.25 and 3.5 ml/kg ethylbenzene in cornoil. The observation period was not given but the test was reported to be by a standard method which implies a 10 or 14 day period. The intraperitoneal LD_{50} in male NMRI mice was 2.624 ml/kg this is equvialent to 2275 mg/kg assuming a density of 0.867. Signs and symptoms following dosing were not reported **(Mohtashamipur et al., 1985)**. The LD_{50} was calculated by the method of **Cavalli-Sforza and Lorenz, 1964.**

Tanii et al. (1995) assessed the effect of metabolism on acute toxicity of aromatic hydrocarbons. Ddy mice were pretreated with carbon tetrachloride at a dose level which did not cause death but which was known to inactivate hepatic microsomal monooxygenases. The mice received either olive oil alone or olive oil containing 20% CCl_4 by intraperitoneal injection 24 hours prior to determination of the intraperitoneal LD_{50} of ethylbenzene. Observation continued over the following week. The ip LD_{50} in control mice was 19.7 mmol/kg = 2100 mg/kg and the ip LD_{50} of CCl_4-pretreated animals 1900 mg/kg = 17.81 mmol/kg. This reduction of toxicity was attributed to reduced metabolism due to carbon tetrachloride treatment.

Human data
No data available.

Conclusion

Human data on the acute toxicity of ethylbenzene are not available. In animals ethylbenzene proved to be harmful by inhalation of the vapours (the inhalation LC_{50} for rats is 17.6 mg/l/4 hours). Oral and dermal toxicity is low with LD_{50} values above 2000 mg/kg: an oral LD_{50} of 3500 mg/kg was determined for rats in general, and an oral LD_{50} of 5460 mg/kg specifically for male rats; the acute dermal toxicity was tested with rabbits and revealed a dermal LD_{50} of 15.5 g/kg. On the basis of these results ethylbenzene has to be classified as "Xn, harmful" and labelled with "R 20, harmful by inhalation". Labelling because of the acute oral and dermal toxicity is not warranted.

Gerarde (1960) hypothesized that pulmonary injury resulting in chemical pneumonitis might be the principal cause of the deaths observed after oral administration of ethylbenzene to rats because necropsy after oral administration revealed hyperaemia and haemorrhage of the lungs. As a result of his experiments he concluded that aspiration of even a small amount of ethylbenzene may cause severe lung injury. And due to its low viscosity and surface tension ethylbenzene would spread over a large area of pulmonary tissue, causing oedema and haemorrhage. Therefore, labelling with "R 65, Harmful: May cause lung damage if swallowed" is necessary.

4.1.2.2 Irritation

and

4.1.2.3 Corrosivity

Animal data
Skin irritation

Data on skin irritation tests according to international test guidelines are not available. On the basis of the two available tests with rabbits a moderate skin irritation potential after single application of the substance and a high defatting potential leading to severe effects after repeated skin contact can be concluded.

A skin reaction graded 4 (within a scale of 6 for undiluted test materials) resulted after a 24-hours uncovered application of 0.01 ml of undiluted ethylbenzene (no data on purity) to a group of 5 rabbits (grade 2 indicates the least visible capillary injection and grade 6 indicates necrosis). Ethylbenzene was considered a moderate skin irritant. Further information is not given **(Smyth et al., 1962)**.

Moderate irritation resulted in a test with ten to twenty times application of undiluted ethylbenzene (no data on purity) to the ear and to the shaved abdomen (occluded) of rabbits over a period of 2-3 weeks. Moderate irritation is characterised by definite erythema and development of oedema and superficial necrosis, resulting in a "chapped" appearance and exfoliation of large patches of skin. Further information is not given **(Wolf et al., 1956)**.

Eye irritation

Corneal necrosis grade 3 (out of 10) was reported after instillation of undiluted ethylbenzene (no data on purity) to the eyes of rabbits. Injury grade 3 was defined as causing injury of up to 5.0 points after instillation of 0.1 ml undiluted test substance or over 5.0 points after instillation of 0.5 ml (points result from a scoring system for injury of the rabbit eye developed by the authors; e. g. 5 points for corneal necrosis of 63-87%, visible after fluorescin staining). No information on recovery time is given **(Carpenter et al., 1946)**.
Within a report on chemical burns caused on the eyes of rabbits (usually 5 animals per test) by 240 chemical substances, ethylbenzene (no data on purity) is mentioned to have caused chemical burns of the cornea characterised by grade 3 out of a scale of 10. Grade 3 signifies that 0.5 ml undiluted substance yielded over 5.0 injury units and 0.1 ml yielded not over 5.0. This injury unit of 5.0 is stated representative of severe injury and corresponds to dense necrosis covering about 3/4 of the area of the cornea. The authors state the any compound with Grade 3 or a higher grade is capable of causing severe corneal injury when a sufficient amount enters the eye **(Union Carbide, 1946)**.
Within a publication of a similar list of toxicological data, for undiluted ethylbenzene (no data on purity) grade 2 corneal necrosis in a scale of 10 is stated as result of an eye irritation test with rabbits (grade 1 indicates at most a very small area of necrosis resulting from 0.5 ml of the undiluted chemical to the eye, grade 5 severe necrosis by 0.005 ml, and grade 10 severe

necrosis by 0.5 ml of an 1% solution in water or propylene glycol). No further data are given (**Smyth et al., 1962**).
The data reported by **Carpenter et al. (1946), Union Carbide (1946)** and **Smyth et al. (1962)** most probably relate to the same or similar experiments using the same scoring system.

Slight conjunctival irritation (perceptible irritation of the conjunctival membranes) but no corneal injury by fluorescein stain was observed in an eye irritation test with rabbits using 2 drops of undiluted ethylbenzene each (no data on the purity of the test substance). Observations were made at 3 minutes, 1 hour and 1, 2, and 7 days after instillation. No more data are given **(Wolf et al., 1956)**. This test procedure comes close to present days' methods, but not to those used by **Carpenter et al (1946), Union Carbide (1946), or Smyth et al. (1962)**.

Irritation by inhalation

Sensory irritation and pulmonary irritation by airborne ethylbenzene (analytical grade) was measured to determine the concentration which causes a 50% decrease in respiratory rate due to sensory irritation (RD_{50}). Sensory irritation of the upper respiratory tract occurs reflectively from stimulation of the trigeminal nerve endings in the nasal mucosa. In the test male Swiss-Webster mice either intact or after cannulation of the trachea were used (4 mice/group). In cannulated mice the trigeminal nerve endings are bypassed, thereby excluding the development of sensory irritation of the upper respiratory tract. Intact animals were treated at doses of 410 ppm, 860 ppm, 1875 ppm, 3970 ppm, or 9640 ppm for 30 minutes after a baseline respiratory rate was recorded. Cannulated animals were treated at doses of 2100 ppm, 4000 ppm, or 7800 ppm. The desired exposure concentrations were obtained by evaporating the substance in a glass nebulizer. Each animal was placed in a body plethysmograph attached to the exposure chambers so that the head of the animal protruded into the chamber and the respiratory pattern of each animal was monitored. A resulting pulmonary irritation in mice is difficult to interpret and can be either observed as escape attempts or as a depression and immobilisation.
The RD_{50} of ethylbenzene was found to be 4060 ppm in mice. No pulmonary irritation was observed in cannulated mice. It was noted that at 7800 to 9640 ppm normal as well as cannulated mice were sedated and anesthetized toward the end of exposure. This general anesthetic effect could have contributed to a decrease in respiratory rate at that time which makes it difficult in interpreting which type of effect, pulmonary irritation or central nervous depression predominated. The RD_{50} of 4060 ppm is equivalent to intense irritation of the eyes, nose and throat. The fast fading of the response in mice is also in agreement with observations in humans. If 0.03 RD_{50} for ethylbenzene (122 ppm) is used to suggest an acceptable TLV, a good agreement was found with the current TLV-TWA of 100 ppm **(Nielsen and Alarie, 1982).**

In a similar experiment 6 Swiss OF1 mice per concentration were exposed for 5 minutes to 4 different concentrations of ethylbenzene (stated to be of high purity) resulting in an RD_{50} of 1432 ppm **(De Ceaurriz et al., 1981)**.

Acute exposure to vapours of ethylbenzene (commercial product) in air in concentrations of 0.1%, 0.2%, 0.5% or 1% (equivalent to 1000–10000 ppm) produced the following effects in guinea pigs (sex and strain not specified): concentrations of 1% and 0.5% (duration of exposure not specified) produced immediate intense irritation to the conjunctiva and nasal mucous membranes. Further symptoms noted in order of occurrence were unsteadiness and staggering on attempting to move about, apparent unconsciousness, intermittent tremors, and twitching of the extremities and changes in the respiration. Animals exposed to 1% developed a rapid,

jerky type of respiration very soon after unconsciousness occurred. Respiration in animals exposed to 0.5% remained apparently normal until unconsciousness occurred, then it became shallow. A concentration of 0.2% produced moderate eye and nasal irritation within one minute and a 0.1% concentration caused only slight nasal irritation. It is mentioned that some guinea pigs died but it is not specified how many and at which concentrations. Gross pathology of the deceased animals and of those which were killed shortly after the end of the test, showed intense cerebral congestion, congestion and oedema of the lungs with signs of passive congestion throughout the abdominal viscera. The blood appeared much darker in colour and gave a cyanotic hue to the organs. It was concluded that the damage to the cerebral tissues and the lungs increases with the severity of the exposure. In animals which did not die on the test or soon after there was not much evidence that the lung irritation was severe enough to produce permanent injury. As regards lethality 10000 ppm was fatal within a few minutes, 5000 ppm dangerous to life within 30–60 minutes, 3000 ppm was tolerated up to 1 hr without mortality, and 1000 ppm for several hrs without serious effects. In only three animals changes in the lungs were observed after an interval of four days following exposure; after eight days none of the animals showed lung damage **(Yant et al., 1930)**.

Human data

It is pointed out by **Gerarde (1960)** that the aspiration of even a small amount of ethylbenzene may cause severe lung injury. Due to its low viscosity and surface tension ethylbenzene was expected to spread over a large area of pulmonary tissue, causing oedema and haemorrhage. (no further information, cited in: **Browning, 1965**). In 1959 **Gerarde (1959)** stated that direct contact of liquid ethylbenzene with pulmonary tissue causes chemical pneumonitis characterised by pulmonary oedema, haemorrhage, and tissue necrosis. Ethlybenzene (no data on purity) was tested for sensory irritation in humans. Six men breathing 0.1% (1000 ppm) ethylbenzene vapour in air found the atmosphere very irritating to the eyes, producing a sensitisation of smarting and burning, accompanied by profuse lacrimation. This irritation gradually decreased on continued exposure until, after a minute or two, it was scarcely notable. Two men upon leaving and re-entering the chamber noticed no eye irritation. It was the opinion of the observers that this atmosphere could be tolerated after the first few minutes. The same six observers found 0.2% (2000 ppm) vapour almost intolerable on first entering the chamber, although it became less irritating upon continued exposure. One observer stayed five minutes and found that the irritation to the eyes and throat gradually disappeared, but vertigo developed. Exposure to 0.2% was accompanied by throat irritation and a feeling of constriction in the chest. Four observers exposed for six minutes while a concentration of 0.2% was “built up”, noticed a moderate nasal irritation and a moderate to strong eye irritation. All complained about dizziness upon leaving the atmosphere. Three observers upon entering a 0.5% (5000 ppm) mixture of ethylbenzene with air found the atmosphere intolerable, being extremely irritating to the eyes, nose and throat. It was the opinion of the exposed men that a 0.2% concentration of vapour would give sufficient warning and would not be tolerated; and that 0.5% would have sufficient irritating properties to render working in this atmosphere impossible **(Yant et al., 1930)**.

The RD_{50} of 4060 ppm obtained in mice is equivalent to intense irritation of the eyes, nose and throat. The fast fading of the response in mice is also in agreement with observations in humans. If 0.03 RD_{50} for ethylbenzene (122 ppm) is used to suggest an acceptable TLV, a good agreement was found with the current TLV-TWA of 100 ppm **(Nielsen and Alarie, 1982)**.

In an accident at workplace skin and eye corrosion was caused by ethylbenzene (no data on purity). This corrosion was detected on eyes and mouth, and in the skin of face, neck, and body of a worker after leakage of a pipeline containing the chemical; clinical treatment was necessary **(BASF AG, confidential information, 1995)**.

Conclusion

In humans, high concentrations of ethylbenzene vapours are irritating to mucous membranes of the eyes, nose and respiratory tract. Chemical burns of the eyes, mouth, face, and trunk after a leakage of a pipeline with ethylbenzene are reported.
Ethylbenzene is moderately irritating to the skin of rabbits after single exposure. After repeated exposure it caused definite erythema and development of oedema and superficial necrosis, resulting in a "chapped" appearance and exfoliation of large patches of skin.
Ethylbenzene caused grade 2-3 injury of the eyes of rabbits out of a scale of 10 based on the degree of corneal necrosis after instillation of various amounts and concentrations of chemical. The authors stated state that a compound listed with grade 3 or higher would be capable of causing severe corneal injury when a sufficient amount enters the eye. In guinea pigs exposure to 5000 and 10000 ppm of ethylbenzene vapour produced immediate and intense irritation of the conjunctiva, while 2000 ppm caused moderate eye and nose irritation within 1 minute.
In different strains of mice RD50 of 1432 or 4060 ppm were determined for sensory irritation.
Overall, a classification and labelling as “Xi, Irritant, R 36/37/38 irritating to eyes, respiratory system and” is warranted.

4.1.2.4 Sensitisation

Animal data

Animal data on skin or inhalation sensitisation tests are not available.

Human data

Kligman conducted a maximisation test with 10% ethylbenzene (no data on purity) in petrolatum on 25 volunteers. Ethylbenzene produced no sensitisation reactions **(Kligman, 1974, cited in Opdyke, 1975)**.

Conclusion

Animal data on skin or inhalation sensitisation tests with ethylbenzene are not available. There are no reports on skin sensitisation or inhalation allergy caused by the substance at the workplace. A human patch test with 10% ethylbenzene in petrolatum revealed no skin sensitisation potential. Taking into account the long time of human experience with this chemical, it can be assumed that skin sensitisation or respiratory allergy is not a hazard that has to be expected when handling ethylbenzene. This assessment is in line with the fact that also for benzene as parent compound of alkylated benzenes there are no reports on sensitisation caused at the workplace. Thus, no sensitisation potential is expected for ethylbenzene.

4.1.2.5 Repeated dose toxicity

4.1.2.6.1 Animal data (*inhalation< oral studies< other routes)*

Conventional toxicological studies

Inhalation route ***(Table 4.1.2.6 A)***

In subacute inhalation studies, B6C3F1 mice, F344 rats and New Zealand White rabbits (five/sex/group) were exposed by inhalation to ethylbenzene vapours for 6 hrs/day, 5 days/week for 4 weeks. Rats and mice received 0, 99, 382 or 782 ppm ethylbenzene, while rabbits received 0, 382, 782 or 1610 ppm ethylbenzene (0, 1.8, 3.7, 7.6 mg/l) **(Cragg et al., 1989)**. The purity of the test substance was 99.7% with toluene (0.15%), isopropanol (0.04%), and benzene (0.005%) as impurities. Vapour concentrations were monitored at hourly interval, Variations of analytical concentrations were within the 10% level of the target concentration. Test design of the studies was similar to that of the previous version of OECD Test Guideline 407 (1981). Animals were examined for mortalities, clinical symptoms, body weight development, final body and organ weights, ophthalmology and standard parameters on hematology, clinical chemistry (except mice), urinalyses (only rats), and histopathology (on 30 organs/tissues for high dose and control groups).
During the course of exposure, none of the animals died. Rats exposed to 382 and 782 ppm ethylbenzene showed sporadic incidences of salivation and lacrimation but the incidence was not sufficient to establish a dose relationship. For all species, no significant difference in body weight gain was observed at the end of the study. There were increases in mean liver weights and liver to body weghts ratios and liver to brain weight ratios, that gained significance for male and female rats exposed at 782 ppm, for male and female mice at 782 ppm (only liver to brain weight ratio for both sexes and absolute weights for females), and for male rats at 382 ppm (only absolute liver weight) and for female rats at 382 ppm (only liver to body weight ratio). Minimal to slight increase in liver weights were found for treated rats and mice that received 99 ppm or more that did not reach significancy. No effect on liver weight has been observed for rabbits at all exposure levels. The authors interpreted the increased liver weights in the absence of histopathological changes or changes in clinical chemistry as an adaptive metabolic response.
Exposure concentration of 782 ppm ethylbenzene induced a statistically significant increase of platelets in male rats and a significant increase in mean leucocyte counts in female rats. No other significant effect on hematology parameters was found in any of the test species. No treatment-related effect was observed for parameters of clinical chemistry in rats and rabbits and for parameters of urinalyses in rats. No gross and microscopic changes could be attributed to ethylbenzene inhalation exposure.
The toxicological importance of some of the findings remains equivocal and may be clarified in the context of data from other studies. The most sensitive treatment-related effects were lacrimation and salivation without a dose relationship and liver weight increase in rats that received 382 ppm or more and mice exposed to 782 ppm but without corresponding effects in histopathology or clinical biochemistry. The NOAEC for rats and mice is 782 ppm and the NOEC 99 ppm for the rat and 382 ppm for the mouse. No adverse effect was attributable to ethylbenzene concentrations up to 1610 ppm (NOAEC) for rabbits.

In another, shortly reported subacute study, F344 rats, B6C3F1 mice (5 males/group each) and New Zealand rabbits (4 males/group) were exposed for 6 hrs/day, on 4 consecutive days at vapour concentrations of 0, 400, 1200 and 2400 ppm ethylbenzene (99.7% purity; concentrations correspond to 0, 1.7, 5.3, 10.6 mg/l) **(Biodynamics, 1986)**. This investigation most

probably was the preliminary "range finding" study for the main 4 week-study mentioned above. The study was not carried out under a standard study design. The report contains only a brief documentation without tables on summary incidences and individual data. Control animals were exposed to room air only. Following the fourth exposure, all survivors were sacrificed and fully necropsied. Brain, heart, lung, liver, kidney, and testes with epididymides were weighed and histopathological examinations were performed on sections from testes, kidneys, liver, lungs and nasopharyngeal tissue. Analytical concentrations were estimated to deviate less than ±2.5% from target concentrations and the means were 390, 1190, and 2360 ppm.
All rats exposed to 2360 ppm were found dead prior to termination (no data on exact timepoint). Increased lacrimation, shallow breathing, prostation, and yellow ano-genital staining were noted. Although these observations were not explicitly attributed to specific dose groups, the rapporteur assumes they occur in rats of the 1190 ppm and 2360 ppm dose groups, since no treatment-related symptoms were reported to occur in rats exposed to 390 ppm. Mean body weight was lower for rats at 1190 ppm (not estimated for dead animals at 2360 ppm). Mean absolute and relative liver weights were increased at concentrations of 390 ppm and above and relative kidney weights were significantly increased at 1190 ppm. In early deaths of the 2360 ppm group, the lungs of were dark red and microscopic examination revealed congestion in the lungs, nasal mucosa, liver and kidneys. No other treatment-related microscopic findings were seen in the other dose groups.
All mice of the 2360 ppm group and four of five mice of the 1200 ppm groups died prior to study termination. Increased lacrimation, shallow breathing, prostation, and yellow ano-genital staining and partly closed eyes were observed. The only observation in mice exposed to 400 ppm was excess lacrimation on day 3 and 4. No effects on body and organ weights were found for mice of this dose group (not estimated for higher dose groups). Red discoloration of the lungs was seen in animals that died spontaneously. When examined microscopically, congestion of the lungs, nasal mucosa, liver and kidney were seen in the premature decendents but not in animals of the 390 ppm group.
All rabbits survived to the end of the study and showed no change in body and organ weights. No treatment-related findings were observed grossly or microscopically.
The NOAEC in rats was 390 ppm with increased liver and kidney weights without histopathological changes and 2360 ppm were lethal. In mice 1190 ppm led to mortality and the NOAEC was 390 ppm. For rabbits the NOAEC was 2360 ppm.

A study with subacute administration was conducted to examine parameters potentially involved in the mode of action for tumorigenicity. Groups of 6-8 male and female Fischer 344 rats and 6-8 B6C3F1 mice were exposed to 0, 75 or 750 ppm (actual means: 75.2 and 738.6 ppm) ethylbenzene vapours 6 hrs/day for 5 consecutive days and to 0 and 750 ppm (actual mean: 761 ppm) for 5 days per week over 4 weeks **(Stott et al., 1999; 2003)**. Besides body weight and clinical signs the target organs of the NTP carcinogenicity study were investigated, i.e. kidney in rats and lung and liver in mice. Cell dynamics, histopathology and enzyme activities were determined by the following parameters in these organs: organ weight, histopathological changes by light and electron microscopy, S-phase DNA synthesis (centrilobular, midzonal, and periportal regions of mouse livers, epithelial cells of the lower airways and alveoli of mouse lungs; cortex, outer and inner medulla of rat kidney), apoptotis (high dose and control animals), and serum chemistry (alanine transaminase, aspartate transaminase, alkaline phosphatase, creatinine, urea nitrogen, and γ-glutamyl transpeptidase). In isolated microsomes the following enzyme activities were analysed in the target organs: Ethoxyresorufin- (EROD reflecting primarily CYP1A1 subfamily activity), methoxyresorufin- (MROD reflecting primarily CYP1A2 subfamily activity, four week study only), pentoxyresorufin- (PROD reflecting primarily CYP2B1/2 subfamily activity), ethyoxyfluorocoumarin-

O-dealkylase (ECOD for several mixed function oxygenases activities including CYP2E1, CYP1A and CYP2B), para-nitrophenol hydoxylase (p-NPH for CYP2E1 activity), and glucuronosyl transferase (UGT) (results see 4.1.2.1.1). In addition α-2μ-globulin was evaluated in the kidney of male rats by immunohistochemical staining.

Results obtained with mice:
There were no treatment related effects on survival, clinical signs, body weight or serum enzymes. After both exposure periods, the relative liver weights were increased approximately 6 and 15% (after 1 week) and 7 and 13%(after 4 weeks) in 750 ppm males and females, respectively. The increase of relative liver weights was reported to be statistically significant ($p=0.05$) at 750 ppm in both sexes. Histopathology examination of livers of mice exposed to 750 ppm ethylbenzene found increased numbers of mitotic figures in the majority of exposed males and females (>6/lobe), most in the midzonal to centrilobular areas, following 1 and 4 week exposure. Accordingly very slight or slight hypertrophy of centrilobular/midzonal hepatocytes was observed in 7/8 males and females, one control animal per sex showed very slight hypertrophy.
The relative rate of S-phase DNA synthesis (LI) in the liver of male mice of the 1 week study exposed to 750 ppm ethylbenzene progressively increased across the liver lobule from a 180% increase in periportal hepatocytes to a 479% increase in midzonal hepatocytes to a 1116% increases in centrilobular hepatocytes. The differential anatomic effect was still evident but less pronounced after 4 weeks of 750 ppm exposure. A similar regional progression was evident in 750 ppm females from the 1 week study. Females had higher LI levels in all hepatic zones than males, however, the relative increase was less due to a much greater control LI level. A relatively high LI was also observed in 4 week study females, however a relatively high degree of interanimal variability confounded interpretation. A maximal 56% increase in LI was noted in centrilobular hepatocytes of female mice exposed to 750 ppm ethylbenzene. Nonsignificant changes in LI were noted in either sex of mice exposed to 75 ppm ethylbenzene for 1 week. No effects were noted for apoptosis.
There was no effect of ethylbenzene exposure upon lung weights or histopathology in either sex of mouse at either necropsy time point. In the terminal bronchioles of the lungs of 750 ppm mice in the 1 week study, LI were increased 180% and 149% over controls in males and females, respectively. After 4 weeks exposure, the LI of 750 ppm males and females was increased 82 and 115% over controls, respectively, but not significantly different. No consistent effect upon S-phase synthesis was found in the alveoli. No effects were note for apoptosis in the lung.
In summary, mice exposed to 750 ppm for 4 weeks exhibited increased liver weights, hepatocellular hypertrophy, increased mitotic figures, S-phase DNA synthesis and enzyme activities (see 4.1.2.1.1). In the lung S-phase synthesis rates in terminal bronchiolar epithelium were elevated and accompanied by loss of MFO activity.

Results obtained with rats:
There were no treatment-related effects on survival, clinical signs, or serum parameters. Rats exposed to 750 ppm weighed slightly less than controls; however, differences were statistically significant only on a few days of the study. The kidney weights of males and females exposed to 750 ppm ethylbenzene were slightly increased (approximately 5-8%; $p=0.05$) following both exposure periods but not at 75 ppm after one week.
After one week of exposure in the kidneys of male rats at 750 ppm there was an increase in number and size of hyaline droplets in the cells of the proximal tubules. 75 ppm led only to an equivocal increase in hyaline droplets. After 4 weeks of exposure at750 ppm male rats had a subtle renal lesion described as nephropathy. This effect was present in multiple foci that were located primarily in the mid-cortical region, similar to the site and distribution of the

areas where hyaline droplet accumulation is typically present in F344 male rats. However no treatment related accumulation of hyaline droplets was evident. No treatment-related renal changes were identified in females at 750 ppm after 5 days or 4 weeks. Evaluation of S-phase DNA synthesis and α-2μ-globulin deposition in 750 ppm males of both studies revealed localized focal effects in the cortical tubular epithelium, relative to controls. Changes coincided with foci of increased hyaline droplet deposition and tubular epithelial degeneration. Analysis of such cortical "hot spots" revealed a 41% and 79% greater LI than controls in 1 and 4 week studies (p=0.05), respectively. Cortical "hot spots" also contained approximately 160% and 66% more α-2μ-globulin than controls following 1 and 4 weeks exposure, respectively. There was no effect on cortical cell S-phase synthesis in males exposed to 75 ppm ethylbenzene for one week. In females the rate of S-phase DNA synthesis in the kidneys was lower than in males and labelled cells appeared randomly distributed in all areas examined. In the 1 week study, a nearly 50% decrease in LI was observed in cortical tubular epithelium of the 750 ppm females (p=0.05) and in the 4 week study, no change in S-phase synthesis was observed at his location. A dose related effect on apoptosis was not found.
In summary, in male rats an initial increase in hyaline droplet deposition (observed following one weeks exposure) was followed by a diminution in α-2μ-globulin deposition at 4 weeks. This was accompanied by continued elevation of S-phase DNA synthesis and histopathological changes suggestive of Chronic Progressive Nephropathy (CPN) and a more chronic regenerative cell proliferation. Female rat kidneys did not show significant histopathological changes or increase S-phase DNA synthesis.

In a study on subacute and subchronic toxicity with specific focus on enzymatic induction and liver cell electron microscopy, groups of five male Wistar rats (body weight 342 ± 29 g) were exposed for 2, 5, 9 or 16 weeks (6 hrs/day, 5 days/week) to concentrations of 0, 50, 300 or 600 ppm (0, 0.2, 1.3, 2.6 mg/l) ethylbenzene (99% pure) **(Elovaara et al., 1985)**. After 2, 5, 9 or 16 weeks of treatment, the animals were killed after a four hour period of exposure, and liver biopsies were taken and prepared for electron microscopy. The activity of several metabolising enzymes was estimated in liver and kidney homogenates (see 4.1.2.1.1). Few standard parameters such as body, liver and kidney weight and serum alanin aminotransferase (ALAT) activity were examined.
No treatment-related effect was observed in body weight, liver to body weight ratio and ALAT activity at all time intervals. The relative weight of kidneys was higher after week 2, and 9 (not at 16) at 600 ppm. In the liver there was a dose-dependent increase in microsomal protein and increased enzyme activities (see 4.1.2.1.1). At 600 ppm the enzyme activities of two enzymes were increased in the kidneys (see 4.1.2.1.1).
Light microscopy of liver cells showed no effect on cell morphology, but electron microscopy revealed cytoplasmatic alterations at all test concentrations after 2, 5, and 9 weeks and at the high concentration after 16 weeks. The smooth endoplasmatic reticulum was proliferated and the rough endoplasmatic reticulim was partly split and shortened with slight degranulation. Some mitochondria were enlarged and branched. The authors interpreted the proliferation of smooth endoplasmatic reticulum as consistent to the induction of metabolising enzymes.
The excretion of urinary thioethers was overall enhanced with dose. At 600 ppm the output was 8 times that of the controls.

The effect of a 3 day (6 hrs/d) inhalation exposure at 2000 ppm of mixed xylenes and their constituents on hepatic and renal enzyme activities was studied in male Sprague Dawley rats (4 animals/group) by **Toftgard and Nilsen (1982)**. Only the results obtained with ethylbenzene are reported here.

Ethylbenzene increased the liver to body weight ratio and relative weight of kidneys. Enzyme activities were examined and reported in 4.1.2.1.1.

In subchronic inhalation studies close to standard test design of B.29 and under GLP conditions, groups of 10 F344 rats and 10 B6C3F1 mice of each sex were exposed to ethylbenzene (purity > 99%) in the chambers for 6 hrs per day, 5 days per week for 92 (female rats), 93 (male rats), 97 (female mice) or 98 (male mice) days, at 0, 100, 250, 500, 750, or 1000 ppm (0.47, 1.18, 2.37, 3.55, 4.74 mg/l) **(NTP, 1992)**. Controls were exposed to filtered air. Ten additional rats/sex were included at each exposure level and killed on day 23 to provide blood samples for clinical pathology.Animals were observed twice daily for moribundity and once weekly for clinical symptoms of toxicity and weighed. Food and water consumption were not measured, ophthalmology and urine analysis were not carried out. Blood for hematology and serum chemistry studies was collected in the satellite rats only on study days 4 and 23, and at week 13 from retroorbital sinus. The weight of heart, right kidney, liver, lung, right testis, and thymus was determined. Necropsy was performed on all core animals. More than 40 organs and tissues were examined histopathologically in control and high dose animals. Tissues examined in all other dose groups of rats included lung, lymph nodes (bronchial and mediastinal), and only in male rats kidney. In addition, sperm morphology and vaginal cytology evaluations were performed for rats and mice exposed to 0, 100, 500, and 1000 ppm ethylbenzene and a series of mutagenicity studies were conducted (reported in chapter 5.6 and 5.8). Achieved concentrations of ethylbenzene in exposure chambers were within a range of 3 percent of the target concentrations.
In rats, there were no deaths in any of the exposure groups, and no significant clinical signs of toxicity were identified. Male rats exposed to 1000 ppm ethylbenzene exhibited a mild (non-significant) weight depression (5-7%). Serum alkaline phosphatase activity was decreased in a dose-related manner for both male and female rats. Hematology and other parameters of clinical pathology were not consistently affected by ethylbenzene exposure. Significant increases in absolute and/or relative weights of kidney (≥500 ppm), liver (≥250 ppm), and lung (≥250 ppm) were seen in exposed rats with the following details: kidney males (absolute: 500 and 750 ppm; relative: 500 – 1000 ppm), kidney females (only absolute at 750 ppm); liver males (absolute: 250 – 1000 ppm; relative: 750, 1000 ppm), liver females (only absolute at 500 – 1000 ppm); lung males (only relative at 1000 ppm), lung females (absolute: 250, 750, 1000, not at 500 ppm; relative only at 250 and 1000 ppm). Enlarged bronchial and/or mediastinal lymph nodes were observed grossly in rats exposed to 250 ppm or higher concentrations. In all groups at concentrations of 250 ppm and higher, there was lymphoid hyperplasia in the bronchial and mediastinal lymph nodes, and inflammatory cell infiltrates around vessels, with foci of inflammatory cells in septae and lumen of alveoli in the lung. Although most rats in these dose groups were affected, the severity of the lesions was not dose related but the findings corresponded to the increased lung weights. There were no microscopic lesions associated with the increased liver and kidney weights. There were no effects observed on sperm, testicular morphology or the length of the estrous cycle.
A reassessment of the kidney sections from this 13-week study was carried out by **Hard (2002)**, in light of the increase in renal tumours observed in the subsequent 2 year bioassay. In addition to bright field microscopy the H&E stained slides were examined under fluorescence at 450-490 wavelength to evaluate lysosomal activity and/or hyaline droplet density and to distinguish between different types of tubule proliferation. There was no evidence of compound-related tubule cell injury, proliferative response or karyomegaly involving proximal tubules in male or females exposed for 13 weeks. The incidence of chronic progressive nephropathy (CPN) was investigated. The incidence of severity moved from grade 1 (minimal) in controls to grade 2 (mild) in 750 ppm and grade 3 (low-moderate) in 1000 ppm males. The

mean grade for these groups was significantly different from controls. There was no statistical difference in females. Under fluoresence microscopy a dose related increase in incidence was observed at 750 and 1000 ppm for hyaline droplets.
In mice, there were no ethylbenzene-related adverse effects on the occurrence of deaths, clinical signs of toxicity, or body weight during the course and at the end of the study period and no exposure-related gross observations. Dose-related increases in both absolute and relative liver weights were seen in both sexes of mice exposed to 750 or 1000 ppm, but significances were only attained for the absolute weights. The relative kidney weight was increased in female mice at 1000 ppm compared to that of controls. The organ weight changes were not accompanied by histopathologic changes, and no ethylbenzene related histopathologic changes were identified in any organs.
The $NOAEC_{local}$ for effects on the respiratory tract proposed by the authors was 1000 ppm for rats and for mice. The authors discussed the lung findings in the rat study not to be attributable to the ethylbenzene inhalation, since the inflammatory effects were distributed without a dose-relationship. Lesions could also not be interpreted as corresponding to the characteristic cranio-caudal distribution of findings that are typical for irritants. Although antibodies to common rodent respiratory tract viruses have not been detected, the most likely causes for the lung effects were virus infections.
Increases in organ weights in the kidney and liver were not accompanied by morphologic abnormalities and were considered to be associated with the induction of metabolic enzymes. The authors concluded that there were no adverse clinical or histopathological changes other than increases of liver and kidney weights. But taking into account the histopathological reinvestigation by **Hard (2002)** the NOAEC for male rats is 500 ppm.
One major purpose of the above mentioned subchronic inhalation studies **(NTP 1992)** was to define the exposure concentrations for a carcinogenicity bioassay according to the protocol of the US National Toxicology Program (**NTP, 1999**). In this carcinogenicity bioassay Fischer rats and B6C3F1 mice (50 animals/sex/dose) were exposed by inhalation at 0, 75, 250 and 750 ppm ethylbenzene (> 99% purity) over 104 weeks (6 hrs/d, 5 d/week). Apart from clinical observations and body weight determination all major tissues were examined microscopically. Findings related to general systemic toxicity are reported here, those related to carcinogenicity in section 4.1.2.8.
Rats: survival of top dose male rats was significantly reduced (animals surviving to study termination was 2/50 for 750 ppm (p<0.001), 13/50 for 250 ppm; 14/50 for 75 ppm; 15/50 in controls). Mean body weights of 250 ppm and 750 ppm males were generally lower than those of the chamber controls (up to 5 and 15%, respectively) from week 20. Mean body weights of all exposed groups of females were generally lower (5-6%) than those of the chamber controls during the second year of the study. Exposure-related histopathological alterations were present predominantly in the kidney and testes. In kidneys the incidence of renal tubule hyperplasia in 750 ppm males was significantly greater than that in the chamber control group (12/50 vs. 2/50 in controls; p=0.01). The findings from an extended evaluation (step section) of the kidney showed a significant increase in the incidence of renal tubule hyperplasia in 750 ppm males and females (17/50 vs. 10/50 in controls for males, p=0.05, and 8/49 vs. 1/50 in controls for females, p=0.05). The severity of nephropathy was significantly increased relative to the chamber controls in 750 ppm male (3.5 vs. 2.3 in controls; p=0.01) and all exposed female rats (2.3, 1.7, 1.6 and 1.3 for 750, 250, 75 ppm and control groups; p=0.05 or p=0.01). The enhanced nephropathy was more severe in males than in females. The associated increase in tumour incidence is described in Chapter 4.1.2.8 carcinogenicity.In the testis, the incidence of interstitial cell and bilateral testicular adenoma was increased in top dose males (see section 4.1.2.8 carcinogenicity for details). However the incidence of interstitial cell hyperplasia was significantly decreased at this concentration level (8/50 vs. 14/50 in controls; p=0.05). In addition in the lungs of the 750 ppm males there was an increased inci-

dence of oedema, congestion, and hemorrhage in comparison to the control animals. The incidences of cystic degeneration of the liver were also increased in 750 ppm males; the biologic significance of this finding in the absence of other hepatotoxic changes was deemed unclear.
In a supplementary study the kidney slides were re-examined for hyaline droplet accumulation, sustained cytotoxicity/cell regeneration, interaction with chronic progressive nephropathy (CPN), and tumors (**Hard, 2002** see chapter 4.1.2.8). Ethylbenzene caused an exacerbation of age-related spontaneous renal disease, CPN, in the 750 ppm animals, markedly so in the male rats, and modestly in the females. In addition, there was a high incidence of high-dose rats with end-stage CPN, a terminal condition where the kidneys are so morphologically altered that renal failure (as well as secondary hyperparathyroidism) occurs. Although there was some evidence of a dose-related increase in hyaline droplet formation in the 13-week NTP study, it was not considered to be of the magnitude indicative of an a-2u-globulin associated mechanism of renal carcinogenesis. Other pathological effects associated with a-2u-globulin were absent in the male rat kidneys from the 2-year NTP study. The author concluded that the re-evaluation of this study provided persuasive evidence that the apparent increase in renal tumors was strongly associated with CPN, a spontaneous age-related disease of rodents with no identical counterpart in humans.
In summary regarding chronic toxicity, there was a decrease in survival and body weight and an increase in kidney pathology (renal tubule hyperplasia and nephropathy) in rats that inhaled >= 75 ppm ethylbenzene for 2 years. The NOAEC was 250 ppm (males) and <75 ppm (females).
Mice: there were no effects on survival and body weights generally were not affected by treatment with ethylbenzene. Test article-related organ pathology was present in the lung, liver, thyroid gland and pituitary gland of ethylbenzene exposed mice. In the lung the incidence of alveolar epithelial metaplasia in 750 ppm males was significantly greater than that in the chamber controls (6/50 vs. 0/50 in controls; p=0.05) but there was no increase in alveolar hyperplasia. There was a spectrum of non-neoplastic liver changes related to ethylbenzene exposure in female and male mice. Females exposed to 750 ppm had an increased incidence of eosinophilic foci (22/50 vs. 5/50 in controls; p=0.01), a lesion which is judged to be a precursor of hepatocellular adenomas. There were increased incidences (p=0.01) of hepatocyte syncytial alteration, hypertrophy and necrosis in the liver of males exposed to 750 ppm ethylbenzene compared to controls (23/50 vs. 0/50, 17/50 vs. 1/50, 10/50 vs. 1/50, respectively) and increased syncytial alteration of hepatocytes of 250 ppm males (8/50 vs. 0/50 in controls; p=0.01). In thyroid positive trends in the incidences of thyroid follicular cell hyperplasia occurred in both males and females, with significant increases in incidences relative to chamber controls in 750 ppm males and females. In the pituitary significantly increased incidences of hyperplasia of the pituitary gland pars distalis were limited to 250 and 750 ppm females.
The lung and liver sections of the mice were re-evaluated by **Brown (2000)**. Apart from the findings reported above the reevaluation revealed decreased eosinophilia in the terminal bronchiolar epithelium of male and female mice exposed to 750 ppm. Additionally there was a dose-related increased incidence in multifocal hyperplasia of the bronchiolar epithelium with extension to the peribronchiolar alveolar epithelium in all male treated groups and mid- and high-exposure females.
In summary liver, lung, thyroid and pituitary pathology was observed in mice that inhaled >= 250 ppm ethylbenzene for 2 years. The NOAEC was 75 ppm.

No adverse effects were contributed to ethylbenzene exposure in another inhalation study on subchronic toxicity **(Clark, 1983)**. Groups of 18 male and female Wistar rats were exposed in $1m^3$ exposure chambers to 0 or 100 ppm ethylbenzene 6 hrs/day, 5 days/week for 12 weeks. During study period, clinical signs of toxicity were observed daily and body weights were recorded weekly. Feed intake was measured in weeks 6 and 7; 4 h samples of urine were ana-

lysed before, during week 6 and at the end of study. Prior to necropsy, blood was taken for analyses of hematology and serum chemistry parameters. 18-22 hrs after last exposure, animals were subjected to a complete necropsy. The weights of liver, kidneys, spleen, brain, heart and testes were determined. Sections of more than 20 tissues and organs including one transverse section of the nasal cavity, one section of the left lobe and the right lower lobe of the lung, two levels of trachea and one section below the bronchial bifurcation were processed for histopathologic examination. No exposure related effects were found. The study was well documented; however, the testing of only a single relatively low concentration of 100 ppm was a major weakness.

Early experiments on chronic toxicity studies in the 1940th and 1950th were summarised by **Wolf et al. (1956)**. Groups of 10-25 male and female Wistar-derived rats and 5-10 albino guinea pigs and 1-2 albino rabbits and 1-2 rhesus monkeys were exposed to ethylbenzene during 7-8 hrs/day on 5 days/week. Animals were exposed on 103-138 exposure days within a study period of 144-214 days. Target concentrations were 400 and 600 ppm for all test species, 1250 ppm for rats, guinea pigs and rabbits; in addition, one group of male rats received 2200 ppm ethylbenzene. The analytical concentrations were claimed to be within a 10% range of the desired concentrations. Room air exposed animals in a chamber and unexposed animals were used as control groups.
In rats, at vapor concentrations of 400 ppm and above slightly increased mean weights of the liver and kidneys were found, and at 1250 and 2200 ppm cloudy swelling of liver cells and renal tubular cells were seen. At 1250 ppm there was a questionable and at 2200 ppm a moderate growth depression. The LOAEL was considered by the authors to be 400 ppm. Growth depression was also seen for guinea pigs at 1250 ppm (only females tested), liver weight was increased in male and female guinea pigs at 600 ppm, but not at 1250 ppm for this species. Monkeys exposed to 600 ppm demonstrated increases in liver weight (both sexes) and degeneration of testicular germinal epithelium, which was the only treatment-related effect seen in rabbits receiving 600 ppm ethylbenzene. The presence/absence of the effect at 1250 ppm was not clearly indicated. The NOEL was for guinea pigs, rabbits, and monkeys 400 ppm and for rats the LOEL was 400 ppm.

A study specifically designed to detect immunological effects according to OPPTS 870. 7800 was carried out by **Stump (2004),** the reference/study report was not available to the rapporteur.
. Groups of 10 female Sprague-Dawley rats inhaled 0, 25, 100, and 500 ppm ethylbenzene for 6 hrs/d daily over 28 consecutive days. A positive control group received cyclophosphamid intraperitoneally (50 mg/kg) on the 4 days prior to sacrifice. All animals were treated with sheep red blood cells intravenously 4 days prior to scheduled sacrifice. Besides determination of body weight and food consumption, clinical observations and hematolgy were carried out. At necropsy brain, kidneys, liver, lungs, spleen and thymus were weighed and speenic tissue was analysed for antibody forming cells (AFC).
Relative liver and kidney weights were significantly increased at 500 ppm. The functional ability of the humoral immune competence as measured by the splenic AFC response to sheep erythrocytes was not affected.

Sakazaki et al. (2001) developed an in vitro assay with C3H/He and BALB/c cells for evaluating immunotoxicological effects of about 255 substances including ethylbenzene. The growth-inhibitory effect of the test materials on mouse splenic lymphocyte mitogenesis using lipopolysaccharide and Con A as the specific mitogens for B and T cells was determined and the effect on humoral and cell-mediated immunity, respectively, was evaluated. After treat-

mant with the test substance the DNA content of the cells was determined as an indicator of lymphocyte growth.

Of the 255 chemicals tested, 173 showed inhibitory effects on mitogenesis. The data were classified into four typical inhibition patterns from the dose-response curves as follows:

A - No inhibition within the dose range assayed
B - Weak inhibition up to the maximum 50%
C - Concentration dependent inhibition
D - Sudden inhibition at certain concentrations, probably due to toxicity.

A was considered negative, B, C and D were considered positive. Results for ethylbenzene indicated type D inhibition.

The authors had carried out a literature search for previous reports relating to immunotoxicity and investigated the concordance between the current test results and published data. In total for the 255 substances tested, the concordance between experimental and reported results is 64.2%. In conclusion, there was some evidence of an inhibitory effect on mitogenesis in splenic B and T cells.

Table 4.1.2.5-1 Inhalation studies with repeated exposure to ethylbenzene

Species No. of animals tested	Study design	Compliance to test guidelines	Results	NOAEC – LOAEC	Reference
F344 rats, B6C3F1 mice, NZW rabbits (5/sex/ group)	0, 99, 382 or 782 ppm (rat/mouse), 0, 382, 782 or 1610 (rabbits) ppm (0, 0.436, 1.684, 3.448, 7.1 mg/l) 6 h/d, 5 d/wk, 4 wk	Similar to OECD 407 (version 1981)	≥382 ppm: sporadically lacrimation, salivation in rats, liver weight↑ in rats, 782 ppm: liver weight↑ in mice, platelet and total WBC counts↑ in rats. Liver weight increases without corresponding changes in histopathology and clinical chemistry	NOEC 99 ppm (rats), 382 ppm (mice); NOAEC 782 ppm (rats and mice), 1610 ppm (rabbits)	Cragg et al., 1989
F344 rats, B6C3F1 mice, (5 males/group); NZW rabbits (4 males/group)	0, 400, 1200, 2400 ppm (0, 1.7, 5.3, 10.6 mg/l) 6h/d, 4 d	Not given	Rats: body weight decrease at 1200 ppm, increase in liver weight at ≥ 400 ppm and in kidney weights at 1200 ppm without histopathological changes; ≥1200 ppm: lacrimation, shallow breathing, prostation, 2400 ppm: deaths of all males before Day 4 with red discoloration of lungs, congestion in lungs, nasal mucosa, liver, kidneys Mice: 400 ppm: excess lacrimation, ≥1200 ppm: deaths of all males at 2400 ppm, 4/5 males at 1200 ppm, lacrimation, shallow breathing, prostation, closed eyes, red discoloration of lungs, congestion of lungs, nasal mucosa, liver, kidneys Rabbits: no toxic signs/deaths/treatment related find-	Rat: NOAEC 400 ppm (see comment in the text) Mice: NOAEC 400 ppm Rabbits: NOAEC 2400 ppm	Biodynamics, 1986

			ings		
F344 rats, B6C3F1 mice, 6/sex/sp (1 w) 8/sex/sp (4 w)	6 h/d, 5 d (0, 75 or 750 ppm); 5 d/w, 4 w (0 or 750 ppm). Investigation of kidneys in rats, liver and lung in mice	Not a guide-line study	Mice: increased liver weight at 750 ppm (m + f) and DNA synthesis (m > f). Increased DNA synthesis in lung (m + f). Some changes in en-zymes in liver and lung. Rats: slight increase of kidney weight (m + f)at 750 ppm; increase of hyaline droplets after 1 wk only, nephropathy, DNA synthesis (males)	NOAEC rats and mice (m + f): 75 ppm.	Stott et al., 1999; 2003
Wistar rats (18/sex/ group)	0, 100 ppm (0.43 mg/l) 6h/d, 5 d/wk, 12 wk	Not given	No adverse effect	NOAEC 100 ppm	Clark, 1983
Groups of 10-25 Wistar rats, 5-10 guinea pigs, 1-2 rabbits, 1-2 Rhesus monkey	0, 400, 600, 1250 (both sexes of rats, guinea pigs and rabbits), 2200 (m rat only) ppm; for mon-keys 400 (f only) and 600 ppm (1.7, 2.6, 5.4. 9.5 mg/l) 7-8 h/d, 5 d/wk, 103-138 exposure days within a period of 144-214 d	Not given	Rats: ≥400 ppm ↑ of liver + kidney weight, ≥1250 growth↓, cloudy swelling of liver cells and renal tubular epithelium Guinea pigs: at 600 ppm liver weight↑, at 1250 growth↓ Rabbits: degeneration of testicular germinal epithelium at 600 ppm Monkeys/600 ppm: liver weight↑, degeneration of testicular germinal epithelium	LOEC 400 ppm (rats), NOEC 400 ppm (rabbits, guinea pigs, monkeys)	Wolf et al., 1956
Wistar rats (5m/group/ interval)	0, 50, 300, 600 ppm, 6 h/d, 5 d/wk, 2, 5, 9, 16 wk	Not given	≥50 ppm: proliferation of SER in hepatocytes and induction of some liver enzymes, 600 ppm: induction of some kidney enzymes,kidney: body ratio↑	Not applicable	Elovaara et al., 1985
Sprague-Dawley rats (4m)	2000 ppm; 6 h/d; 3 d	Not given	Increase of relative liver and kidney weights; enzyme induction in liver and kidney,	Not applicable	Toftgard and Nilsen (1982)
F344 rats, B6C3F1 mice (10/sex/ group)	0, 100, 250, 500, 750, 1000 ppm (0, 0, 0.47, 1.18, 2.37, 3.55, 4.74 mg/l) 6h/d, 5 d/wk, 92-98 days	Close to B.29	Rats:≥250 ppm: liver weight↑ ≥500 ppm: kidney weight↑ Mice:≥ 750 ppm liver weight ↑; 1000 ppm: kidney weight↑ Histopathological reevaluation of kidneys (Hard, 2002): increased incidence of CPN and hyaline drop-lets at :≥750 ppm in male rats	Rats and mice: $NOAEC_{local\ +\ sys}$ 1000 ppm Male rat kidney: NOAEC 500 ppm	NTP, 1992; Hard, 2002
F344 rats, B6C3F1 mice (50/sex/ group)	0, 75, 250, 750 ppm; 6 h/d; 5 d/wk; 104 weeks	NTP carcino-genicity bio-assay	Rats: chronic nephropathy in males at ≥ 250 ppm and females at ≥ 75 ppm (with no relevance for hu-mans);decreased survival and de-creased body weight at 750 ppm (males). Slightly decreased body weight for females at all exposure concentrations. Mice: histopathological effects in lung (alveolar epithelial metaplasia in males, 750 ppm), liver (eosino-philic foci, syncytial alteration, hy-pertrophy, necrosis in females, 750 ppm; syncytial alteration of hepato-cytes in males 250 ppm), thyroid	NOAEC rats: males 250 ppm, females below 75 ppm NOAEC mice:75 ppm	NTP 1999; Hard, 2002; Brown, 2000

			(thyroid cell hyperplasia in males and females, 750 ppm), pituitary (hyperplasia pars distalis in females ≥250 ppm)		
Sprague-Dawley rats (10 females / group)	0, 25, 100, 500 ppm; 6 h/d over 28 consecutive days	OPPTS 870.7800	Increased liver and kidney weights at 500 ppm; no effect on humoral immunological response	NOAEC 100 ppm	Stump, 2004

m male; f female
NZW New Zealand White,
WBC white blood cell
SER smooth endoplasmatic reticulum
CPN chronic progressive nephropathy

Oral studies *(Table 4.1.2.6 B)*

The early report of **Wolf et al. (1956)** also included some data on the oral route. Groups of 10 female Wistar-derived rats were administered by gavage with 13.6, 136, 408 or 680 mg/kg/d ethylbenzene on 5 days/week for six months. 20 rats served as controls that received 2.5 ml of the vehicle (olive oil). Besides determination of clinical signs, mortality, body weight and food consumption, hematology examinations on selected parameters (RBC, total and differential WBC, Hb concentration) were performed after 20, 40, 80 and 130 doses. Lung, heart, liver, kidney spleen and testes were inspected macroscopically and weighed.
After 130 applications (after day 182 of the study), ethylbenzene doses of 408 mg/kg and above induced increases in the mean weight of the liver and the kidney. Slight histopathological findings were observed in the livers and the kidneys of treated rats of these groups consisting of cloudy swelling of hepatocytes and of renal tubular epithelium. No effect was observed in rats receiving 136 mg/kg. No other data were reported.

In a range finding study similar to OECD TG 407 **Mellert et al. (2003; 2007)** (the study report by **Mellert et al. (2007)** was not available to the rapporteur).treated male and female Wistar rats by gavage in corn oil over 28 days at dose levels of 75, 250 and 750 mg/kg/d. Split doses of 37.5, 125 and 375 mg/kg were given twice daily 8 h apart. Animals were investigated for clinical signs, mortality, body weight, food and water consumption, hematology, biochemistry, urine analysis, organ weights (11 organs) and histopathological changes (liver and kidney). In addition to H&E staining, the kidneys were stained with Mallory-Heidenhein to visualise hyaline droplet nephropatahy.
There was no treatment related mortality and salivation was observed in high and mid dose rats of both sexes. Body weight gain was significantly reduced in high dose males at day 7. Water consumption was increased in high and mid dose rats of both sexes (+21 to + 40%), attaining statistical significance over several time periods. There was a slight increase in serum ALT, bilirubin and cholesterol in high dose animals of both sexes. Increase in bilirubin did not attain statistical significe in males. Increased serum urea was observed in high dose males and reduced sodium levels in high dose females. By urinalysis an increased incidence of granular and eptihelial cell casts in top and mid dose males together with an increased number of degenerated transitional epithelial cells in high dose males were found. The absolute and relative liver weights in both sexes were significantly increased at the high dose levels and at the mid dose in males. In addition, absolute and relative kidney weights in top and mid dose males were significantly increased (the absolute weight change at the high dose did not reach statistical significance). Significant differences in epididymal and spleen weights were regarded as incidental.
Histopathology revealed centrilobular hepatocellular hypertrophy in the liver of top dose males and females and mid dose males. Hyaline droplet nephropathy visualised by Mallory-

Heidenhain stain occurred with higher incidence in the male kidney at mid and high dose levels in comparison to controls.
The NOAEL in this range finding study was 75 mg/kg based on increased liver weight and hepatocellular hypertrophy at higher dose levels in males and 250 mg/kg in females. There was evidence of male rat specific nephropathy in high and mid dose groups which is not considered of relevance to man.

Mellert et al. (2004; 2007) (the study report by Mellert et al. (2007) was not available to the rapporteur).carried out a 90d oral gavage study in corn oil (OECD TG 408) in male and female Wistar rats (10 animals /sex/dose) on the basis of the above mentioned range finding study at dose levels of 75, 250 and 750 mg/kg by the same dosing regime. The parameters investigated corresponded to those prescried by the test guideline.
No animals died during the test period and salivation was observed at 250 and 750 mg/kg. The value of the landing foot-splay test was significantly decreased in 750 mg/kg males but this finding may have been related to the decreased body weight of this group. Motor activity was significantly increased in high dose females..
In males at 750 mg/kg, body weight was significantly decreased. Water consumption was significantly increased at most time points in 750 mg/kg males from day 7 to 84 and in 750 mg/kg females between days 7 and 77. In 250 mg/kg males increased water consumption with statistical significance was achieved on days 56 and 70.
Slight but statistically significant increases in alanine aminotransferase and gamma-glutamyltransferase were found in sera of high and mid dose males at the end of the study. In females, alanine aminotransferase activity was increased only at 750 mg/kg. Some further changes in clinical chemistry paramerters (potassium, total bilirubin, albumin, cholesterol, magnesium, calcium, urea, creatinine, total protein, globulin, sodium) were considered to be due to induction of the hepatic microsomal enzyme system and/or secondary to effects on feed and water consumption.
Significantly increased mean corpuscular volume was observed in 750 mg/kg animals of both sexes ($p<0.01$) and 250 mg/kg females and decreased platelet counts were present in 750 mg/kg females. Both findings were considered treatment-related and possibly due to a minimal transitional regenerative anemia. Prothrombin times were shorter in 250 mg/kg animals of both sexes, however, the reduction of prothrombin times in 750 mg/kg males occurred only as a tendency towards shorter clotting times.
Increased numbers of degenerated transitional epithelial cells and granular epithelia cell casts were present in the urine of 250 and 750 mg/kg males. The findings indicated mild damage or functional impairment to the kidneys.
In the liver, a significant increase in absolute and relative weights was recorded in both sexes at ≥ 250 mg/kg bw/day. At 75 mg/kg a very slight increase (5%) in relative liver weights in the males was found but was not accompanied by histopathological change and hence considered of no biological relevance. In the kidneys of 750 and 250 mg/kg males, a statistically significant increase in absolute and relative weights was noted. Females receiving >= 250 mg/kg bw/day ethylbenzene exhibited slight but significant increases in relative kidney weight that was not correlated with histopathological changes and hence was considered of no biological relevance. A significant decrease of absolute and relative thymus weights was present in ≥ 250 mg/kg females. Althouth it was not correlated with histopathological changes it might indicate mild thymus atrophy.
Histopathology showed in liver mimimal to slight centrilobular hypertrophy of hepatocytes in males and females at the two highest dose levels and correlated with the increased liver weights reported above. This was considered an adaptive response. In the kidney the only

histopathologic finding attributed to treatment was an increase of hyaline droplet storage in the tubular epithelium of male rat kidneys (visualised by Mallory-Heidenhain stain) considered to come from an increase in production of the male specific protein a-2u-globulin. Morphologic signs of Chronic Progressive Nephropathy (CPN) were similar in the control and treatment groups.

Changes in kidney weights and histopathology in male rats were indicative of male rat specific alpha 2u-globulin nephropathy. In the absence of histopathological changes in the female kidney the increased kidney weights were not considered of biological significance.

Various changes in electrolytes, urea and creatinine were probably associated with the effects of treatment on food and water consumption and are considered secondary effects.

The NOAEL for this 13 week rat gavage study was considered to be 75 mg/kg/day with a LOAEL of 250 mg/kg/day. This was based on changes in haematology indicative of a mild regenerative anaemia together with changes in clinical chemistry parameters, increased liver weights and centrilobular hepatocellular hypertrophy indicative of hepatic microsomal enzyme induction.

Table 4.1.2.5-2 **Studies with repeated oral exposure to ethylbenzene**

Species (No. of animals tested)	Study design	Compliance to test guidelines	Results	NOAEL – LOAEL	Reference
Wistar rats (10 f/group)	13.6, 136, 408 or 680 mg/kg/d, 5 d/wk, 6 mo	Not given	≥408 mg/kg/d: weight↑ in liver and kidneys, slight cloudy swelling of hepatocytes and renal tubular epithelium	NOAEL 136 mg/kg/d	Wolf et al., 1956
Wistar rats (5m, 5f/group)	Gavage, 0, 75, 250, 750 mg /kg/d; 4 weeks	Range finding study similar to OECD TG 407	≥ 250 mg/kg: Increased liver weight with centrolobular hypertrophy in high and mid dose males and high dose females. Elevated activity of ALAT, TBil and Chol. Increased kidney weight in high and mid dose males with histopathological evidence for male rat specific hyaline droplet nephropathy.	NOAEL 75 mg/kg/d	Mellert et al. (2003; 2007)
Wistar rats (10m, 10f/group)	Gavage; 75, 250, 750 mg/ kg/d; 3 months	OECD TG 408	≥ 250 mg/kg: Changes in haematology indicative of mild regenerative anemia, elevated activity of ALAT, TBil and Chol, increased liver weight with centrilobular hypertrophy. Changes in male rat kidney indicative of male specific alpha-2uglobulin nephropathy.	NOAEL 75 mg/kg/d	Mellert et al. (2004; 2007)

f: female
m: male

A 13 week oral study has been carried out with 1-phenylethanol for dose selection for a carcinogenicity bioassay **(NTP, 1990)**. As 1-phenylethanol is the major metabolite of ethylbenzene in rats the results are briefly reported here. Based on a 16 day range finding study, rats (10 /sex/dose) were orally dosed by gavage with 93, 187, 375, 750, and 1500 mg/kg/d in corn oil for 5 days/week over 13 weeks and mice (10/sex/dose) with 46.9, 93.8, 187.5, 375, and 750 mg/kg/d. Clinical signs, mortality and body weight were recorded. The only organ weight determined was that of the liver. Histopathology included all top dose and control animals, all premature descendents and in rats the spleen was investigated in addition for all animals at 750 mg/kg/d and males receiving 375 mg/kg/d.
In rats deaths of 1/10 top dose males and 3/10 top dose females were considered to be treatment related. Rats receiving 750 or 1500 mg/kg/day exhibited ataxia, rapid breathing and lethargy for up to 30 minutes after dosing. At the top dose level (1500 mg/kg/day) final mean body weight was decreased in both sexes. The relative liver to body weight ratio was increased in all treated female groups and for males receiving 375, 750 and 1500 mg/kg/day. A minimal to mild increase in deposition of brown pigment characteristic of haemosiderin was found in splenic macrophages in males receiving 750 and1500 mg/kg/day, similar findings were observed in 6/10 top dose females. It is not possible to assign a NOAEL to this study as liver weights were increased at lower dose levels and histopathological examination was not undertaken at these levels.
Mice receiving 375 or 750 mg/kg/day exhibited ataxia, rapid breathing and lethargy for up to 30 minutes after dosing and no further substance related effects were noted. Therefore, based on the limited parameters investigated the NOAEL is 750 mg/kg/d.

Dermal application

Although percutaneous resorption is demonstrated no valid studies with repeated dermal applications are available.

In secondary references, the skin irritant property of undiluted ethylbenzene was mentioned. However, no data on dosages, responses or time of occurrence was given. **Wolf et al. (1956)** described in their data collection over 17 years moderate irritation and necrosis of the epidermis after 10 to 20 applications of undiluted ethylbenzene onto the ear or the shaved abdomen in white rabbits. The data were not further specified.

Other application routes

In a subacute study on potential nephrotoxicity of 20 industrial solvents, groups of 5 female Sprague-Dawley rats were given 5 intraperitoneal injections of 0, 350 or 750 mg/kg ethylbenzene per week for 2 weeks **(Bernard et al., 1989)**. Urinary excretion of N-acetyl-β-D-glucosaminidase, β_2-microglobuline and albumin was determined. Mean values for β_2-microglobuline and albumin were slightly (non-significant) higher than that of controls at 750 mg/kg, no effect was seen at 350 mg/kg.

4.1.2.6.1 Human data

see specific effects on nervous system as no other relevant effects are quoted in the public literature

Specific effects on the nervous system:

Human data

There are no specific data on neurotoxicity in humans by monoexposure to ethylbenzene, but for other aromatic solvents there is evidence for such effects in humans and especially in animals, e.g. for toluene, xylenes or styrene. This relates to depressive and narcotic effects and there is strong experimental evidence for ototoxicity. These data suggest that the findings in animals are relevant for humans, too. Data from toluene exposed workers that showed hearing loss accompanied with vestibular impairment give concern that ethylbenzene effects on the inner ear may not be limited to the cochlea region (cited from **Morata et al., 1994**).

Sulkowski et al. (2002) found symptoms of vestibular dysfunction (by electronystagmography) and sensorineural high frequency hearing loss in workers involved in the production of paints and varnishes for 2 to 34 years. In the abstract it was mentioned that the most significant exposure could be attributed to the following mixture constituents: ethylbenzene, xylene and trimethylbenzene isomers. But this can hardly be verified by the exposure data given: ethylbenzene was not found by environmental monitoring (ethyltoluene is mentioned in table 3; possibly a mistake?) or in blood and for urine only an excretion rate but no concentration of mandelic acid is given (the meaning of this data is unclear). Therefore it is not possible to verify the extent of exposure to ethylbenzene.

Animal data

Depending on the exposure concentration and duration, ethylbenzene can produce a variety of neurofunctional disorders and neurotoxic effects.

A. Effects on neuroendocrine and neurotransmitter systems:

The effect of ethylbenzene on dopamine and noradrenaline nerve terminal systems in the di- and telencephalon was examined **(Andersson et al., 1981).** Sprague-Dawley rats (6 males/group) were exposed to 2000 ppm ethylbenzene (purity ≥99%) on 6 hrs/day on three consecutive days, control animals were air-exposed. Animals were sacrificed 16-18 h after the end of exposure. Additional groups of rats were injected with the tyrosine hydroxylase (TH) inhibitor (alpha-methyltyrosine methyl ester H44/68) prior to sacrifice. Depletion of catecholamine (CA) stores following TH inhibition may indicate a change in CA turnover. Catecholamines (dopamine-DA and noradrenaline-NA) were visualised in brain sections (at least 2 sections/regions) using fluorescence histochemistry and quantified using microfluorimetry.
Analytical vapor concentration was reported to deviate less than 15% from target concentration. Ethylbenzene exposure produced no change in dopamine levels in various parts of the hypothalamus and the median eminence and a decrease in noradrenaline concentration in paraventricular hypothalamic nucleus was found 16-18 hours following the last exposure. Ethylbenzene accelerated the turnover of dopamine and noradrenaline in various parts of the hypothalamus and the median eminence. Reduced prolactin serum levels indicated selective reduction of prolactin secretion, but ethylbenzene had no effect on the level of the other hormones investigated such as TSH, GH, and FSH and corticosterone. In conclusion, inhalation of 2000 ppm of ethylbenzne produced effects on nor-adrenaline levels and turnover of noradrenaline and dopamine in the forebrain and hypothalamus.

In this context the result of an in vitro screening assay for estrogenic activity is mentioned **(Nishihara et al., 2000)**. 517 chemicals including ethylbenzene were investigated in a yeast two-hybrid assay which is based on the ligand-dependent interaction of two proteins, a hormone receptor (ER alpha receptor) and coactivator (TIF2). Hormonal activity is detected using a beta-galactosidase reporter gene. The results were evaluated by relative activity expressed as REC10 (10% relative effective concentration). This is the concentration of the test chemical showing 10% of the agonist activity of 10-7 M estradiol (this is the optimum concentration for estradiol). The REC10 for ethylbenzene was greater than the higest concentration tested (> 1 mM). Ethylbenzene was considered inactive in this assay.

Ethylbenzene as well as some of its putative metabolites caused a significant depletion of dopamine and increase of homovanillic acid levels in the striatum and tubero-infundibular region of the hypothalamus. Groups of 8 male adult New Zealand rabbits (2200g) were exposed to 750 ppm (3.3 mg/l) ethylbenzene vapor during 12 hrs daily on 7 consecutive days **(Romanelli et al., 1986; Mutti and Franchini, 1987; Mutti et al., 1988)**. Consistent effects were seen in groups of 8 rabbits given i.p. injections of 4 mmol/kg phenylglyoxylic acid (PGA) in pH 7.4 adjusted saline solution for 3 days. Same, but less prominent effects were seen when 4 mmol mandelic acid (another product of ethylbenzene metabolism) was injected to another group of 8 rabbits. No effects were obseved by the other metabolites investigated, namely hippuric acid, methyl hippuric acid and 7-methyl mandelic acid. The control group of the inhalation study was exposed to fresh air, those of the injection study received 5 ml of saline solution (NaCl 0.9%). Animals were killed 12 hrs after inhalation exposure and 24 hrs after administration of the last i.p. dose, respectively. Catecholamines and their metabolites were measured in brain homogenates by a HPLC analysis system. Neither ethylbenzene nor its metabolites affected norepinephrine levels in these brain areas.
Mutti et al. (1988) additionally investigated the ability of glyoxylic acid and phenylglyoxylic to condense non-enzymatically with dopamine in vitro. The authors conclude that the effects on brain dopamine are due to metabolic interference of metabolites with dopamine catabolism. Only those metabolites whose side chain can be transformed to an alpha-ketoacid caused dopamine depletion.

B. Depressive and narcotic neurofunction effects:

Barnett et al. (2006) (the reference/study report was not available to the rapporteur) investigated the subchronic neurotoxicity of ethylbenzene by a specifically designed guideline study (EPA OPPTS 870.6200, similar to OECD 424). Sprague-Dawley rats were given ethylbenzene in corn oil twice daily with three-hr intervals by gavage of total daily doses of 50, 250 and 500 mg/kg/d over 90 days. Control and high dose groups consisted of 16 male and 16 female animals, the other groups of 10 animals /sex. The selection of the top dose was based on the results of previous repeated dose toxicity studies and PBPK modeling comparing oral doses with inhalation exposures. Besides detailed general clinical observations, opthalmological examinations and determination of body weight and feed consumption, liver and kidney weights were determined at necropsy. The following specific investigations for neurobehavioural assessment were carried out: a functional observational battery (FOB: lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to light, piloerection, respiration, urination, defecation as indications for autonomic functions; sensorimotor responses to visual, acoustic, tactile and painful stimuli as indications for reactivity and sensitivity; reactions to handling and behavior in the open field as indications for excitability; gait pattern in the open field, severity of gait abnormalities, air righting reaction, visual placing response and landing foot splay as indications for sensorimotor coordination), forelimb and

hindlimb grip strength, motor activity, body temperature. At necropsy 9 to 11 rats/sex/dosage group were perfused in situ with neutral buffered 10% formalin. Liver and kidneys and brains of rats selected for neurohistological examination were weighed. The kidneys, livers, eyes, brain, spinal cord, hindlimb peripheral nerves and muscle from control and high dosage groups were examined histologically.
There were no treatment related deaths and body weight gains in the two top doses in comparison to the control group were decreased in males but increased in females, both not to a statistically significant extent. At 500 mg/kg bw/day, there were slight increases in the numbers of male and female rats observed with slight to moderate excess salivation and marginal increases in urine-stained abdominal fur. The majority of observations of excess salivation occurred around the time that the daily doses were administered.
There were no differences between the dosage groups in a large majority of the FOB measures and those few occurring were not considered treatment related. There were no further indications for treatment related neurobehavioural effects. The absolute organ weights for the liver and paired kidneys were increased for both the male and female rats at 250 and 500 mg/kg /day, and relative weights of these organs were numerically or significantly increased ($p<0.05$ to $p<0.01$) in male and female rats at these dose groups. These weight changes are considered effects of ethylbenzene. The histological examination of tissues from the control and 500-mg/kg bw/day dosage male and female rats did not reveal any test substance-related microscopic lesions in the neural and muscle tissues evaluated nor in the livers and kidneys.
In conclusion, 91-day oral exposure of rats to ethylbenzene at dosages ranging from 50 to 500 mg/kg /day did not cause treatment-related effects on the FOB, motor activity or histopathology of the central and peripheral nervous system. The 250 and 500 mg/kg bw/day dosage of ethylbenzene increased liver and kidney weights in male and female rats but without histopathological correlates. Additionally at the 500 mg/kg bw/day dosage increased incidences of clinical signs in both males and females were observed. The NOAEL (including that for neurotoxicity) is 500 mg/kg/d and the NOEL 50 mg/kg/d.

High concentrations of inhaled ethylbenzene induce CNS depression and neuromuscular impairment. Using a standard battery of neurobehavioral tests, so-called ‘functional observation battery (FOB)’, in CFW mice (8 males/group), inhalation periods of 20 minutes to 2000 to 8000 ppm ethylbenzene produced abnormal postures, decreased arousal and rearing, increased ease of handling, disturbances of gait, mobility, and righting reflex, decreased forelimb grip strength, increased landing foot splay, impaired psychomotor coordination, and decreased reactivity to various external sensory stimuli **(Tergeris and Balster, 1994)**. No effects were seen on measures of the autonomic nervous system such as urination, defecation, and piloerection. The minimal effective concentration was not determined. Animals recovered within minutes after removal from exposures. The effects observed were similar to those seen with other alkylbenzenes and pentobarbital and they were dose related. These acute effects of ethylbenzene were short lived with recovery beginning within minutes of removal from the exposure chamber. The authors stated that the depressant effects during high concentration short exposures can be related to the rapid clearance from the central nervous system and is probably independent from delayed or chronic neurotoxic effects.

The group motility was continuously estimated in an earlier study on 8-week old CFY male white rats (8 males/group) during a 4 hr inhalation exposure to six concentrations up to those inducing anaesthesia (100-2180 ppm ethylbenzene vapour) **(Molnar et al., 1986)**. Enhanced motility associated with highly incoordinated movements has been observed at concentrations of 400-1500 ppm, whereas 2180 ppm was calculated to be the minimal narcotic concentration in this test system. Decreased mobility occurred at and above 2000 ppm. The central excita-

tion at lower concentration and central depressive effect at high concentration of ethylbenzene corresponded to the characteristical biphasic effects of other organic solvents.

In-vitro tests:

There are some investigations on the primary cellular targets in the central nervous system. In isolated rat synaptosomal membranes ethylbenzene at 2, 4, 6 and 8 mM linearly increased synaptosomal membrane fluidity while acetylcholinesterase and ATPase activities decreased. The authors speculate that this is caused by disturbance of lipid/protein interactin induced by ethylbenzene and other test solvents like benzene, styrene and toluene **(Naskali et al., 1993)**. A concentration-dependent inhibition of enzyme activity of membrane-bound total ATPase in neural membranes preparations from astrocyte cultures was induced by ethylbenzene at the concentrations tested (3, 6, 9 mM) **(Naskali et al., 1994)**. **Vaalavirta and Tähti (1995)** confirmed the data and they found significant inhibitory effects at 3, 6 and 9 mM ethylbenzene on Na^+, K^+-ATPase and on Mg^{2+}-ATPase in astrocytic cell membrane preparations. It was concluded that ethylbenzene like other organic solvents exert their toxic effects on the central nervous system, at least in part, by disturbing ATPase-dependent astrocytic functions.

C. Ototoxicity:
There are a number of animal studies on the ototoxic potential of ethylbenzene (to facilitate understanding some explanations are given in *italics* within brackets).

In a reflex modification for audiometry (RMA) testing (*behavioural audiometry test, indicating effects on the central and peripheral parts of the auditory pathway without discrimination between affected localisation*) at 1 and 4 weeks postexposure, 200 g male Wistar-derived rats (Wag/Rij/Cpb/Hsd) exposed to ethylbenzene concentration of 800 ppm for 8 hrs/day on 5 days had persistently increased auditory thresholds of a noise-evoked startle response **(Cappaert et al., 1999)**.
The RMA thresholds increased significantly about 25 dB in the entire investigated 4-24 kHz region and did not change between 1 and 4 weeks post exposure indicating that neither recovery nor further deterioration of auditory thresholds occurred.
Severe hearing loss was recorded in ethylbenzene-exposed rats at week 8 to 11 after the end of exposure. Electrocochleography at the apex of the cochlea *(reflecting exclusively effects at the periphery of auditory pathway*) demonstrated significantly increased thresholds (shift of stimulus level 10-30 dB) of recorded auditory-evoked responses (compound action potentials (CAP)) at all frequencies tested (1-24 kHz). Immediately after electrocochleography, cochleas were fixed by a perfusion technique and hair cell counting at 5-6 subsequent and representative sections of the organ of Corti revealed a significant loss of outer hair cells (OHC, *required for normal hearing sensitivity, hearing loss of 40-50 dB can occur due to their absence*) (52.1%±9.7) in the upper part of the basal turn and in the lower part of the middle turn (65.6%±7.3). These two cochlear turns correspond with the mid-frequency region (11-21 kHz). Inner hair cells were present at 100% in the exposed and control animals, and spiral ganglion cell appeared normal in both groups. As the threshold shifts were very similar for RMA and CAP the authors concluded that ethylbenzene primarily exerts its effects on the peripheral part of the auditory system.

In a second study on lower ethylbenzene concentrations, auditory function in 9-week old Wag/Rij rats (8/group, average weight 200 g) was tested by measuring compound action potentials (CAP) in the frequency range of 1-24 kHz and distortion product otoacoustic emissions (DPOAEs, *a very sensitive, noninvasive test of cochlear function, detecting OHC im-*

pairment; measurement of emitted sounds produced as stimulus-induced active OHC response) in the frequency range of 4-22.6 kHz three to six weeks after the end of exposure **(Cappaert et al., 2000)**. Inhalation exposure to 400 and 550 ppm ethylbenzene for 8 hours/day for 5 consecutive days increased auditory thresholds (significant increase of the threshold for CAP at 8kHz after 550 ppm, at 12 kHz after 400 and 550 ppm and at 16 kHz after 550 ppm), whereas significantly decreased DPOAE amplitude growth curves were observed after 550 ppm dose at 5.6, 8, and 11.3 kHz, but not at other frequencies. OHC loss was found in two of five examined localisations in the cochlea. At 400 ppm, 25% OHC loss was found at the 11- and 21-kHz region; a small OHC loss was found in the 21 kHz location (-12%). The 550 ppm concentration evoked 40% and 75% OHC loss at the 11- and 21-kHz regions. No significant effect on measures of hearing function and no statistically significant loss of cochlear hair cells were seen at 300 ppm ethylbenzene, the lowest concentration tested.

Simultaneous exposure to noise and ethylbenzene had synergistic effects on the loss of OHCs especially of the 3rd row. The effects of 0, 300 or 400 ppm ethylbenzene and three noise levels (95 or 105 dB mostly in the frequency range of 1.5 and 12.5 kHz or background noise below 65 dB) and all their combinations were investigated for a 5 day exposure at 8 hrs/day in albino Wag/Rij rats (8 animals/group) **(Cappaert et al., 2001)**. Ambient noise was below 50dB over most of the frequency range. At very low frequencies, where rats were reported to be very insensitive, ambient noise probably produced by the air supply system was highly variable and could reach levels up to 60 dB, but did not exceed 65 dB. Data on hearing function were generated by the measurement of distortion product otoacoustic emissions (DPOAEs) and by the estimation of compound action potentials (CAP) in electrocochleography. Morphological abnormalities were determined by quantitative estimation of hair cells of the organ of Corti (in representative sections of the mid-frequency sections and in whole cytocochleograms) in perfusion fixed cochleas from both sides. Measurements and cochlea harvesting were conducted between 3 and 7 weeks after exposure. The reason for this time point was that noise induced hearing loss that still exists 20 to 30 days after the last exposure is considered to be permanent. DPOAEs and compound action potentials were affected after 105 dB noise alone, and after 105 dB noise in combination with 300 and 400 ppm ethylbenzene. However, the amount of hearing loss with these combinations did not exceed the loss for 105 dB noise alone. A slight not significant OHC loss after exposure to 300 ppm ethylbenzene was located in the third row of OHCs. Significant hair cell loss was observed at 400 ppm spreading to the 2nd and first OHC row. Noise alone hardly affected the OHC counts except for a minor loss in the first row of OHCs after 105 dB. Noise at 105 dB in combination with ethylbenzene at 300 and 400 ppm, however, showed OHC loss greater than the sum of the losses induced by noise and ethylbenzene alone. The row of inner hair cells was not affected by either agent. The authors located the outer hair cell loss in the mid-frequency region of the cochlea as the target region of ethylbenzene action and found concentration-dependent expansion of findings. In conclusion, by using DPOAE and CAP measurements there was no evidence of a synergistic effect between the two ototoxic agents. However using reduction of hair cells as an indicator of ototoxicity there was a statistically significant synergistic effect between exposure to noise at 105 dB and ethylbenzene exposure at levels of 300 and 400 ppm.

Guinea pigs were less susceptible to the ototoxic effect of ethylbenzene than rats. A study on species differences was conducted with exposure of eight female guinea pigs (200 g) to high concentrations of ethylbenzene (2500 ppm, 8 hrs on the first day, thereafter 6 hrs/day, over the following 4 days) **(Cappaert et al., 2002)**. Duration of exposure was reduced for guinea pigs after the first day from 8 to 6 hrs because of severe toxicity. A group of eight rats (200 g)

were exposed to 550 ppm ethylbenzene for 8 hrs/day on 5 days. Control groups were exposed to ambient air alone. In a supplementary study, blood concentration at 500 ppm ethylbenzene (8hrs/day, 3 days) were estimated in four animals of each species at the end of day 1 and 3, three air exposed animals/species were used as controls. Hearing function in both species was tested by electrocochleography and histopathology of perfusion-fixed standard sections of the cochleas of all animals was carried out (identical to the methods reported in the above mentioned studies).
Shifts of CAP thresholds indicated a mid-frequency hearing loss in rats exposed to 550 ppm ethylbenzene, at 8, 12, 16 and 24 kHz. Mean thresholds were not affected in guinea pigs at 2500 ppm ethylbenzene. Significant loss of OHC was observed in rats in the 11- and 21-kHz regions of the cochlea, the average percentage of remaining OHC was only 25% in the 21-kHz region. In guinea pigs there was only little OHC loss at any frequency.
Ethylbenzene concentrations in blood showed significant differences between species. On day 1, the level was 8.3 times higher in rats than in guinea pigs and 4.3 times higher in rats than in guinea pigs on day 3. Absolute concentration was lower on day 3 than on day 1 indicating that metabolic transformation rose with increase in treatment duration. It was concluded that species differences in ototoxic potency can be related to the blood concentrations of ethylbenzene and might be explainable by differences in uptake, distribution, metabolism and excretion.

Gagnaire et al. (2007) exposed groups of male Sprague-Dawley rats (14/group) to ethylbenzene for 6 hrs daily, 6 days/week over 13 weeks at 0, 200, 400, 600 and 800 ppm. The animals were maintained for a recovery period of 8 weeks before being sacrificed. Electrophysiological measurements (brainstem auditory-evoked response, BAER) were made at the end of the 4th, 8th, and 13th weeks of exposure and at the end of the 8th week of recovery (week 21) at 2, 4, 8 and 16 kHz. Morphological examinations were carried out following the recovery period. The cochleae were prepared and cytocochleograms (total cell count by histopathology) were constructed from the surface preparation, with the frequency coordinates of the organ of Corti.
There was no exposure related effect on body weight. The audiometric thresholds of the animals exposed to 400, 600 and 800 ppm ethylbenzene were higher than those of controls at the 4 frequencies studied (note: results were provided in figures, hence specific group data values are not presented). The highest hearing losses were observed in the groups exposed to 600 and 800 ppm. They ranged from 44 dB at 2 kHz to 49 dB at 16 kHz. They did not increase significantly throughout the exposure period. No recovery was observed 8 weeks after the end of exposure, when the losses were 43 dB at 2 and 4 kHz, 49 dB at 8 kHz and 53 dB at 16 kHz. The hearing losses were smaller in the 400 ppm exposed groups, ranging from 23 to 27dB depending on the frequencies studied at the end of the recovery period. No shift in audiometric thresholds was observed in the controls and in the group exposed to 200 ppm.
Morphological examination revealed that exposure to 800 and 600 ppm caused nearly complete hair cell loss in the three OHC rows of the organ of Corti. Only the basal part of the cochlea, which transcribes the high frequencies, was partly spared. There were also inner hair cell (IHC) losses in the basal part of the organ of Corti. Quantitative evaluation of the IHC losses indicated that, on average, they reached 32 and 14% in the 800 and 600 ppm ethylbenzene exposed groups, respectively. Exposure to 400 ppm ethylbenzene caused considerable OHC losses, mainly in the apical and the upper and lower medium parts of the organ of Corti. The highest losses occurred in the third row and the lowest in the first row. Occcasional IHC losses were observed in the basal part of the organ of Corti. Exposure to 200 ppm caused significant losses (up to 30% losses in the mid frequency range) in the third row of the OHC in four of the eight animals. The average loss in the third row of the OHC of the animals exposed to 200 ppm was 4%. The theoretical lowest adverse effect levels (TLAELs) calculated

from the three statistical upper confidence limits, i.e. 95, 99 and 99.9%, were 114, 120, and 130 ppm, respectively.
In conclusion, ethylbenzene produced slight to severe ototoxicity in rats exposed for 4 to 13 weeks to concentrations ranging from 200 to 800 ppm. 400 ppm and greater produced significantly increased audiometric thresholds that did not normalise within 8 weeks after exposure ceased. Following the 8 week recovery period, OHC losses were present with increasing severity (4% to nearly 100%, respectively) in the rats at 200 to 800 ppm ethylbenzene. No NOAEL was identified in this study the LOAEL being 200 ppm, the lowest dose level tested and the theoretical lowest adverse effect level was calculated to be around 120 ppm.

Fechter et al. (2007) studied the interaction of noise and solvent exposure on hearing loss. Groups of 6 male Long-Evans rats were exposed inhalationally to a hydrocarbon mixture of 400 ppm toluene and 660 ppm ethylbenzene for either one or two weeks (5 days/week, 6 hrs/day). The groups of rats were exposed in the presence and absence of an octave band of noise at 93-95 dB. Untreated and noise only exposed control groups were included. Impairment of auditory function was assessed using distortion product otoacoustic emissions (DPOAE) and compound action potential (CAP) at 3 days, 1 week and 4 weeks post exposure. The organs of Corti were then dissected to evaluate hair cell loss and a cochleogram was prepared. The uptake and elimination of the solvents was assessed in additional rats (3-4/group) not used for auditory testing by measuring tissue hydrocarbon (blood, liver, cochlear) levels immediately after exposure and 1 hr after a single 6 hr exposure. Glutathione levels in the liver, brain and lung were measured between 0 and 3 hrs after a 4 hr exposure.
A significant amount of ethylbenzene was present in blood and liver 1 hr post exposure and there was no evidencew of glutation depletion.
The combined exposure to toluene and ethylbenzene produced no effects on DPOAE or CAR at any of the postexposure time points after a 5 or 10 day exposure. The combination with noise over 5 or 10 days produced a deficit in DPOAE amplitude most pronounced 3 days post-exposure but even 4 weeks post-exposure there remained a deficit. These effects showed statistical significance. There also was a statistically significant loss in pure tone auditory threshold. Noise alone over 5 days produced a deficit of 10-20 dB 3 days post-exposure, however complete recovery was observed by 1 week post-exposure. Rats experiencing noise for 10 days did show some persistent impairment of DPOAE amplitude even at 4 weeks post-exposure but this was less marked than in the rats exposed to mixed solvents and noise. There was no effect of noise alone on auditory threshold.
By histopathology solvent exposure alone did not lead to a significant loss of OHC cells. Noise alone showed an increase of OHC death in relation to controls. This was quite limited but particularly apparent in rows 1 and 2. The OHC loss did not exceed 5% in any area of the cochlea. The combined treatment with solvent and noise showed after 5 days exposure clear OHC death at 12-24 kHz, greatest in row 1, intermediate row 2 and very limited row 3. Loss of OHC did not exceed 25% at any locus. A similar pattern of effect was seen after 10 days exposure.
In summary, inhalation exposure of rats to a mixture of ethylbenzene (600 ppm) and toluene (400 ppm) for 5 or 10 days did not have an adverse effect on DPOAE amplitude or auditory threshold. There was no significant loss of outer hair cells. Coexposure to these mixed solvents and noise 93-95 dB resulted in a potentiation of permanent auditory damage.
It should be pointed out that in this study high solvent exposures alone (660 ppm ethylbenzene plus 400 ppm toluene) did not result in adverse hearing effects. This is in clear contrast to the studies referenced above of Cappaert et al. and Gagnaire et al. who observed marked effects even at much lower concentrations of ethylbenzene.

Gagnaire and Langlais (2005) compared the ototoxic potency of 21 aromatic solvents after gavage application to Sprague–Dawley rats. 8 male animals received 8.47 mmol/kg (corresponding to 900 mg/kg for ethylbenzene) 5 days/week for 2 weeks. 10 days after treatment the cochleae of 6 rats were prepared for histopathology and hair cell death was quantitatively determined. The severity of hair cell death in the different rows was taken as an indication for ototocix potency. With increasing potency hair cell death moved from OHC row 3 to row 2 and 1 and finally also the inner hair cells (IHC) became affected to a minor extent.
Treatment did not affect clinical signs or body weight gain of ethylbenzene treated animals. Ethylbenzene treatment led to a nearly complete OHC loss in all 3 rows and to a minute loss of IHC. By quantitative comparison, ethlybenzene belonged to the solvents with the highest ototoxic potency together with styrene and clearly more potent than toluene or p-xylene and a little less potent than allylbenzene.

In a very sophisticated experimental design **Campo et al. (2007)** studied the effect of aromatic solvents on the middle ear *(mediated by the middle ear muscles)* and inner ear *(by recording cochlear microphonic potential during exposure to noise)* acoustic reflexes elicited by the auditory efferent system. These reflexes are mediated by the auditory efferent system and protect the inner ear against acoustic insults by noise exposure. The solvents investigated were administered by intracarotid bolus injection to rats (100 or 200 µl over 30 or 60 sec at a concentration of 101.2 mM) and the protective reflexes during exposure to noise were measured before, during and after injection of the solvent. Thereby it was demonstrated that ethylbenzene after intracarotid administration inhibits the action of the middle ear reflex. This finding has no direct relevance for the ototoxic action per se but it may have implications for the interaction of noise and ethylbenzene.

Summary and discussion on Repeated dose toxicity

General toxicity

Inhalation route
A number of conventional toxicology studies on rats, mice and rabbits with repeated inhalation exposures are available. Few data of deficient quality were reported on inhalation exposure in rhesus monkeys and guinea pigs.

Clinical signs indicative of irritation were reported in rats starting at about 400 ppm with a NOEC of 100 ppm **(Cragg et al., 1989; Biodynamics, 1986)** with similar signs of irritation in mice at 400 ppm **(Biodynamics, 1986)**. Slight body weight depressions in rats started at about 750 ppm **(Stott et al.,1999; 2003; NTP, 1992)** in studies up to 3 months of duration, while after an exposure period of 2 years **(NTP, 1999)** such slight effects on body weights were already observed at 250 ppm in rats but not in mice. Mortality was increased after 4 days of exposure in rats at 2400 ppm and in mice at 1200 ppm **(Biodynamics, 1986)** while after a 2-year exposure there was a decreased survival of male rats at 750 ppm (but not in female rats or mice), most probably related to chronic progressive nephropathy **(NTP, 1999).**

The most consistent effect is an increase in liver weight of rats and mice without histopathological alterations by standard procedures in studies with duration up to 3 months **(Cragg et al., 1989; Biodynamics, 1986; Stott et al., 1999;2003; Wolf et al., 1956; NTP, 1992,**

Stump, 2004). Such effects were also noted in studies dealing with fertility (cf section 4.1.2.9), e.g. in parental animals of a 1- **(Stump, 2003)** and 2-generation study **(Stump, 2005)** and in a non guideline investigation on female fertility **(Andrew et al., 1981; Hardin et al., 1981)**. Liver changes most probably are related to enzyme induction as has been demonstrated by several authors **(Stott et al., 1999; 2003; Elovaara et al., 1985; Toftgard, Nilsen, 1982)**. This is supported by a proliferation of smooth endoplasmatic reticulum **(Elovaara et al., 1985)**. Increase in kidney weight to be indicated also, to be linked to the re-evaluation of Hard. A detailed histopathological reevaluation of the kidneys of rats exposed for 3 months in the NTP (1992) study revealed a dose-related increase of the severity of chronic progressive nephropathy (CPN) in male rats at 750 and 1000 ppm, but not in females. In addition, an increase in incidence of hyaline droplets was observed at these dose levels in male rats **(Hard, 2002)**. Chronic progressive nephropathy in rats has no specific relevance for humans and liver enzyme induction is not to be considered as a toxicological relevant effect for human risk assessment. The NOEC for all of these findings is 100 ppm and the NOAEC in the most relevant 90 day **NTP study (1992)** for extrapolation to humans is 1000 ppm (4.74 mg/l).

Similar organ-related systemic toxicity was observed after 2 years of exposure **(NTP, 1999)**. In rats a detailed histopathological reexamination again revealed chronic progressive nephropathy at 750 ppm markedly in male and modestly in female rats in all treated groups. There was a high incidence of severe CPN with kidney alterations that may lead to renal failure. Cystic degeneration of the liver was increased in 750 ppm males, but the biological significance in the absence of other hepatotoxic changes is unclear. In mice there was a spectrum of non neoplastic liver changes for both males and females. Histopathological findings related to lung tumor formation will be reported in the section on carcinogenicity. Hyperplastic changes were also reported for the thyroid in males and females at 750 ppm and for the pituitary in females starting at 250 ppm. In summary, as chronic progressive nephropathy has no toxicological relevance for human risk assessment, the NOAEC for rats was 250 ppm in males and 750 ppm in females if small reductions (5-6%) in body weight (males at 250 ppm and females at all exposure levels) for females are not taken into account. In mice the NOAEC was 75 ppm for males and females.

In a study specifically designed to detect immunological effects no such findings were reported for female rats after an exposure to 500 ppm over 28 consecutive days **(Stump, 2004).**

Oral route
Data obtained after oral exposure support the main findings of inhalation studies. The most comprehensive investigation is that of **Mellert et al. (2004, 2007)** exposing rats by gavage at levels of 75, 250 and 750 mg/kg bw/d over 90 days. Body weight was significantly decreased in males at 750 mg/kg bw/ d. Liver and kidney weights were increased at 250 and 750 mg/kg bw/d accompanied by slight centrolobular hypertrophy, those in the kidney are characterised as chronic progressive nephropathy and accumulation of male specific protein α-2μ-globulin. In addition at the two top dose levels there was a slight increase of serum alanine aminotransferase and gamma-glutamyltransferase in males and signs for a minimal regenerative anemia. Increases in total bilirubin and mild increase in hemosiderosis (observed for 1-phenylethanol only) might indicate on a mild hemolysis as possible cause of anemia. Overall, the NOAEL was 75 mg/kg bw/d.

No information is available for systemic toxicity after repeated **dermal exposure**.

Organ-specific toxicity (effects on the nervous system)

In experimental animals ethylbenzene exposure induced various effects on the central nervous system:

A. Effects on neurotransmitters
   Two studies reported modulation of neuronal transmitters at 2000 ppm or 750 ppm, respectively, over a few days. Furthermore, at 2000 ppm a decrease of prolactin in serum was observed. However, the significance of these effects is unclear.

B. Depressive and narcotic effects
   Although depressive or narcotic effects have been observed in humans by aromatic solvents in general, the animal data are less consistent showing transient effects for ethylbenzene only at very high acute exposures.

   In a 90 day oral guideline study specifically designed for the detection of neurotoxic effects dose levels up to 500 mg/kg bw/d did not lead to findings indicative of neurotoxicity in rats.

   The results of in vitro tests indicate that ethylbenzene may affect the regulatory functions of astrocytes.

   It may be suspected that ethylbenzene could lead to lesions in the central nervous system similar to other organic solvents. But no indications for such morphological alterations of the central nervous system have been reported in other animal experiments including the 2-year bioassay with exposures up to 750 ppm. But because of the limited reliability of standard H&E staining for detecting neurological disorders this is not sufficient proof for the absence of minor morphological abnormalities.

C. Ototoxicity
   Ethylbenzene leads to ototoxicity in rats. Persistent hearing loss in the mid-frequency range was confirmed in a series of auditory tests in rats (sound-evoked electrical responses, otoacoustic emissions, behavioral auditory tests) corresponding to a concentration dependent death of sensory cells (outer hair cells - OHC) in the upper basal and middle turns of the cochlea. Outer hair cell death determined by histopathology is the most sensitive endpoint for auditory effects **(cf Cappaert et al., 2000; Gagnaire et al., 2007)**. For death of outer hair cells 200 ppm was a LOEC and the NOEC was calculated to be 114 ppm (95% confidence limit) **(Gagnaire et al., 2007)**. With increasing ethylbenzene concentrations the other endpoints for ototoxicity become affected, too, and OHC death spreads over the frequency range in the cochlea and from row 3 to row 2 and 1 of the outer hair cells. OHC death as confirmed by histopathology indicates that hearing loss is irreversible. This is substantiated by **Cappaert et al. (1999)** since hearing loss did not change between 1 and 4 weeks post exposure to 800 ppm over 5 days. On the other hand, electrophysiological investigations did not show any further deterioriation of auditory function when exposure to 400 ppm was prolonged from 4 over 8 up to 13 weeks **(Gagnaire et al., 2007)**. Auditory loss remained at the same level following additional 8 weeks of recovery. This might indicate that OHC loss at this concentration was already complete at week 4 and was irreversible.
   Comparable ototoxic effects were observed in rats that were repeatedly receiving etyhlbenzene via the oral route **(Gagnaire and Langlais, 2005)**, which is in line with the experience gained from other aromatic solvents (e.g., styrene). Since only one concentration of ethylbenzene was examined, (900 mg/kg, 5 d/wk/2 weeks), a N/LOAEL could not be estimated. Other oral tests, also those following OECD standard study designs, did not include specific auditory examinations.

By comparison between behavioral and electrophysiological methods **Cappaert et al. (1999)** concluded that ethylbenzene primarily exerts its effects on the peripheral part of the auditory system.

In a comparative study with different aromatic solvents given orally by gavage ethylbenzene belonged to the most potent ototoxicants together with styrene by means of OHC death. The potency was higher than that of toluene and p-xylene. **Gagnaire et al. (2007)** found that ototoxicity in mixed exposure to xylenes and ethylbenzene mainly depends on the concentration of ethylbenzene and auditory loss was higher in combined exposure than after single exposure to same ethylbenzene concentrations.

Experiments with combined exposure to noise and ethylbenzene indicated to a synergistic effect of both **(Cappaert et al., 2001)**. In the other experiment of **Fechter et al. (2007)** a very high exposure to the solvent mixture used (660 ppm ethylbenzene + 400 ppm toluene) (without noise) did not result in adverse hearing effects in contrast to all the other studies with ethylbenzene, while hearing loss was reported for the combination of this solvent mixture with noise exposure of 93-95 dB. Actually no explanation for this unexpected result could be given. As synergistic effects were also known from mixed exposure to other aromatic solvents, the outcome of this study appears questionable.

By comparison with rats, guinea pigs are very insensitive against ethylbenzene-induced ototoxicity **(Cappaert et al., 2002)**. The low sensitivity of guinea pigs was attributed to the low blood concentration of ethylbenzene in comparison to that of rats.

The critical question is whether ototoxicity of ethylbenzene in humans is best comparable to that in the rat rather to that seen in the guinea pig. Until the exact position of humans within the inter-species ranking of susceptibility to ethylbenzene-induced ototoxicity is actually known, data from the rat are to be taken as relevant for humans. This assumption is supported by a number of reports on hearing deficits in humans occupational exposed to organic solvents or from people after solvent abuse (for review cf Risk Assessment Reports on toluene and styrene).

Ethylbenzene belongs to the most ototoxic aromatic solvents, its potency being comparable to that of styrene but higher than those of toluene or p-xylene. Comparing rat data on the lowest effective concentrations for ethylbenzene and toluene, the risk of ototoxicity is expected to be higher for ethylbenzene.

Irreversible damage of auditory function and of sensory cells of the cochlea is a serious health damage. After 13 weeks of exposure minimal effects were still observed at 200 ppm (0.88 mg/l) and the NOEC was extrapolated to 114 ppm (0.5 mg/l). Thus, the classification limit for R48/20 (0.25 mg/l) is formally not attained. Nevertheless, such a classification is proposed taking into account that the experimental ototoxicity of ethylbenzene is comparable to that of styrene and less than that of toluene and that both of these chemicals have been assigned R48/20.

4.1.2.6 Mutagenicity

In vitro tests: bacterial gene mutation and yeast

Three bacterial gene mutations investigations and two mitotic recombination studies in yeast have been conducted with ethylbenze (see Table 4.1.2.6-1).
In the first of the studies 17 substances, including ethylbenzene, were examined for the ability to produce mutagenicity in 5 strains of *Salmonella typhimurium*, (strains TA1535, TA1537, TA1538, TA98 and TA100) and mitotoic recombination in *Sachchromyces cerevisiae* D3 **(Stanford Research Institute, SRI Project Number LSC-4378, 1976)**. Treatment with ethylbenzene both in the presence and absence of S-9 mix gave no evidence of mutagenic effects. Bacterial gene mutation tests with ethylbenzene reported by **Shell Oil Co. (1981)** and **Zeiger et al., (1992)** were also negative at doses up to 2000 ug/plate with and without S-9 mix. A standard plate test and a preincubation test were conducted; toxic effects were induced in both assays.
A yeast test (mitotic recombination) was negative with and without S-9 mix at doses up to 5000 ug/plate **(Shell Oil Co., 1981)**. No toxic effects were induced.

In vitro tests: Mammalian cell systems

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In an *in vitro* mouse lymphoma mammalian mutation assay conducted by **McGregor et al. (1988)** treatment with ethylbenzene produced a weakly positive response at the highest tested dose of 80 µg/ml (done only without S-9 mix). The positive result was reproducible but paralleled by strong cytotoxicity.
The ability of ethylbenzene to induce mutations at the thymidine kinase locus was examined in L5178Y mouse lymphoma cells both in the presence and absence of rat liver S-9 mix. Weak increases in frequency of mutant colonies were only observed at the highest doses tested with S-9 mix (825 and 900 µg/l) which were also strongly cytotoxic. Without S-9 mix ethylbenzene was not mutagenic at doses up to 100 µg/ml; higher doses were strongly cytotoxic. The negative results at non-toxic concentrations were confirmed in further experiments with and without S-9 mix **(RCC, 2000)**.

An *in vitro* chromosomal aberration study carried out as part of the National Toxicoloical Program examining the toxicology of ethylbenzene **(NTP, 1992)** reported that CHO cells treated with ethylbenzene in a dose range of 75-125 µg/ml showed an equivocal increase of chromosomal aberrations in the absence of S-9 mix. The effect was marginal and without statistical significance. In the presence of S-9 mix no evidence of clastogenic effects was seen at all dose levels tested. No data on toxicity were provided.

An *in vitro* chromosomal aberration test with a cell line derived from rat liver, conducted without S-9 mix only, was negative at doses up to 100 µg/ml **(Shell Oil Co., 1981)**. Higher doses had "inadequate cell survivals".
The result of an *in vitro* micronucleus test in SHE cells without metabolic acitvation was positive at all tested doses ranging from 25 up to 200 µg/ml **(Gibson et al., 1997)**. The 2- to 3-fold increases of micronucleated cells over the negative control were without clear dose-dependency and paralleled by moderate toxicity.

An investigation to examine the induction of sister chromatid exchanges (SCE) in CHO cells was negative up to 150 µg/ml with S-9 mix and up to 125 µg/ml without S-9 mix. The highest doses induced precipitations; data on toxic effects were not given **(NTP, 1992)**.

In-vitro-tests: Cell transformation test

Kerckaert et al. (1996) studied cell transformation in Syrian hamster embryo cells (SHE cells) treated with ethylbenze, (in absence of S-9 mix), for 24 hrs or 7 days. The authors reported that 24 hr exposure at doses up to 500 µg/ml, (causing cytotoxicity), failed to produce an increase in cell transformation while a 7-day exposure with doses between 150 to 200 µg/ml (causing cytoxicity), caused an increase in morphological transformation frequencies: highest effect 1.38% as compared to 0.59% in the negative control

In-vivo-tests: Micronucleus tests (Table 4.1.2.6-5)

No increase in the frequency of micronuclei was measured in bone marrow cells of mice which had received two intraperitoneal injections of ethylbenzene at doses from 109 up to 322.5 mg/kg bw **(Mohtashamipur et al., 1985)**. The doses were injected with an interval of 24 hrs. Information on local cytotoxicity or toxic signs were not provided in the report but the highest dose corresponded to 70% of the LD_{50}.
Another *in vivo* micronucleus test examining bone marrow cells obtained from mice exposed to ethylbenzene by inhalation, (doses 500 to 1,000 ppm), also produced a negative result. (NTP, 1992). The highest dose, (1,000 ppm), was reported to have produced local cytotoxicity as indicated by the PCE:NCE ratio. Information about other toxic effects were not provided in the report.

In-vivo-tests: Test for induction of unscheduled DNA synthesis (Table 4.1.2.6-6)

Ethylbenzene was negative for induction of UDS in rat liver after 6-h inhalative exposure of up to 1000 ppm in males and up to 750 ppm in females **(CTL, 2000)**. The highest tested doses, which are in accordance with the maximum tolerated concentrations, induced clinical signs in both genders; the local cytotoxicity was described to be "not excessive".

Conclusion
Ethylbenzene produced consistently negative results in bacterial gene mutation tests and in the yeast assay on mitotic recombination. In mouse lymphoma mammalian mutation assays a weak positive response was reported but only at doses with strong cytotoxicity. No clear conlusion can be drawn regarding *in vitro* chromosomal aberration. Without S-9 mix there were equivocal increases in chromosomal aberration frequencies and micronuclei in CHO and SHE cells, respectively, or a negative result in a rat liver cell line. With S-9 mix ethylbenzene did not cause chromosomal aberrations in CHO cells. An *in vitro* SCE test was clearly negative with and without S-9 mix.
In vivo, ethylbenzene was clearly negative in two micronucleus assays and in an mouse liver UDS assay.
In conclusion, on the basis of various mutagenicity tests *in vitro* and *in vivo*, there is currently no relevant indication that ethylbenzene is a germ cell mutagen. Based on the available data ethylbenzene should not be classified as a germ cell mutagen.

Table 4.1.2.6-1. In vitro tests: bacterial gene mutation and yeast

Test system	Concentration range	Result	Toxicity	Remarks	Reference

	with S-9 mix	without S-9 mix				
Gene mutation, Salm. typh. TA 97, TA 98, TA 100, TA 1535	10 - 1000 µg/plate	10 - 666 µg/plate	negative	with S-9 mix: 1000 µg/plate without S-9 mix: 333 µg/plate and higher	preincu-bation test	Zeiger et al., 1992
Gene mutation, Salm. typh. TA 98, TA 100, TA 1535, TA 1537, TA 1538; E. coli WP2; E. coli WP2 uvrA	0.2 - 2000 µg/plate	0.2 - 2000 µg/plate	negative	without S-9 mix: 2000 µg/plate	standard plate test	Shell, 1981
Gene mutation, Salm. typh. TA 97, TA 98, TA 100, TA 1535	10 - 1000 µg/plate	10 - 1000 µg/plate	negative	No information provided	standard plate test	Stanford Re-search Instiute 1976
Gene conversion; Sacch. cerevisiae JD1 Gene conversion; Sacch. cerevisiae D3	10 - 5000 µg/ml 10 - 1000 µg/plate	10 -5000 µg/ml 10 - 1000 µg/plate	negative negative	no toxic effects No information provided		Shell, 1981 Stanford Re-search Instiute 1976

Table 4.1.2.6-2. In vitro tests: mouse lymphoma assays

Test system	Concentration range		Result	Description of result	Reference
	with S-9 mix	without S-9 mix			

Mouse lymphoma assay (L5178Y cells; Tk-locus)	25 - 900 µg/ml	8.6 - 100 µg/ml	Positive	weakly positive with S-9 mix only at doses with strong cytotoxicity with S-9 mix: weak positive in two out of three tests at the highest tested doses of 825 µg/ml (1. test) or 900 µg/ml (2. test); increase of mutant frequency 4.2-fold (1. test) or 2.4-fold (2. tests); strong toxicity at these doses: relative total growth 17.9 % (1.test) or 9.5 % (2. test); a third test was negative up to 150 µg/ml (toxicity: 33.8% relative total growth) without S-9 mix: overall negative; only in one out of three tests increase of mutant frequency at the highest tested doses of 34.4 µg/ml (2.2-fold) and 68.5 µg/ml (4.6-fold); strong toxic effect at 68.5 µg/ml: 27.5 % relative total growth; two tests were negative up doses of 100 ug/ml; toxic effects (relative total growth) at 90 (up tp 39.4 %) and 100 µg/ml (up to 25.0 %)	RCC, 2000
Mouse lymphoma assay (L5178Y cells; Tk-locus)	not done	10 - 80 µg/ml	positive	weak positive effect only at the highest tested dose of 80 µg/ml; reproducible effect; increase of mutant frequency 9.8-fold (1. test) and 4.8-fold (2. test); strong toxicity at 80 µg/ml: relative total growth 24.0 % (1. test) and 12.5 % (2. test)	Mc Gregor et al., 1988

Table 4.1.2.6-3. In vitro tests: chromosomal aberrations and micronuclei

Test system	Concentration range		Result	Description of result	Reference
	with S-9 mix	without S-9 mix			

Chromosomal aberrations, CHO cells		75 - 125 µg/ml	equivocal	without S-9 mix: equivocal treatment/sampling: 2-3h / 10.5 h dose-range (µg/ml): (neg. cont.) 75 100 125 aberrant cells (%; excl. gaps): 3.0 1.0 3.0 5.0	NTP, 1992
	75 - 125 µg/ml		negative	with S-9 mix: negative treatment/sampling: 2h / 10.5 h toxicity: with and without S-9 mix no data for the tested dose-range (at 150 µg/ml apparently entirely toxic)	
Chromosomal aberrations, rat hepatocytes	not done	25 - 100 µg/ml	negative	treatment/sampling: 24h / 24h toxicity: no data for the tested dose-range ("cell survival was inade-quate" > 100 µg/ml)	Shell, 1981
Micronuclei, SHE cells	not done	25 - 200 µg/ml	positive	positive at all tested doses treatment/sampling: 24h / 24h dose-range (µg/ml): (neg. cont.) 25 50 100 200 micronucleated cells (%): 2.8 5.4 5.8 7.1 6.0 toxicity (rel. cell count, %): 63 58 49 42	Gibson et al., 1997

Table 4.1.2.6-4. In vitro tests: test for induction of sister chromatid exchanges (SCE)

Test system	Concentration range		Result	Toxicity	Remarks	Reference
	with S-9 mix	without S-9 mix				

CHO cells	125 - 150 µg/ml	75.5 - 125 µg/ml	negative	no data	precipitations with and without S-9 mix at the highest tested doses	NTP, 1992

Table 4.1.2.6-5. In vivo tests: micronucleus tests with mice

Test system	Doses	Exposure regimen	Sampl. times	Result	Local cytotoxicity	Remarks	Reference
NMRI mice; bone marrow erythrocytes	109 - 322.5 mg/kg bw	2 x i.p. with an interval of 24 h	30 h	negative	no data	use of males only 645 mg/kg = 70% of LD_{50}	Motashamipur et al., 1985
$B6C3F_1$ mice; bone marrow erythrocytes	500 - 1000 ppm	inhalative for 13 weeks (6 h per day, 5 days per week)	after 13 weeks	negative	at 1000 ppm	use of males and females no data on general toxicity	NTP, 1992

Table 4.1.2.6-6. In vivo tests: test for induction of unscheduled DNA synthesis (UDS)

Test system	Doses	Exposure regimen	Sampling times	Result	Local cytotox.	General toxicity	Remarks	Reference

B6C3F1 mice; liver	500 - 1000 ppm (males) 375 - 750 ppm (females)	6 h inhalative	directly after exposure	negative	no "excessive" cytotox.	highest tested dose = MTC (based on a preliminary study) clinical signs in both gender at the highest tested dose	autoradiography	CTL, 2000

4.1.2.7 Carcinogenicity

Inhalation studies (see Tables 4.1.2.8-1 and -2)

- In two NTP cancer bioassays on rats and mice (**Chan et al., 1998; NTP, 1999**; the following summary was overtaken and modified from the SIDS Initial Assessment Report, 3/2002), groups of 50 male and 50 female F344/N rats were exposed by inhalation to 0, 75, 250 or 750 ppm ethylbenzene (0, 0.33, 1.1, 3.3 mg/l), 6 hrs/day, 5 days/week for 104 weeks. Individual-housing animals were observed twice daily, clinical findings were recorded monthly, body weights were recorded initially, weekly for the first 13 weeks and monthly thereafter. Necropsy and complete histopathologic examinations were performed on all surviving and spontaneously dying animals of the chamber control and exposed groups. Sections of gross lesions, tissues masses and of more than 40 tissues/organs were examined. From the kidneys, four step sections in addition to the single standard section were prepared and examined.
  The survival rates in all male dose groups were decreasing with increasing dose, at 750 ppm the survival in males was significantly reduced, while in females survival was increased (not significant). No adverse clinical findings were attributed to the ethylbenzene exposure. In males exposed to 250 or 750 ppm, body weights were reduced (up to 5 and 15%, respectively) from week 20 to the end of the study. In females all exposed groups weighed up to 5% less than the controls during the second year, but there was no dose-response. In both males and females exposed to 750 ppm, but not to 75 or 250 ppm, there was increased renal tubule hyperplasia and increased severity of nephropathy (Table 4.1.2.8.A). A higher incidence of transitional cell hyperplasias (renal pelvis) was present in the 750 ppm male group compared to the control group (68% vs. 24%). In this male group, there was an increased incidence of renal tubule adenomas and combined renal tubule adenoma/carcinomas based on combined original kidney sections and additional step-sectioning of the kidneys (Tumor data in Table 4.1.2.8 A). The incidence of renal tubule carcinomas alone was not significantly elevated. In females, no renal tubule carcinomas were found. However, at 750 ppm, there was an increased incidence of renal tubule adenomas. The preneoplastic finding attributable to the tumors of the renal tubule was tubular hyperplasia observed with significantly increased incidences in male and female groups

exposed at 750 ppm. This finding was distinguished from regenerative epithelial changes commonly seen as a component of chronic progressive nephropathy. In males exposed to 750 ppm, there was a slight, but significant, increase in the incidence of testicular interstitial cell adenoma; incidences of bilateral interstitial cell adenomas were also significantly increased, whereas interstitial cell hyperplasias were significantly lower in 750 ppm male rats.

Identified NOAEC for non-neoplastic and non-preneoplastic toxicity was 250 ppm for the rat.

- The kidney slides from this study, as well as the NTP 13-week study were re-examined (**Hard, 2002**). It was concluded that the apparent increase in renal tumors was strongly associated with chronic progressive nephropathy (CPN), a spontaneous age-related disease of rodents with no identical counterpart in humans. CPN occurred in the 750 ppm animals, markedly so in the male rats, and modestly so in the females. In addition, there was a high incidence of high-dose *male* rats that had end-stage CPN, a terminal condition where the kidneys are so morphologically altered that renal failure (as well as secondary hyperparathyroidism) occurs and that was the most plausible cause of deaths. Although there was some evidence of a dose-related increase in hyaline droplet formation in the 13-week NTP study, it was not considered to be of the magnitude indicative of an α2u-globulin associated mechanism of renal carcinogenesis **(Hard, 2002)**.

- B6C3F1 mice (50 mice/sex/group) were exposed by inhalation to 0, 75, 250 or 750 ppm ethylbenzene (0, 0.33, 1.1, 3.3 mg/l), 6 hrs/day, 5 days/week for 103 weeks. Exposure to ethylbenzene had no meaningful effect on survival or body weight gain. In the lung at 750 ppm, male rats exhibited increased alveolar epithelial metaplasia, but there was no increase in alveolar hyperplasia. In females, no significant increase in the incidence of either hyperplasia or metaplasia was observed. There was increased incidence of alveolar/bronchiolar adenomas and of combined alveolar/bronchiolar adenoma/carcinomas in male mice exposed to 750 ppm. Incidences of lung tumors at 75 and 250 ppm were not significantly different from the control incidences. There were, however, increased incidences of centrilobular hypertrophy, syncytial alteration, and necrosis in the liver of males exposed to 750 ppm ethylbenzene. In female mice exposed to 750 ppm, there was a significantly increased incidence of hepatocellular adenomas and combined hepatocellular adenoma/carcinomas. The incidence of tumors in females exposed to 75 and 250 ppm were not significantly different from the control incidences. In males there was no increase in liver tumors at any exposure concentration. Syncytial alteration was defined as greatly enlarged hepatocytes containing multiple nuclei, generally five or more, either randomly scattered throughout the liver lobule or with a tendency to cluster in centrilobular areas. Hepatocellular necrosis was evident as random single cell necrosis, generally of hypertrophied cells.
  There were also increased follicular cell hyperplasia in the thyroid gland in both the 750 ppm males and females; and hyperplasia in the pituitary gland in the 250 and 750 ppm females.
  Identified NOAEC for non-neoplastic and non-preneoplastic toxicity was 250 ppm for the mouse.

- The lung and liver sections of mice from the National Toxicology Program (NTP) two-year bioassay were re-evaluated by **Brown** (**2000, cited from SIDS document, draft version 3/2002)**. This re-evaluation revealed an increased incidence of male and female mice

of the 750 ppm exposure group with decreased eosinophilia of the terminal bronchiolar epithelium. Also, a dose-related increased incidence in multifocal hyperplasia of the bronchiolar epithelium with extension to the peribronchiolar alveolar epithelium was observed in all male treated groups and mid- and high-exposure females. The author noted that the necrotic hepatocytes in the high-dose males were usually that of a coagulation-type necrosis of single or small groups of cells, usually the enlarged, hypertrophied centrilobular hepatocytes. The morphology of this necrosis was histomorphologically different from apoptosis. Also the syncytial cells were not the predominant cell type with necrosis.

Table 4.1.2.7-1

Incidences of neoplasms, preneoplastic and nonneoplastic lesions in rats in the 2-year inhalation study (extracted table from NTP, 1999, Chan et al. 1998)

Organ	Male				Female			
	Control	75 ppm	250 ppm	750 ppm	Control	75 ppm	250 ppm	750 ppm
	50	50	50	50	50	50	50	50
Kidney								
Nephropathy [a]	47 (2.3[b])	43 (2.4)	47 (2.3)	48 (3.5**)	38 (1.3)	42 (1.6*)	43 (1.7**)	46 (2.3**)
Renal Tubule Hyperplasia[c]	11 (2.0)	9 (2.3)	11 (2.1)	**23**** (2.5)	1 (1.0)	2 (1.0)	4 (2.2)	**10**** (1.8)
Renal Tubule Hyperplasia, Oncocytic[c]	2 (3.0)	3 (2.3)	0	1 (2.0)	0	0	0	0
Transitional epithelial hyperplasia [a]	12	14	15	34	0	0	0	**2**
Renal Tubule Adenoma[c,d]	0	5	7	**20****	0	0	1	**8****
Renal Tubule Carcinoma[c,e]	0	0	1	3	0	0	0	0
Renal Tubule Adenoma or Carcinoma[c]	3	5	8	21**	0	0	1	8**
Oncytoma[c]	0	1	1	2	0	0	0	0
Testis								
Interstitial cell hyperplasia	14	19	12	8*				
Interstitial cell adenoma[f, g]	36	33	40	**44****				
Bilateral adenomas	27	23	32	40**				

[a] Standard evaluation of the initial single section
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Combined incidences of single sections and 4 step sections
*, ** Significantly different (*P≤0.05, **P≤0.01) from the chamber control group by the logistic regression test (incidence) or by the Mann-Whitney U test (severity)
[d] Historical incidence (of the test laboratory) for 2-year inhalation studies with chamber male control groups (mean ± standard deviation): 6/652 (0.9% ±1.3%); range, 0-4%
[e] Historical incidence in male groups 0/652
[f] Historical incidence (of the test laboratory) for 2-year inhalation studies with chamber male control groups (mean ± standard deviation): 450/655 (68.7% ± 8.7%); range 54%-83%
[g] Overall rates: number of animals with neoplasm per number of animals with testis examined microscopically

Table 4.1.2.7-2 **Incidences of neoplasms and nonneoplastic lesions in mice in the 2-year inhalation study (extracted table from NTP, 1999)**

Organ	Male				Female			
	Control	75 ppm	250 ppm	750 ppm	Control	75 ppm	250 ppm	750 ppm
No. of animals	50	50	50	50	50	50	50	50
Lung								
Alveolar Epithelium, Hyperplasia[a]	1 (1.0)[b]	5 (2.6)	2 (1.5)	4 (2.0)	0	1 (2.0)	3 (2.0)	1 (3.0)
Alveolar Epithelium, Metaplasia	0	1 (1.0)	2 (1.0)	**6*** (1.2)	0	0	0	1 (2.0)
Alveolar/Bronchiolar Adenoma	5	9	10	**16****[c]	4	4	5	8[e]
Alveolar/Bronchiolar Carcinoma	2	1	5	3 (1.2)	0	2	0	0
Alveolar/Bronchiolar Adenoma or Carcinoma	7	10	15	**19**** [d]	4	6	5	8[f]
Liver								
Hepatocyte, Hypertrophy[a]	1 (1.0) [b]	0	0	**17****				
Hepatocyte, Necrosis	1 (1.0)	1 (2.0)	3 (1.3)	**10**** (1.8)				
Hepatocyte, Syncytial Alteration	0	5 (1.0)	**8**** (1.4)	**23**** (1.1)				
Eosinophilic Focus					5 (1.8)	7 (1.4)	6 (1.5)	**22**** (2.0)
Hepatocellular Adenoma[g]	22	24	34	34	6	9	12	**16***
Hepatocellular Carcinoma	34	16	22	20	7	4	3	12
Hepatocellular Adenoma or Carcinoma[h]					13	12	15	**25***
Thyroid								
Follicular Cell Hyperplasia	21	21	29	32	18	23	25	35
No. of animals	44	45	45	47	48	49	47	49
Pituitary								

Pars distalis, Hyperplasia	1	1	1	0	10	12	23	22

*, ** Significantly different (*P≤0.05, **P≤0.01) from the chamber control group by the logistic regression test

[a] Number of animals with lesion

[b] Average severity grade of lesions in affected animals: 1= minimal; 2=mild; 3=moderate; 4=marked

[c] Historical incidence for 2-year inhalation studies with chamber control groups (mean ±standard deviation): 141/946(14.9%±7.0%); range 6%-36%

[d] Historical incidence: 205/947 (21.7%±8.0%); range 10-42%

[e] Historical incidence: 61/939 (6.5%±3.2%); range 0-14%

[f] Historical incidence: 97/939 (10.3%±8.0%); range 10-42%

[g] Historical incidence: 114/937 (12.20.3%±9.7%); range 0-40%

[h] Historical incidence: 200/937 (21.3%±11.9%); range 3-54%

Oral studies

- Within a series of cancer bioassays on aromatics, 40 male and 40 female Sprague-Dawley rats received by gavage administration a dose of 500 mg/kg ethylbenzene in olive oil on 4-5 days per week for 104 weeks and were then kept under observation until spontaneous deaths **(Maltoni et al., 1985)**. Olive oil alone were given to 50 male and 50 female control animals. Animals were 7 weeks old at the study begin. After 141 weeks, ethylbenzene induced an increase in total numbers of malignant tumors (35% in male and 45.9% in female rats of dosed groups versus 26.7% in male control and 22.4% in female controls), the types, sites and incidences of tumors were not specified. No thymomas occurred in the 500 mg groups and in control groups. The incidence of lymphoreticular neoplasms were reported to be 8.1% in male rats and 0% in female rats at 500 mg/kg in comparison to 6.7% in male control and 2% in female control animals. The study design and results were very briefly reported in this reference, contained no data on statistical evaluation, no further details than cited here were available.

Carcinogenicity – Animal data

Summary and discussion

In carcinogenicity inhalation studies ethylbenzene was carcinogenic in F344 rats and B6C3F1 mice **(NTP, 1999)**. Exposure to 750 ppm ethylbenzene resulted in increased tumor rates in male (kidney and testis) and in female rats (kidney), in male mice (lung) and in female mice (liver).

Since genotoxicity is not considered to be the responsible mode in the initiation of tumors, other (non-genotoxic) mechanisms may be active in ethylbenzene carcinogenicity:

Kidney tumors/male and female rats

Hypothesis: Tubular cell adenomas in male and female rats and tubular cell carcinomas in male rats were discussed to be associated to chronic progressive nephropathy (CPN), a spontaneous lesion of the old-aged rat **(Hard, 2002).**

Pros:

- CPN is commonly observed spontaneous disease in F344 rats, progressing with age and with preference in male rats **(Montgomery and Seely, 1990)**.
- There was a significant ethylbenzene-related increase in mean severity of CPN in high dose male and female rats, and a nonsignificant increase in incidence in high dose female rats.
- Higher grades of mean severity including secondary effects of CPN were evident in males than in females of high dose groups. This was parallel with the higher tumor incidence in males of the high dose group compared to that in the female high dose group. This supports an association of CPN and tumor growth.
- Chemically-exacerbated nephropathy can occur in either sex of F344 rats, but is more common in males **(Chandra and Frith, 1993/94)**. This lesion can result in shorter life spans in dosed animals **(Montgomery and Seely, 1990).**

- The renal tubular hyperplasia was identified at increased rates in male and female rats exposed to 750 ppm and was probably the preneoplastic lesion in tumor development. The increase in kidney weight seen in subacute-subchronic repeat-dose studies possibly reflect tubular hyperplasia or hypertrophy or both, but CPN can also induce increase in kidney weight. The exact cause of altered weight e.g. changes in cytoplasmic and subcellular structures were not examined by appropriate methods.
- In rats, ethylbenzene has no direct toxic effect on renal tubular cells up to 750 ppm after 2-year exposure and up to 1000 ppm after 92-98 days of treatment. This also suggests that the increase in mean severity grades of CPN reflects a ethylbenzene-related exaceration of a spontaneous lesion with uncertain toxicological significance.
- The absence of kidney tumor in the mouse gives support on the assumption of a species-specific phenomenon. Rats with moderate to severe graded CPN may differ significantly in their response to xenobiotics from rats unaffected by this lesion.

Cons:
Some uncertainty remains on the postulated causal relationship of tumor induction with the increase in mean severity of CPN is unknown:

- In male rats, the incidence of CPN did not increase with dose. It was already high in control male rats (94% in males vs. 96% in males rats exposed to 750 ppm). Being already at a very high level of incidence, an increase in tumor response could not be associated to the increase in incidence of CPN.
- Uncertainty remains on the possible outcome in a carcinogenicity rat study on ethylbenzene using a strain with lower spontaneous rate of CPN (e.g., in Wistar rats).
- To our knowledge from literature, there is no report that spontaneous CPN in non-treated animals is associated to significantly increase in spontaneous tumor rates in the kidneys.
- Hyperplasia of the renal tubule, the putative preneoplasia followed by tubular adenoma and carcinoma, was reported as a finding distinct from the foci of CPN with regenerative tubules. This raises the uncertainty about a causal relationship between CPN and renal cell tumors.

The cons arguments did not represent significant contradictions against the hypothesis. They are judged as some remaining uncertainties on the postulated mode of action. Taking all arguments mentioned above the rapporteur agree that ethylbenzene carcinogenic action on the rat kidney can be attributed to its mediation of the CPN. It is suggested that ethylbenzene enhances the development of CPN in F344 rats and thereby enhances a more rapid progression to renal tubular tumors.

There are some rat kidney tumors associated with ethylbenzene exposure that were oncocytomas. These are benign tumors that occur spontaneously at extremely low incidences in rats and humans and that are characterised by abnormal mitochondria. Ethylbenzene affected metabolism in liver mitochondria **(Michiewicz and Rzeczcki, 1988)**. However, an association of ethylbenzene-induced increased/suppressed enzyme activity in mitochondria with the development of oncocytomas is currently, to our knowledge, unknown. If new data on ethylbenzene are generated, a concern for a presumable health risk from this rare tumor type may raise and should not be overlooked.

Testicular tumors/male rats
The interstitial cell tumor is a common tumor type in control F344 rats if they live their natural life span. The increased rates at 750 ppm (88%) slightly exceeded that reported for historical controls of the laboratory (mean 69%, range 54%-83% **(NTP, 1999)**). Significantly increased rates in rats of the 750 ppm group support an association with ethylbenzene treatment. However, the relevance of this increase of tumor rates at such a high level of spontaneous incidence not accompanied by a similarly increased hyperplasia incidence remains equivocal: Ethylbenzene appeared to enhance its development in F344 rats, but not in mice.

Lung tumors/male mice
Alveolar/bronchiolar tumors are the most common lung tumor type occurring spontaneously or chemically induced in B6C3F1 mice.
In 750 ppm-exposed male mice, the incidences of alveolar/bronchiolar adenoma and adenoma or carcinoma (combined) were significantly greater than those in the control group but were within the historical control ranges of the laboratory **(NTP, 1999)**. In agreement to other literature data **(Dixon and Maronpot, 1991)** which reported spontaneous incidences of adenomas and carcinomas (combined) of 19 % in males and 7.3% in females of this strain, it seems questionable whether the lung tumors can be attributed to ethylbenzene. No significant increase in lung tumor rates was found in the female mice and in male and female rats.

Liver tumors/female mice
Similarly, there is uncertainty on the association of liver tumors in female mice with the ethylbenzene exposure. The occurrence of liver tumor in one sex of one species is a weak argument for a tumor with relatively high spontaneous rates in B6C3F1 mice **(Haseman et al., 1994)**. The incidences of hepatocellular adenoma and adenoma or carcinoma (combined) increased in exposed female mice only with a positive trend gaining significance at 750 ppm ethylbenzene, whereas in male mice all liver tumors were at comparable high levels without a treatment-related effect (16-34%). Again, the incidences in females did not exceed the historical control ranges of the laboratory. Early induction of liver tumors associated with higher and early mortality could support a chemical-induced tumorgenicity **(Maronpot and Boorman, 1982)**. Since increased tumor rates did not influence the survival of female mice, liver tumors in females of this bioassay are considered a late life process.

A possible role of increased levels of reactive oxygen species (ROS) produced by ethylbenzene-mediated induction of particular cytochrome P450 enzymes has been discussed **(Serron et al., 2000)**. For male F344 rats, increased levels of ROS, such as hydrogen peroxide, has been shown in liver microsomes after single ip injection of 10 mmol/kg ethylbenzene. No data on ROS generation are available for the mouse liver. Whether this mechanism contributes to toxicity and/or carcinogenic effect in experimental animals and its relevance for humans, is actually unknown.

Pars distalis hyperplasia/female mice
Ethylbenzene affected the hypothalamus-pituitary axis of neuroendocrine regulation. Pituitary hormones were shown to interact with the cytochrome P450 expression profile in ethylbenzene exposed animals. Hypophysectomized rats had lower CYP2C11 expression than intact rats **(Bergeron et al., 1998)** and growth hormone supplementation in hypophysectomized rats returned CYP2C11 protein levels towards to control levels **(Serron et al., 2001)**. Whether the

hyperplastic effect on the pituitary reflects is mediated by any of these findings, is currently unknown.

It is concluded, that:

- Long-term inhalation exposure to ethylbenzene was carcinogenic in F344 rats and B6C3F1 mice.
- A significant increase of tumor incidences has been observed in the kidneys (renal tubule adenoma and carcinoma), testis (interstitial cell adenoma), liver (adenoma and carcinoma) and lung (alveolar/bronchiolar adenoma and carcinoma).
- There was no concordance in carcinogenic response between rats and mice. Elevated rates of kidney tumors were seen in male and female rats. Each of other tumors occurred in one sex and in one species only.
- Genotoxicity data did not indicate a direct DNA damaging effect.
- The rapporteur concluded that sufficient evidence exists that kidney tumors in the male and female rats are associated with the high strain-specific incidence of chronic progressive nephropathy (CPN) that is unknown for humans.
- For tumors in the testis, liver and lung high or very high spontaneous rates occur in the mouse and rat strains used. Ethylbenzene may exert its carcinogenic action by enhancement of tumor development in genetically disposed animals or by reduction in latency periods in tumor development.
- Although the detailed mechanisms underlying the increases in tumor rates are presently not clarified, it appears likely that the mode of carcinogenic action of ethylbenzene possesses species and strain specificity.
- Therefore the toxicological significance and relevance to human health of these findings is uncertain.
- It appears unlikely from the data available that ethylbenzene poses a carcinogenic risk for humans exposed.
- The evidence is insufficient to fulfil the EU criteria for classification for carcinogenicity.

4.1.2.8 Toxicity for reproduction

4.1.2.9.1 Fertility impairment

Human data

No data available.

Animal data

A 1-generation rat reproductive toxicity study (the original study is not available to the rapporteur, the description was taken from the industry version of the RAR) was carried out by

Stump (2003, unpublished) as a pilot investigation for dose and exposure decisions for a subsequent 2-generation reproductive toxicity study. 20 Sprague-Dawley rats / sex / dose level were exposed to 0, 100, 500 and 1000 ppm by inhalation **(Faber et al., 2007)**. The minimal premating exposure period was 4 weeks for males and 2 weeks for females. Animals were paired on a 1:1 basis after treatment for at least 2 weeks. Mating was confirmed by the presence of a copulatory plug or presence of sperm in a vaginal smear (assigned gestation day 0). Females continued exposure from GD 0-20. Before delivery, 50 % of the F0 females were selected for an inhalation/gavage phase and the remaining females were assigned to inhalation only phase. Inhalation exposure in both phases was suspended from GD 21 through LD 4. One-half of the F0 dams were treated by gavage on postnatal day 1-4 at dose levels of 0, 90, 342 and 621 mg/kg bw/d (divided into 3 equal doses, approximately 2 hours apart). The oral dosages were calculated using the ethylbenzene PBPK model **(Tardif et al., 1997)** to produce equivalent doses to the inhalation exposures. After postnatal day 4, inhalation exposure was continued for F0 females until the day prior to sacrifice. On postnatal day 4, litter size was reduced to 10 pups per litter (5/sex where possible). The F1 offspring were potentially exposed to the test article in utero and through nursing during lactation until weaning. On lactation day 21, respectively on lactation day 28, pups were weaned and selected (one pup/sex/litter) for inhalation exposure to the same concentration of the test article as their dam beginning on postnatal day 22 or 29 and lasting through postnatal day 33. This approach served to investigate the feasibility of gavage dosing of dams during lactation days 1-4 and to determine whether to begin inhalation exposure of F1 weanlings on PND 22 or 29.
All F0 and F1 were observed twice daily for clinical signs, behavior and mortality and a more detailed examination was carried out weekly. Body weight for F0 animals was recorded weekly and 8 times for the dams during pregnancy. Each pup was subject to a detailed physical examination and body weight determination on postnatal days 1, 4, 7, 14, 21 and 28. All parental animals were subject to gross examination at sacrifice which was after mating for males and after weaning for females. For females that delivered the number of former implantation sites were recorded. For females that failed to deliver, non-gravid uteri were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. Organ weights were recorded for livers and kidneys. A gross necropsy was performed on all pups on PND 28 for unselected pups and on PND 34 for pups selected for further toxicity evaluation.
The mean exposure concentrations achieved were within +/- 1% of the target concentration.
Postnatal survival of F1 pups was slightly reduced from birth to postnatal day 4 in the 500 and 1000 ppm groups (inhalation phase) and in the 1000 ppm/621 mg/kg bw/day group (inhalation/gavage phase) compared to concurrent and historical controls. For F1 weanlings exposed from PND 22-33 several exposure-related deaths were observed in the 500 (1/9M) and 1000 ppm (2/9M) group (inhalation phase) and the F1 1000 ppm/621 mg/kg bw/day (inhalation/gavage) phase weanlings (3/9 M and 1/9F). No deaths were noted in the F1 weanlings exposed to ethylbenzene beginning on postnatal day 29 (postnatal days 29-33) at any dose level. In F1 weanlings exposed from PND 22-33 exposure-related clinical signs were observed one hour following exposure to 500 ppm, 1000 ppm or 1000 ppm/621 mg/kg and included labored respiration, eyelids half-closed, prostration, righting difficulty, rocking, lurching, and swaying while ambulating. These findings were generally noted in the first several days of exposure only. There were no adverse effects clinical signs in all F1 weanlings exposed from PND 29-33.
F0 body weight gain was decreased in the 500 and 1000 ppm group males and females during the first week of exposure ($p<0.05$ or $p<0.01$) and continued to be reduced in the 1000 ppm group males during the second week of exposure (not statistically significant). As a result mean body weights in the 1000 ppm F0 males were reduced 4.2-4.7% during study weeks 2-4 ($p<0.05$). In the F0 females the reduction was not of sufficient magnitude to produce a sig-

nificant reduction in mean body weight relative to control. F1 weanlings exposed to 500 and 1000 ppm beginning on postnatal day 22 showed a reduction in mean body weight gain in both the inhalation and inhalation/gavage groups ($p<0.05$ or $p<0.01$). A slight but mostly not significant reduced mean body weight was noted in the F1 weanlings exposed to 100 ppm beginning on postnatal day 22 in both the inhalation (4-14%) and inhalation/gavage (6-16%) groups. F1 weanlings exposed from PND 29-33 exhibited reduced mean body weight gain at 500 or 1000 ppm in both the inhalation and inhalation/gavage groups being statistically significant for the inhalation only animals. By postnatal day 34, body weights were reduced by 6-13% at 1000 ppm and at 500 ppm by 5-9%. Mean body weights and body weight gains in the F1 weanlings exposed to 100 ppm of ethylbenzene beginning on postnatal day 29 were similar to control group values. Pup weight reductions in the 1000 ppm inhalation exposure groups were more pronounced in the inhalation/gavage phase (14.8 and 10.9% in male and female pups, respectively, on postnatal day 28) ($p<0.05$ or $p<0.01$) than in the inhalation phase (3.4 and 4.3% in the male and female pups, respectively, on postnatal day 28) during the pre-weaning period. These results suggest that at the top dose level the gavage dosing of the dams on lactation days 1 to 4 affected the growth of the offspring.
Ethylbenzene did not adversely affect reproductive performance in either sex.
In F0 animals absolute and relative liver (males and females) and kidney (males only) weights were increased in the 500 and 1000 ppm (generally statically significant).
In conclusion, ethylbenzene at an exposure level of 1000 ppm did not adversely impact reproduction (F0). Based on evidence of reduced weight gain at 500 ppm and 1000 ppm in the F0 generation and on reduced pup survival (F1) through lactation, reductions in pup weights at 1000 ppm and to a lesser extent at 500 ppm, the top dose level for the subsequent 2-generation study was chosen as 500 ppm. These effects were observed with and without gavage administration on PNDs 1-4; therefore, it was decided to use gavage dosing of F0 dams during this period with a top dose level of 342 mg/kg/day. Clinical signs of toxicity and reduced survival and reduction in bodyweight gain were observed only in the F1 generation exposed from PND 22 particularly at the top dose level and to a lesser extent at 500 ppm; therefore, the age at which to commence exposure of the F1 generation was determined as PND 22. The NOAEC for this study is considered to be 1000 ppm for reproductive toxicity and 100 ppm for parental systemic toxicity and for developmental effects.

Based on the data obtained by the above mentioned pilot test, a 2-generation rat reproductive toxicity study according to OECD TG 416 was carried out **(Faber et al., 2006; Stump, 2005)**. This study investigated the effects of whole body exposure by inhalation to ethylbenzene on the reproductive capabilities of rats over 2 generations. In addition to the standard observations, a functional observational battery examination (FOB) was carried out on F1 females during gestation and lactation. A developmental neurotoxicity study was conducted on 2 subsets of the F2 offspring. The details of these supplementary neurotoxicity and developmental neurotoxicity components are reported in the subsequent section on developmental toxicity.
Parental Sprague-Dawley rats (30/sex/dose for the F0 and 25/sex/dose for the F1 generation) inhaled 0, 25, 100 and 500 ppm during the premating exposure period of 70 consecutive days. Males were sacrificed following parturition. Inhalation of females continued after mating through gestation day 20 and from lactation day 5-21. On postnatal day 1-4, the F0 and F1 females were treated by gavage with 0, 26, 90 and 342 mg/kg bw/d (divided into three equal doses, approximately 2 hours apart). The oral dosages were calculated using the ethylbenzene PBPK model **(Tardif et al., 1997)** to produce equivalent doses to the inhalation exposures. F2 offspring potentially exposed in utero and through nursing during lactation were sacrificed on PND 22 or assigned to developmental neurotoxicity study.

For mating daily vaginal lavages were performed for determination of estrous cycles, beginning 21 days prior to pairing. Animals (30 per sex per dose group for F0 and 25 per sex per dose group for F1 generations) were paired in a 1:1 basis (adjustments were made only to avoid sibling pairings) after treatment for at least 70 days. Each pair was examined daily and mating was confirmed by the presence of a copulatory plug or presence of sperm in a vaginal smear. Females with no evidence of mating after 14 days (or three estrus cycles) were paired with another male of the same exposure group that had successfully mated for an additional 7 days. If no evidence of copulation was obtained after 21 days, the female was placed in a plastic maternity cage with nesting material. On postnatal day 4, the litters from both generations (F1 and F2 pups) were reduced to 10 pups per litter (5/sex, where possible).
The following parameters were assessed in the parental F0 and F1 animals: clinical signs including detailed physical examinations, body weight and food consumption (weekly and on 6 occasions during pregnancy), estrous cycle, sperm examination (motility, morphology, sperm count), functional observational battery evaluations on all F1 (P) females on gestation days 6 and 12 and lactation days 10 and 21 (details reported in section developmental toxicity). At necropsy for females that delivered the number of former implantation sites were recorded. For females that failed to deliver, nongravid uteri were opened for detection of implantation sites. Organ weights were recorded for pituitary, thymus, thyroid, epididymides (total and cauda), ovaries, prostate, seminal vesicles (with coagulating glands and accessory fluids), testes and uterus with oviducts and cervix. By histopathology ovarian primordial follicle counts were recorded for all control and 500 ppm F1 females and for 25 and 100 ppm F1 females that failed to mate or produce offspring.
The following parameters were assessed in the F1 and F2 offspring: Survival was checked daily and each pup was subjected to a detailed physical examination on postnatal days 1, 4, 7, 14 and 21. Anogential distance was measured on postnatal day 1, and pups were individually sexed on postnatal days 0, 4, 7, 14 and 21. F1 and F2 body weights were recorded on postnatal days 1, 4, 7, 14 and 21 and F2 body weights were recorded on postnatal days 1, 4, 7, 11, 13, 17, and 21. The following developmental landmarks were evaluated in each pup: Pinna detachment beginning on postnatal day 4 and continuing until both auricles of the pinnae were fully detached or until scheduled euthanasia. Incisor eruption beginning on postnatal day 7 until both upper and lower incisors had erupted. Hair growth beginning on postnatal day 8 until hair growth was considered normal. Eyelid separation beginning on postnatal day 12 until both eyelids were fully open or until scheduled euthanasia. Balanopreputial separation was assessed in males (30 per dose group for F1 males and 20 dose group for F2 males) from postnatal day 35 onwards. Vaginal opening was assessed in females (n = 30 per dose group) from postnatal day 25 onwards. Body weight was recorded for all pups on the day of acquisition of each developmental landmark. Gross necropsy was performed on all F1 and F2 weanlings not selected for the F1 and F2 generations on postnatal day 21 and on any pups dying on postnatal days 0 and prior to weaning, and tissues were preserved for histological examination. Brain, spleen, pituitary, thymus, thyroid, uterus and testes weights were recorded for three randomly selected F1 pups/sex/litter and from all F2 weanlings not selected for neuropathology evaluation. 15 tissues and all gross lesions were collected and retained for histopathology.
Blood was collected for determination of ethylbenzene levels as follows: On lactation day 4 approx. one hour following the third gavage dose from 4 F1 dams/group. On day 22 post partum approx. one hr following completion of the 6 hr inhalation exposure from the same dams. On postnatal day 4 from culled pups from the litters of the F1 dams that had blood collected for analyses approx. 1 hour following the third gavage dose of their mothers. Blood was pooled from within each litter to obtain approx. 0.5 mL. On postnatal day 22 approximately 1

hour following completion of a 6 hour inhalation exposure from 1 weanling/sex/litter from the same litters that were used in the postnatal day 4 collections.
The mean measured inhalation exposure concentrations nearly exactly matched the target concentrations. Significant treatment related effects on body weight gain and body weight were noted only at a few time points for parental F0 males and females and F1 males at 500 ppm during the pre-mating period. There were no treatment related effects for the parental F0 and F1 animals for mating index, fertility index, precoital interval, mean gestation length, sperm parameters, number of implantations and corpora lutea, ovarian primordial follicles, or by macroscopy or microscopy after sacrifice. The cycle length in F0 500 ppm females was slightly lower than controls ($p<0.1$). However all females in this group were cycling normally and there was no impairment of mating or fertility. As the F1 females showed no statistically significant difference in cycle length this finding in F0 females was not considered of biological significance. As regards organ weight changes at 500 ppm there were statistically significant increases in relative and absolute liver and kidney weights in F0 and F1 males and in relative and absolute liver weight in F0 and F1 females. These changes were considered treatment related but adaptive in the absence of histopathological alterations. In F0 males at 100 and 500 ppm absolute and relative thyroid weights were slightly and statistically significantly increased, however as this was not seen in the F1 generation these increases were not considered to be substance related but rather due to biological variation. The only other statistically significant changes were decreases in absolute lung and prostate weight at 500 ppm in F0 males. Relative lung and prostate weights were not significantly affected and the effect was not seen in the F1 males. With the additional absence of histopathological findings these changes were considered unlikely to be treatment related.
For F1 and F2 offspring there were no statistically significant treatment related differences in litter parameters in either generation (litter size, live pups, % males, pup survival), pup body weight, pup necropsy findings, pre-weaning developmental landmarks (pinna detachment, hair growth, incisor eruption, eye opening,). Time to balanopreputial separation was statistically significant higher ($p<0.05$) in the 500 ppm F1 but not F2 males. However the value was equivalent to the mean lab historical control value and therefore was not considered treatment related. Vaginal patency was statistically significantly lower in all F1 but not F2 exposure groups compared to concurrent control. However, this was most likely due to a slightly elevated F1 concurrent control mean value when compared to the mean value of the lab historical control and hence was not considered treatment related.
Mean blood levels on postnatal day 4 were in dams (control, 26, 90 and 342 mg/kg dose groups) 0, 0.49, 3.5, and 18.3 mg/l and undetectable in pups (detection limit 0.006 mg/l). On postnatal day 22 the blood levels (0, 25, 100, 500 ppm) were in dams 0, 0.11, 0.56, 11.0 and in pups 0, 0.023, 0.281, 12.06 mg/l.
In summary, there were no adverse effects on reproductive or developmental endpoints at dose levels up to 500 ppm. Parental toxicity was minimal with treatment related effects confined to transient decreases in bodyweight gain in top dose males and increased liver and kidneyweights in males and females of both generations. In the absence of histopathological change the organ weight changes were considered adaptive. The NOAEC for this study is considered to be 500 ppm for parental systemic toxicity, reproductive toxicity and developmental effects.

Further information related to possible impairment of organs of the reproductive system can be derived from repeated dose toxicity studies with oral and inhalative exposure mentioned above (c.f. 4.1.2.6).

In their multispecies pre-guideline inhalation study, Wolf et al. (1956) investigated effects on testes weight and histopathology. Animals were exposed 7 hrs/day for 5 days/week at concentrations of 400, 600, 1250 and 2200 ppm (10-25 male rats/group over 144 days for the top dose, 214 days for the 1250 ppm and 186 days for the other groups), 400 and 600 ppm (5-10 male guinea pigs/group or 1-2 male rabbits/group over 186 days) and 600 ppm (1 or 2 male rhesus monkeys/group over 186 days). No testicular effects were noted in rats (NOAEC 1250 ppm over 214 days) or in guinea pigs (NOAEC 600 ppm over 186 days). In rabbits and monkeys there were slight degenerations of the germinal epithelium at 600 ppm over 186 days, but the significance is unclear as the group sizes were very small. No effects were noted in rabbits at 400 ppm.

In a 12 week rat inhalation study, **Clark (1983)** exposed 18 Fischer 344 rats/sex/dose to 0 and 100 ppm over 6 h/d, 5 d/week. At termination testicular weight was determined and ovaries, testes, uterus and prostate were investigated histopathologically. No adverse findings were noted (NOAEC 100 ppm over 12 weeks).

In the 4 week repeated inhalation toxicity study **(Cragg et al., 1989)**, Fischer 344 rats, B6C3F1 mice, and New Zealand white rabbits (five/sex/group) were exposed to ethylbenzene (99.7% purity) vapours for 6 h /day at 5 day/week. Rats and mice received 0, 99, 382, or 782 ppm ethylbenzene, while rabbits received 0, 382, 782, or 1610 ppm. At termination weights of the major organs were recorded. Over 30 tissues including gonads (ovaries or testes with epididymides) and uteri (horn and cervix) from each of the high-exposure and control animals of all species were subjected to histopathological examination. In addition testicular tissues from the intermediate-exposure rabbits were also examined.
No effects were noted on reproductive organ weights and also no gross or microscopic changes were noted in any of the 30 tissues from each of the three species exposed to ethylbenzene vapours at the high concentration.
A NOAEC/reproductive organ toxicity of 782 ppm (3449 mg/m^3) ethylbenzene for rats and for mice and of 1610 ppm (7100 mg/m^3) for rabbits can be derived from this study.

In the 13 week repeated inhalation toxicity study **(NTP, 1992)**, Fischer 344 rats and B6C3F1 mice (10/sex/group) were exposed (whole body) to ethylbenzene vapours for 6 h /d at 5 d/week for 92 (female rats), 93 (male rats), 97 (female mice), or 98 (male mice) days at 0, 100, 250, 500, 750, or 1000 ppm ethylbenzene. At termination weights of the major organs including right testis and left epididymis were recorded. At necropsy tissues and organs including prostate, testes, epididymis, seminal vesicles, clitoral and preputial glands, ovaries and uteri were taken, examined for gross lesions and from all 1000 ppm exposed and control animals subjected to histopathological evaluation. Sperm morphology and motility, sperm and spermatid counts, and vaginal cytology evaluation for oestrus cyclicity were performed for both species for animals exposed to 100, 500 and 1000 ppm and for the controls.
No effects were noted on absolute and/or relative testes and epididymal weights of rats and of mice after exposure to ethylbenzene. There were no histopathological alterations. Furthermore, no effects were observed on sperm, testicular morphology, or length of oestrus cycle in the animals exposed to ethylbenzene.
A NOAEC for reproductive organ toxicity of 1000 ppm (4410 mg/m^3) ethylbenzene can be derived from this study.

In the 2-year carcinogenicity bioassay (**NTP 1999**, cf section 4.1.2.8), Fischer 344 rats and B6C3F1 mice (50 animals /sex/dose) were exposed at 0, 75, 250 and 750 ppm over 6 h/d, 5

d/week. Microscopic examination was done for clitoral gland, mammary gland, ovary, preputial gland, prostate, testis, epididymis and seminal vesicle. Organs were not weighed.
In male rats, the incidence of interstitial cell adenoma in 750 ppm males (44/50 [88%]) was significantly greater than in the chamber control group (36/50 [72%]) and slightly exceeded the NTP historical control range for inhalation studies of 69% (54-83%). The incidence of bilateral testicular adenoma was also significantly increased at 750 ppm (40/50 [80%] vs. 27/50 [54%] in controls), whereas the incidence of interstitial cell hyperplasia (ICH) was significantly decreased at this concentration level (8/50 vs. 14/50 in controls; p=0.05). Although testicular adenoma will develop in nearly all aged Fischer rats, ethylbenzene appeared to enhance its development since 92% (22 of 24 rats) of the 750 ppm rats that died between day 400 and day 600 had testicular adenoma, whereas only 33% (3 of 9 rats) of the controls that died early had testicular adenoma. No other histopathological changes were reported in the other reproductive organs examined in rats and mice. Apart from findings related to Leydig cell tumor development the NOAEC for effects on reproductive organs was 750 ppm in rats and mice.

In a 6 month oral gavage study **(Wolf et al., 1956)**, male Wistar rats were treated with 0 (vehicle only), 13.6, 136, 408 and 680 mg/kg bw in olive oil for 5 d/week over 182 days. Testes were examined by weight and histopathology. No adverse findings were noted, the NOAEL for testes effects was 680 mg/kg bw.

In a 4-week oral gavage study **(Mellert et al., 2003; 2007)** Wistar rats (5/sex/group) received total daily doses of 0 (vehicle only, corn oil), 75, 250 and 750 mg/kg bw. The doses were divided into two equal parts and given twice daily 8 h apart. At necropsy the weights of testes, epididymis, ovaries and uterus were determined; histopathology was not performed. No treatment related changes were noted; the NOAEL was 750 mg/kg bw based on reproductive organ weights only.

The subsequent 13-week oral gavage study in Wistar rats (10 animals/sex/dose) (generally met the requirements of OECD Guideline 408) was carried out at the same dose levels with the same exposure scheme **(Mellert et al., 2004; 2007)**. At necropsy testes, epididymes, ovaries and uterus were weighed. Microscopic examination of organs from control and top dose animals was carried out on testes, epididymides, prostate and seminal vesicle, ovaries, oviducts, uterus and vagina and mammary gland. There were no treatment related weight or histopathological changes in the reproductive organs examined. Thereby a NOAEL of 750 mg/kg bw was obtained.

Tab 4.1.2.9.1 Summary on studies for fertility assessment

Inhalation			
Type of study	NOAEC	Observed effects	Reference
1-generation reproduction toxicity study	**NOAEC/fertility: ≥ 1000 ppm**	**--**	**Faber et al. (2007) Stump (2003)**
Sprague Dawley rat 100/500/1000 ppm	**NOAEC/syst. tox.: 100 ppm**	**stat. sign. ↓ body weight during first week of exposure; stat. sign. ↑ organ weights (liver, kidney) at 500 ppm**	

		(LOAEC);	
	NOAEC/devel. tox.: 100 ppm LOAEC/devel. tox.: 500 ppm	↓ pup survival until PND 4, dose-related mortality and signs of CNS depression in PND 22 exposed offspring, ↓ weanling body weight on PND 34 in the PND 22 and in the PND 33 exposed offspring at 500 (LOAEC) and 1000 ppm	
2-generation reproduction toxicity study **Sprague Dawley rat** **25/100/500 ppm**	**NOAEC/fertility:** **≥ 500 ppm** **NOAEC/syst. tox:** **500 ppm** **NOAEC/devel. tox.:** **≥ 500 ppm**	**--** **--** **--**	**Faber et al. (2006)** **Stump (2005)**
sub-chronic non-guideline **rat, guinea pig, rabbit, rhesus monkey** **400/600/1250/2200 ppm**	**NOAEC/testes tox.:** **rat: 1250 ppm** **guinea pig: 600 ppm** **rabbit: 400 ppm**	**rabbit: slight degenerations in germinal epithelium at 600 ppm** **rhesus monkey: slight degenerations in germinal epithelium at 600 ppm**	**Wolf et al. (1956)**
sub-chronic non-guideline; **F 344 rat** **100 ppm**	**NOAEC/reproductive organ tox.:** **≥ 100 ppm**	**--**	**Clark (1983)**
sub-chronic **similar to OECD 407** **F 344 rat, B6C3F1 mouse, NZW rabbit** **99/382/782 ppm (rat, mouse)** **382/782/1610 ppm (rabbit)**	**NOAEC/reproductive organ tox.:** **≥ 782 ppm (rat, mouse)** **≥ 1610 ppm (rabbit)**	**--**	**Cragg et al. (1989)**
sub-chronic **similar to OECD 408** **F 344 rat, B6C3F1** **100/250/500/750/1000**	**NOAEC/reproductive organ tox:** **≥ 1000 ppm** **(rat, mouse)**	**no effects observed on organs of the reproductive system, on sperm pa-**	**NTP (1992)**

ppm		**rameters or on estrous cyclicity**	
carcinogenicity bioassay F 344 rat, B6C3F1 inhalation 75/250/750 ppm	**NOAEC/reproductive organ tox.: 250 ppm (rat) NOAEC/reproductive organ tox.: ≥ 750 ppm (mouse)**	**rat: ↑ incidences of interstitial cell adenoma and bilateral testicular adenoma, ↓ incidences of interstitial cell hyperplasia at 750 ppm**	**NTP (1999)**

Oral gavage

Type of study	NOAEL	Observed effects	Reference
sub-chronic non-guideline rat 13.6, 136, 408, 680 mg/kg bw	**NOAEL/testes tox.: 680 mg/kg bw**	**--**	**WolfWolfe et al. (1956)**
Sub-chronic OECD 407 Wistar rat 75, 250, 750 mg/kg bw	**NOAEL/reproductive organ tox.: 750 mg/kg bw**	**--**	**Mellert et al. (2003, 2007)**
Sub-chronic OECD 408 Wistar rat 75, 250, 750 mg/kg bw	**NOAEL/reproductive organ tox.: 750 mg/kg bw**	**--**	**Mellert et al. (2004, 2007)**

4.1.2.9.2 Developmental toxicity:

In a non-guideline study female, Wistar rats as well as New Zealand rabbits were exposed by inhalation to 0, 100 and 1000 ppm **(Andrew et al., 1981; Hardin et al., 1981)**.
For the rat species female animals were exposed either 3 weeks before fertilisation or from days 1-19 of gestation or combined during premating and gestation at the above mentioned concentrations. Males were not exposed. 3 days after completion of pregestational exposures females were caged in groups of about 4 females to 1 male. The following morning the males were removed and the females examined for the presence of sperm. Each day sperm positive females were assigned to gestational exposure groups (GD1). Mating and initiation of gestational exposures continued until about 38 sperm positive females were assigned to each experimental group. The final group size consisted of 29-33 females.
Body weight and food consumption determinations and clinical observations were carried out regularly. The uterine content was investigated for implantations, dead and resorbed fetuses, corpora lutea, pre- and post-implantation losses and number and percent of live offspring were recorded. Placental, but not gravid uterine weights recorded. Fetal data collected included sex ratio, body weights (sexes combined), crown-rump lengths, and external observations. The heads of approximately one-half of the fetuses in each litter were removed, preserved in Bouin's fixative, sectioned, and inspected. The decapitated fetuses were subjected to a fresh visceral examination (Staples technique). The fetuses not decapitated were eviscerated

and processed for skeletal examination. Full necropsy on all dams was carried out and liver, lung, spleen and kidneys were weighed. Ovaries, uterus, liver, lungs (with trachea) and kidneys were processed and histopathological examination was carried out on about 25% of dams (approx 7/group).
The actual mean exposure concentrations were between the target concentration and -5% thereof. In the maternal animals' food intake, body weight and survival were not affected by treatment. Increased absolute and relative weights of liver, kidneys, and spleen were present in 1000 ppm dams exposed during gestation only or during both premating and gestation. There were no treatment related gross or microscopic changes.
Most of the reproductive parameters did not show an exposure related effect, such as mating success (of females exposed before mating), litter size and weight, number of viable offspring, sex ratio, grossly visible abnormalities, external abnormalities and soft tissue abnormalities. The incidence of extra and rudimentary ribs was statistically increased in some ethylbenzene treated groups, but the interpretation is difficult in the absence of a clear dose-response relationship. When gestational exposure only is considered, there appears to be an increase in this effect as a result of exposure to ethylbenzene at 1000 ppm. The skeletal variants in this study are considered marginally adverse. A significant decrease of crown-rump length occurred in rat which received 1000 ppm during gestation only, however as no similar effect was seen in any other treatment group including that exposed to 1000 ppm both during premating and gestation period this observation is considered spurious.
In conclusion, in female rats exposed inhalationally to 1000 ppm ethylbenzene prior to mating and/or during gestation there was an increase in extra ribs in the offspring in the presence of slight maternal toxicity evidenced by increased liver, spleen, and kidney weights. No maternal or foetal effects were observed at 100 ppm.

As for the rabbits the females were inseminated artificially and groups of 21-24 pregnant females were exposed (whole body inhalation, 7 hrs daily) to vapour concentrations of 0, 100, and 1000 ppm ethylbenzene for days 1-24 during gestation. Animals were observed for evidence of toxicity, food consumption and body weight gain. At sacrifice on g.d. 30 does were examined for organ weight (lung, liver, kidney, spleen) and histopathological investigations were performed (ovaries, uterus, liver, lungs with trachea, kidney) of 6 does of each group. The uterine contents were examined for implantations, dead and resorbed fetuses, and corpora lutea counts. Pre- and post-implantation losses and number and percent of live offspring were recorded. Placental, but not gravid uterine weights were recorded. Foetal data collected included sex ratio, body weights (sexes combined), crown-rump lengths, and external observations. All fetuses were subjected to a fresh visceral examination (Staples technique) and a skeletal examination. The heads of approximately one-half of the fetuses in each litter were removed, preserved in Bouin's fixative, sectioned, and inspected.
Actual mean exposure concentrations achieved in the chambers throughout the study were 99 and 962 ppm ethylbenzene. There were no treatment-related effects on maternal survival, clinical signs of intoxication, maternal body weight or food consumption. Relative liver weights were significantly increased (16%) in 1000 ppm does, but in the absence of histopathological change in the liver this finding was not considered biologically relevant. There was no evidence of histological damage in any of the other organs examined.
There were no treatment-related developmental toxic effects. For live foetuses there was a slight, but statistically significant decrease in the number of live fetuses/litter in the 1000 ppm group (8 ± 3 in the control versus 7 ± 3 in the 1000 ppm group). The finding was considered equivocal due to no corresponding increases in other parameters (implantations, resorptions, dead fetuses, etc.). There were no significant changes in the incidence of variations or malformations in the pups.

From the investigations with rabbits a NOAEC for maternal and developmental toxicity of 1000 ppm ethylbenzene can be derived.

In a poorly reported study on investigations of benzene and several of its alkyl derivatives **(Ungvary and Tatrai, 1985)** CFY rats, CFLP mice, and New Zealand white rabbits were exposed to ethylbenzene via inhalation at different times during gestation. Due to scarce information on test details, evaluated parameters and resulting quantitative and qualitative data, this study is of limited usefulness only and is not considered for quantitative risk assessment with respect to developmental toxicity.
From the investigation on rats it is reported that females had been exposed to inhalation of ethylbenzene (no further specifications provided) at 600, 1200, and 2400 mg/m³ atmospheric concentrations (138, 276, and 552 ppm) for 24 hrs/day and to 600 mg/m³ for 6 hrs/day during days 7 to 15 of gestation. The group size was 17-20 dams. 20 females had been used as controls, however no information is given on their treatment. Animals were terminated on day 21 of gestation and fetuses were studied (no further details provided). Maternal toxicity was reported to be moderate and dose-dependent (no data provided). No developmental effects were reported for animals exposed 6 hours/day to 600 mg/m³. Slightly increased (statistically not significant) percentages of "dead or resorbed" (8-9%) in comparison to the controls (5%) were observed in all of the ethylbenzene exposed 24-hour groups, but without any dose relationship. Further, increases (statistically not significant) in the percentage of fetuses with retarded skeletal development (not further specified) were reported for all of the ethylbenzene exposed 24-hour groups. At 2400 mg/m³ a statistically significantly higher percentage of weight retarded fetuses and of fetuses with skeletal variants (extra ribs) was found. In this group skeletal and urinary tract malformations were also significantly increased.
For mice 20 females were exposed to inhalation of ethylbenzene (no further specifications provided) at 500 mg/m³ atmospheric concentrations for 3x4 hrs/day (or 24 hrs/day; contradictions) during days 6 to 15 of gestation. A total of 115 females were used as controls, however no information is given on their treatment. Animals were terminated on day 18 of gestation and fetuses were studied (no detailed information provided). No data on maternal toxicity is given. For mice fetuses, no effects were seen for percentage of "dead or resorbed", percentages of weight or skeletally retarded fetuses or of percentage of minor abnormalities in comparison to the controls. However, a significant increase in the incidence of skeletal and urinary tract malformations (not further explained or defined) was reported.
For rabbits 9, respectively 3 females were exposed to inhalation of ethylbenzene (no further specifications provided) at 500, respectively 1000 mg/m³ atmospheric concentrations for 24 h/day during days 7 to 20 of gestation. A total of 60 females were used as controls in the investigations with rabbits, however no information is given on their treatment. Animals were terminated on day 30 of gestation and fetuses were studied (no detailed information provided). Maternal toxicity (maternal weight gain decreased) was reported for the higher (1000 mg/m³) exposure concentration, whereas the lower exposure concentration (500 mg/m³) was reported as not toxic for mothers. One out of nine does of the lower exposure level aborted, whereas at the higher exposure level all three does aborted. For the live offspring of the lower level exposure group there was a significantly lower mean fetal weight. No differences in percentages of skeletal retardation, minor abnormalities and skeletal, internal or external malformations were observed in comparison to the controls.
The authors concluded that ethylbenzene has a mild teratogenic potential in mice and rats but not in rabbits.

Saillenfait et al. (2003) investigated the developmental toxicity of ethylbenzene, o-, m-, p- and technical xylene in Sprague Dawley rats by inhalation using the OECD guideline 414.

Only the data for ethylbenzene are reported here. 21-25 Pregnant rats/group were exposed to 100, 500, 1000 and 2000 ppm (whole body) on gestation days 6-20 (6 hrs/day) and sacrificed on day 21 of gestation. Body weight gain, food consumption and clinical observations were recorded. The uterus was removed and weighed and the number of corpora lutea, implantation sites, resorptions, dead and live fetuses were determined. Live fetuses were weighed, sexed, and examined for external anomalies including those of the oral cavity. Half of the live fetuses were preserved in Bouin's solution and examined for internal soft tissue changes by the method of Wilson. The other half were fixed in 70% ethanol, eviscerated, and then processed for skeletal staining with Alizarin Red S for subsequent skeletal examination (Staples technique).
The actual exposure concentrations were within +/-1% of the target concentrations. In the dams ethylbenzene did not cause maternal death. Clinical signs of toxicity (ataxia, decreased motor activity) were seen at 2000 ppm and maternal body weight was significantly reduced on gestation day 21 at 1000 ppm and on gestation day 13 and 21 at 2000 ppm. Dams exposed to 1000 or 2000 ppm showed significant decreases in food consumption and maternal weight gain throughout exposure also reflected in corrected weight gain (1± 21 g; respectively 18 ± 11 g in comparison to 35 ± 10 g in the control group). The number of corpora lutea and implantations was comparable between treated and control groups. Although the difference was not statistically significant, the incidence of resorptions (mean of 20.2 versus 5.2 for controls) was higher at 2000 ppm than in the control group. Similarly, the incidence of non-live implants (mean of 21.4 versus 5.2 for controls) was higher at 2000 ppm than in the control group, although the difference was not statistically significant. This was likely due to the 100% post-implantation loss seen in 3 of the 21 pregnant females exposed to 2000 ppm (0 in other groups).
Litter size, number of viable pups and sex ratio were comparable between treated and control groups. Ethylbenzene produced a concentration-related reduction in foetal weights (of 7 %, respectively of 18 %) that achieved statistical significance at 1000 and 2000 ppm. No evidence of teratogenic effects was found at any exposure level for external, soft tissue or skeletal malformations. There was an increased number (statistically significant) of fetuses with skeletal or any variations at 1000 and 2000 ppm. The mean percentage of fetuses per litter with skeletal or any variations was also significantly increased at 2000 ppm.
In conclusion, ethylbenzene was not teratogenic to rats at dose levels up to 2000 ppm administered from gestation day 6-20. There was however evidence of slight developmental toxicity indicated by reductions in foetal body weight and a higher incidence of skeletal variations at 1000 and 2000 ppm. This was in the presence of maternal toxicity evidenced by clinical signs of toxicity at 2000 ppm and reduced body weight gain and food consumption at 1000 and 2000 ppm. The NOAEC for fetotoxicity and maternal toxicity in this study is 500 ppm.

Saillenfait et al. (2006) investigated the combined effect of ethylbenzene and methyl ethyl ketone (MEK) on developmental toxicity in Sprague Dawley rats by inhalation using a procedure similar to OECD TG 414. Two groups with exposure to ethylbenzene only at 250 and 1000 ppm (whole body) and a zero exposure group were included and only the results obtained herewith will be reported here. 15-19 pregnant rats/group were exposed on gestation days 6-20 (6 h/d) and sacrificed on day 21 of gestation. Body weight gain, food consumption and clinical observations were recorded. The uterus was removed and weighed and the number of corpora lutea, implantation sites, resorptions, dead and live fetuses were determined. Live fetuses were weighed, sexed, and examined for external anomalies. Half of the live fetuses were examined for internal soft tissue changes and the other half were stained for skeletal examination.

In addition, to further characterise the possible interaction between ethylbenzene and MEK an investigation was undertaken with extra satellite groups of 6 non-pregnant females exposed to MEK and ethylbenzene alone and in combination for 15 days under the same experimental conditions as the pregnant rats. At the end of the first and last exposures the females were placed in individual metabolism cages and the urine collected over 16 hours. After the last urine collection the rats were sacrificed by bleeding from the aorta. Liver and kidney were removed weighed and processed for histological examination. Biochemical parameters measured by automated analyser in the serum included ASAT, ALAT, urea and creatinine. Urine parameters determined included volume, creatinine, proteins, GGT, ALP and LDH. Mandelic acid was measured in the urine.
As regards to maternal effects, there were no treatment related changes for mortality, number pregnant or aborting animals, number of resorptions or implantations or pre and post implantation loss. Maternal body weight gain corrected (minus gravid uterine weight) for the period GD 6-21 was significantly lower than controls (18 $\pm$ 18 g in comparison to 34 $\pm$ 8 g in the control group) for the 1000 ppm group. Food consumption was reduced at 1000 ppm (GD 6-13). As regards to fetal data, there were no effects on litter size, number of viable pups, sex ratio, malformations or variations. The body weight of foetuses was significantly lower (8.1 %) than controls following exposure to 1000 ppm. As regards the non-pregnant females from the satellite groups there were no changes in clinical biochemistry and urine parameters. There was no effect on absolute or relative kidney weights, but liver weights (absolute and relative) were significantly increased in rats treated with 250 or 1000 ppm. There were no treatment related pathological changes in histopathology. Urinary mandelic acid excretion was 2-5 fold higher after 15 exposures than after 1 exposure at 1000 ppm.
In conclusion, slight maternal toxicity (body weight) and fetal toxicity (body weight) was seen at 1000 ppm, the NOAEC being 250 ppm.

Saillenfait et al. (2007) further investigated the combined effect of ethylbenzene and butyl acetate on developmental toxicity in Sprague Dawley rats by inhalation using a procedure similar to OECD TG 414. Two groups with exposure to ethylbenzene only at 250 and 1000 ppm (whole body) and a zero exposure group were included and only the results obtained herewith will be reported here. 15-18 pregnant rats/group were exposed on gestation days 6-20 (6 hrs/day) and sacrificed on day 21 of gestation. Body weight gain and clinical observations were recorded. The uterus was removed and weighed and the number of corpora lutea, implantation sites, resorptions, dead and live fetuses were determined. Live fetuses were weighed, sexed, and examined for external anomalies. Half of the live fetuses were examined for internal soft tissue changes and the other half were stained for skeletal examination.
As regards to maternal effects, there were no treatment related changes for mortality, number of pregnant or aborting animals, number of resorptions or implantations or pre and post implantation loss. Maternal body weight gain absolute and corrected (minus gravid uterine weight) for the period GD 6-21 was significantly lower than controls (17 $\pm$ 15 g in comparison to 30 $\pm$ 17 g in the control group) for the 1000 ppm group. As regards to fetal data, there were no effects on litter size, number viable pups or malformations. The body weight of foetuses was significantly lower (6.8 %) than controls following exposure to 1000 ppm. There was a statistically significant difference for the overall % of fetuses with skeletal variations between the control and the 250 ppm group, but not at 1000 ppm. Because of the missing dose response relationship this finding is not considered to be of toxicological relevance.
In conclusion, slight maternal toxicity (body weight) and fetal toxicity (body weight) was seen at 1000 ppm, the NOAEC being 250 ppm.

In combination with a 2-generation rat reproductive toxicity study (c.f. 4.1.2.9.1), an assessment of developmental neurotoxicity according to the draft OECD TG 426 was carried out using F1 and F2 generation animals **(Faber et al., 2007; Stump, 2005)**. Sprague-Dawley rats were exposed by whole body inhalation exposure at concentrations of 0, 25, 100 and 500 ppm. F1 female rats (from ethylbenzene exposed parents) were exposed by inhalation for 6 hours daily for at least 70 consecutive days prior to mating, beginning at weaning (postnatal day 22). Inhalation exposure of the F1 females continued throughout mating and gestation through gestation day 20 and was resumed on PND 4 and continued until weaning. Exposure on PND 1-4 was by gavage in corn oil at dose levels of 0, 26, 90 and 342 mg/kg bw/day (divided into three equal doses, approx. 2 hours apart). F2 pups born to parents treated with ethylbenzene over 2 generations were exposed indirectly in utero and during lactation and were observed until pnd 72. The group sizes were for F1 females 25/group and for F2 pups 40/sex/group (2 pups/sex/litter, if possible).
The following aspects were assessed:
-In the F1 and F2 offspring: Survival was checked daily and each pup was subjected to a detailed physical examination on postnatal days 1, 4, 7, 14 and 21. Anogenital distance was measured on postnatal day 1, and pups were individually sexed on postnatal days 0, 4, 7, 14 and 21. F1 and F2 body weights were recorded on postnatal days 1, 4, 7, 14 and 21 and F2 body weights were recorded on postnatal days 1, 4, 7, 11, 13, 17, and 21. The following developmental landmarks were evaluated in each pup: Pinna detachment beginning on postnatal day 4 and continuing until both auricles of the pinnae were fully detached or until scheduled euthanasia. Incisor eruption beginning on postnatal day 7 until both upper and lower incisors had erupted. Hair growth beginning on postnatal day 8 until hair growth was considered normal. Eyelid separation beginning on postnatal day 12 until both eyelids were fully open or until scheduled euthanasia. Balanopreputial separation was assessed in males (30 per dose group for F1 males and 20 dose group for F2 males) from postnatal day 35 onwards. Vaginal opening was assessed in females (n = 30 per dose group) from postnatal day 25 onwards. Body weight was recorded for all pups on the day of acquisition of each developmental landmark. Gross necropsy was performed on all F1 and F2 weanlings not selected for the F1 and F2 generations on postnatal day 21 and on any pups dying on postnatal days 0 and prior to weaning, and tissues were preserved for histological examination. Brain, spleen, pituitary, thymus, thyroid, uterus and testes weights were recorded for three randomly selected F1 pups/sex/litter and from all F2 weanlings not selected for neuropathology evaluation. 15 tissues and all gross lesions were collected and retained for histopathology.
- Functional Observational Battery (FOB) in the F1 females: In addition to the standard assessments included in the 2-generation component of the study, FOB evaluations were performed for F1 females on gestation days 6 and 12 and lactation days 10 and 21.
- Developmental neurotoxicity in the F2 pups: In addition to the standard litter and developmental landmark assessments included in the 2-generation component of the study, neurobehavioral evaluations were conducted on 2 subsets (each of 20/sex/group) of F2 offspring. Subset A: FOB evaluations on postnatal days 4, 11, 22, 45 and 60, locomotor activity evaluations on postnatal days 13, 17, 21 and 61, acoustic startle response evaluations on postnatal days 20 and 60, and learning and memory evaluations in a Biel water maze task initiated on postnatal day 62. Subset B: learning and memory evaluations in a Biel water maze task beginning on postnatal day 26.
- Brain weight and brain morphometrics in the F2 pups: A further subset (C) of F2 pups (10/sex/dose level) were selected and following in situ perfusion, brain weights and brain dimensions (length and width) were measured on postnatal day 21 (Subset C). Additionally on postnatal day 72 (10 pups/sex/dose from Subset A) were subjected to the same evaluations.

- Neuropathology (microscopic) F2 pups: A microscopic examination was conducted of the brains of control and top dose F2 pups sacrificed at PND 21 (Subset C). Additionally 10 pups/sex from subset A (controls and top dose) were subject to microscopic examination of representative samples of the central and peripheral nervous systems on PND 72.
- Necropsy of F2 pups: Pups not selected for neurobehavioral testing were necropsied on PND 21. All other subset A and B pups were subjected to gross necropsy on PND 72 and 33 respectively.

Results: The mean measured inhalation exposure concentrations were within the +/-1% range of the target concentrations. There were no substance related adverse findings on survival, clinical findings, or body weight in the F1 generation dams or F2 pups, macroscopic investigations in the F1 generation dams and F2 offspring or organ weights and microscopic pathology in F2 offspring. For F1 and F2 offspring there were no statistically significant treatment related differences in litter parameters in either generation (litter size, live pups, % males, pup survival)and in pup body weights and pup necropsy findings). Developmental landmarks (pinna detachment, hair growth, incisor eruption, eye opening,) were not affected in F1 male or female pups exposed to 25-500 ppm; however, in the F2 generation, statistically significant delays in hair growth were observed in all males and females in all exposure groups and eye opening was significantly delayed in males in the 25 and 100 ppm group, but not the 500 ppm group. Time to balanopreputial separation was statistically significant higher ($p<0.05$) in the 500 ppm F1 but not F2 males. However the value was equivalent to the mean lab historical control value and therefore was not considered treatment related. Vaginal patency was statistically significantly lower in all F1 but not F2 exposure groups compared to concurrent control. However, this was most likely due to a slightly elevated F1 concurrent control mean value when compared to the mean value of the lab historical control and hence was not considered treatment related. No statistically significant differences were apparent between the control and test-article exposed groups on maternal F1 FOB evaluations on gestation days 6 and 12 and lactation days 10 and 21, nor for the FOB F2 pups on postnatal days 4, 11, 22, 45 and 60. Brain weight, morphometry and histomorphology did not show any statistically significant treatment related effects in F2 pups. In the Biel Maze Swimming Trials (F2 pups) on PND 26 (Subset B) and PND 62 (Subset (A) there were no biologically meaningful differences between the exposed and control animals in swimming ability or for the learning and memory trials. The findings on locomotor activity and and acoustic startle reflex warrant a more detailed discussion:

- Locomotor activity in F2 pups: In the preweaning observations (postnatal days 13, 17 and 21) there were no statistically significant differences among the groups in activity parameters. It was noted that the overall pattern of total session activity counts appeared to suggest acceleration in the standard developmental activity pattern for offspring from parentally-exposed rats, as indicated by somewhat higher counts on postnatal days 13 and 17 relative to the concurrent controls. This apparent alteration was considered due to an unusual within-session pattern of activity, normally considered habituation, in the control groups on postnatal days 13 and 17, at ages prior to the normal development of habituation in the rat. Therefore, the apparent differences in the overall preweaning activity pattern of activity were attributed to an abnormal activity pattern in the control animals and not to parental exposure to ethylbenzene.

In postweaning observations there was an effect ($p<0.05$) on mean total activity for the low dose females on postnatal day 61. A post-hoc Dunnett's test determined that activity for the 25 ppm group females was significantly increased ($p<0.05$) compared to the control group. Similar increases in total activity were not observed in the 100 and 500 ppm group F2 females. Therefore, the 21.6% increase in mean total activity observed in the 25 ppm group females on postnatal day 61 was not attributed to parental ethylbenzene exposure. Overall, the findings described above are not considered treatment related.

- Acoustic startle test in F2 pups: On preweaning PND 20 there were no exposure-related trends were apparent in the 25, 100 and 500 ppm groups males and females on performance measured in the acoustic startle habituation test (peak amplitude [Vmax], latency to maximum response amplitude [Tmax], and average response amplitude [Vave]).
On postweaning PND 60, an effect was observed in the F2 males with all groups exhibiting lower mean Vmax values (37-49% lower than the control group). The differences in the 25 and 500 ppm group males were statistically significant ($p<0.05$) in spite of the high variability noted in all groups tested at this age. No statistically significant effects were obtained on postnatal day 60 in the F2 females, although mean peak response amplitude in animals from the high exposure group was decreased approximately 34% compared to the controls. Mean peak response amplitude values for concurrent control males fell in the upper quartile of the historical control range of the laboratory at this age, unlike the very low values obtained in these same animals at postnatal day 20. Also in the males, there was no indication of a dose-response relationship in startle amplitude, as a similar magnitude of response was obtained in all three ethylbenzene-dosed groups. Additionally, there were no indications of decreased re-activity, arousal, sensory or motor deficits in relevant components of the FOB conducted on these same males also on postnatal day 60. If, indeed, the apparent decreases in peak startle amplitude in all male exposure groups were related to parental ethylbenzene exposure, some corroborative evidence of alterations in reactivity in the FOB would likely have been apparent, at least in males from the 500 ppm group. Therefore, the differences noted in males at this age were attributed to unusual control values and were not considered to be related to parental ethylbenzene exposure.
No morphometric or histological effects in brains of the F2 animals were observed from any exposure group on PND 21 and 72.
In summary, ethylbenzene at an exposure level of 500 ppm did not adversely affect physical development of F1 and F2 offspring or neurodevelopment in either F1 parental animals or in F2 offspring. There were no toxicologically significant changes in pre or post weaning FOB, motor activity parameters, startle parameters, or in the Biel maze performance. The mean brain weights, morphology and histopathology were unaffected by treatment.

Tab 4.1.2.9.2 Summary on studies for developmental toxicity assessment

Type of study	NOAEC	Observed effects	Reference
Prenatal developmental toxicity; guideline according Wistar rat, New Zealand rabbit 100, 1000 ppm	**rat: NOAEC mat tox: ≥ 1000 ppm; NOAEC dev tox: 100 ppm**	**-- stat. sign. ($p \geq 0.05$) ↑ extra and rudimentary ribs at 1000 ppm (LOAEC)**	**Andrew et al., 1981; Hardin et al., 1981**
	rabbit: NOAEC mat tox: ≥ 1000 ppm; NOAEC dev tox: ≥ 1000 ppm	**-- --**	
Prenatal developmental toxicity; non-guideline	**NOAEC dev tox: 138 ppm**	**stat. sign. ↑ in extra ribs and in weight retarded**	**Ungvary and Tatrai, 1985**

CFY rat 138, 276, 552 ppm		**fetuses at 552 ppm (LOAEC)**	
Prenatal developmental toxicity; OECD 414; Sprague Dawley rat 100, 500, 1000, 2000 ppm	**NOAEC mat tox: 500 ppm**	**↓ food consumption; ↓ maternal body weight and corrected maternal weight gain at 1000 ppm (LOAEC)**	**Saillenfait et al. (2003)**
	NOAEC dev tox: 500 ppm	**↓ foetal body weight; ↑ incidence of skeletal variations at 1000 ppm (LOAEC)**	
Prenatal developmental toxicity study; OECD 414 according; Sprague Dawley rat 250, 1000 ppm	**NOAEC mat tox: 250 ppm**	**↓ corrected maternal weight gain at 1000 ppm (LOAEC)**	**Saillenfait et al. (2006)**
	NOAEC dev tox: 250 ppm	**↓ foetal body weight at 1000 ppm (LOAEC)**	
Prenatal developmental toxicity; OECD 414 according; Sprague Dawley rat 250, 1000 ppm	**NOAEC mat tox: 250 ppm**	**↓ corrected maternal weight gain at 1000 ppm (LOAEC)**	**Saillenfait et al. (2007)**
	NOAEC dev tox: 250 ppm	**↓ foetal body weight at 1000 ppm (LOAEC)**	
Peri-/postnatal developmental toxicity; OECD 415 according; Sprague Dawley rat 100/500/1000 ppm	**NOAEC/parental tox: 100 ppm**	**stat. sign. ↓ body weight during first week of exposure; stat. sign. ↑ organ weights (liver, kidney) at 500 ppm (LOAEC)**	**Faber et al. (2007) Stump (2003)**
	NOAEC/dev tox: 100 ppm	**↓ pup survival until PND 4; dose-related mortality and signs of CNS depression in PND 22 exposed offspring; ↓ weanling body weight gain and ↓ body weights by pnd 34 at ≥ 500 (LOAEC)**	

Peri-/postnatal developmental toxicity; OECD 416, Sprague Dawley rat 25/100/500 ppm	**NOAEC/parental tox: ≥ 500 ppm** **NOAEC/dev tox: ≥ 500 ppm**	**--** **--**	**Faber et al. (2006) Stump (2005)**
developmental neurotoxicity; OECD 426, Sprague Dawley rat 25/100/500 ppm	**NOAEC/parental tox: ≥ 500 ppm** **NOAEC/developmental neurotox: ≥ 500 ppm**	**--** **--**	**Faber et al. (2007) Stump (2003)**

Summary and conclusion on toxicity for reproduction:

No human data are available. In a guideline 2-generation study in rats by inhalation, no effects on reproduction or developmental endpoints were noted at exposure levels up to and including 500 ppm with minimal parental toxicity at this exposure level (decreased body weight, increased liver weight). In the preceding 1-generation study no effects on reproduction were found up to and including 1000 ppm, whereas effects on the offspring (reduced postnatal survival and reductions in mean body weight gain) development were observed at levels ≥ 500 ppm. A slight but mostly not significant reduced mean body weight was even noted in the F1 weanlings exposed to 100 ppm beginning on postnatal day 22 in both the inhalation (4-14%) and inhalation/gavage (6-16%) groups. The findings from the functional tests on fertility with the 1- and 2-generation studies are supported with the results from repeated dose toxicity studies without adverse findings by weight and histopathology of reproductive organs, sperm parameters and estrous cyclicity. Thereby no such adverse effects were found in guideline studies after 13 weeks of inhalation at 1000 ppm, after 2 years of inhalation at 750 ppm (apart from possibly neoplastic related testicular effects) and after 13 weeks of oral gavage application at 750 mg/kg bw. A NOAEC/fertility of 1000 ppm (4410 mg/m^3) should be used for quantitative risk assessment, which is derived from both the available 1-generation study **(Faber et al., 2007)** as well as the available guideline according 13 week study **(NTP, 1992)**. For the oral route of exposure a NOAEL/reproductive organ toxicity of 750 mg/kg/d is derived from the available guideline according 13 week study **(Mellert et al., 2007)**. As regards potential developmental toxicity of ethylbenzene, data from guideline according generation studies, prenatal toxicity studies and a developmental neurotoxicity study are available. From the results of theses studies there is no indication for substance induced teratogenicity (up to and including 2000 ppm) or developmental toxicity (up to and including 500 ppm). From the prenatal developmental toxicity studies there is indication for slight fetotoxicity (reduced fetal body weight and occasional increases in skeletal variations) in the presence of maternal toxicity (significantly reduced maternal net weight gain) with a NOAEC for fetotoxicity and maternal toxicity of 500 ppm. Postnatal viability and pup, respectively weanling body weight gain, however, revealed to be more sensitive to substance-induced changes. Based on increased postnatal mortality and body weight gain depression in the offspring of the 1-generation study **(Stump 2003; Faber et al., 2007)**, a NOAEC of 100 ppm (441 mg/m^3) is derived for developmental toxicity, which should be used for quantitative risk assessment.

From overall assessment of the available animal data there is no indication for a specific toxic potential adverse to fertility and/or (physical and neurological) development. Thus there is no need for classification and labelling.

4.1.3 Risk characterisation

4.1.3.1 General aspects

Toxicokinetics and metabolism

Ethylbenzene is absorbed from the lungs, gastrointestinal tract and through the skin. For risk assessment purposes, inhalation absorption percentages of 65 % for humans and 45 % for animals should be taken into consideration. Furthermore, 100 % oral absorption can be assumed for animals and humans. Concerning dermal absorption, percentages of 45 % for humans and 70 % for animals should be taken for risk characterization purposes.
Ethylbenzene is rapidly distributed through the body (e.g. into liver, gastrointestinal tract and adipose tissue). There was, however, no evidence of ethylbenzene accumulation in fat or fat-rich tissues. Species differences have been shown concerning the metabolism of ethylbenzene. Major metabolites result from side-chain oxidation (e.g. mandelic acid, phenylglyoxylic acid (major metabolites in humans), hippuric acid and benzoic acid (major metabolites in rats)). Metabolism by ring oxidation represents a minor pathway.
Ethylbenzene is rapidly metabolized and metabolites are eliminated rapidly from the body, primarily as urinary metabolites. Exhalation and excretion via feces represent minor pathways of elimination. Excretion is almost complete within 24 hrs after exposure.

Acute toxicity

Human data on the acute toxicity of ethylbenzene are not available. In rats ethylbenzene proved to be harmful by inhalation of vapour with an LC_{50} of 17.6 mg/l/4 hours. Oral and dermal toxicity is low. Oral LD_{50} of 3500 mg/kg or 5460 mg/kg were determined for rats in general or male rats, respectively. The acute dermal toxicity was tested with rabbits and resulted in a dermal LD_{50} of 15.5 g/kg. Due to its low viscosity and surface tension, ethylbenzene is suspected to cause lung injury after swallowing even small amounts of the substance.

In humans, high concentrations of ethylbenzene vapours are irritating to mucous membranes of the eyes, nose and respiratory tract. Chemical burns of the eyes, mouth, face, and trunk after a leakage of a pipeline with ethylbenzene are reported.
Ethylbenzene is moderately irritating to the skin of rabbits after single exposure. After repeated exposure it caused definite erythema and development of oedema and superficial necrosis, resulting in a "chapped" appearance and exfoliation of large patches of skin.
Ethylbenzene caused grade 2-3 injury of the eyes of rabbits out of a scale of 10, based on the degree of corneal necrosis after instillation of various amounts and concentrations of chemical. The authors stated that a compound listed with grade 3 or higher would be capable of causing severe corneal injury once a sufficient amount enters the eye. In guinea pigs ethylbenzene vapour of 5000 and 10000 ppm produced immediate and intense irritation of the conjunctiva, while 2000 ppm caused moderate eye and nose irritation within 1 minute.
In different strains of mice RD_{50} of 1432 or 4060 ppm were determined for sensory irritation.

Ethylbenzene is not expected to cause skin or respiratory sensitization.

Repeated dose toxicity

Repeat-dose or prolonged exposure to ethylbenzene specifically affected the nervous system, but did not induce overt toxicity of any other organ system.
In rats and mice, organ weight increase in the liver and kidney was interpreted to be associated to metabolic enzyme induction. Data from several repeat-dose inhalation studies and some oral data did not reveal consistent findings indicating ethylbenzene causes cell damage or dysfunction in the liver or kidneys. The identified NOAEC for adverse effects on the liver and kidneys was 1000 ppm (4.74 mg/l, 92-98 days) for both species. The NOAEL in a guideline oral 90 day study with rats was 75 mg/kg bw/d (LOAEL 250 mg/kg bw/d) based on indications for a mild regenerative anemia and liver changes indicative of microsomal enzyme induction.
At high vapour concentrations of ≥1200 ppm ethylbenzene can reduce body weight gain or lead to early deaths.

The most relevant toxic effects of ethylbenzene are those on the nervous system that may correspond to the toxic profile known from other organic solvents.

Repeated inhalation exposure to ethylbenzene vapor (and putative other routes of administration, shown for the oral route) was irreversibly ototoxic in rats. Auditory dysfunction was localised in the mid frequencies and corresponded to the loss of cochlear outer hair cells, the sensory cells in the inner ear, most sensitive are those of row 3. Hearing loss and cell damage increased with concentrations exposed. The NOAEC for ototoxicity in a 13 week inhalation study was extrapolated to be 114 ppm. At coexposure with a high level of noise or mixed exposure to organic solvents synergistic effects on hearing loss and cell damage of outer hair cells occurred.

Data from other organic solvents give concern that ethylbenzene might also affect the vestibular system of the inner ear and balance function. However, no repeat-dose studies including specific examinations on vestibular function are currently available.

There were indications for neuroendocrine modulation. Short-term exposure to high concentrations above 2000 ppm induced CNS depression, narcosis and neuromuscular dysfunctions.

Mutagenicity

Ethylbenzene produced consistently negative results in bacterial gene mutation tests and in the yeast assay on mitotic recombination. In mouse lymphoma mammalian mutation assays a weak positive response was reported but only at doses with strong cytotoxicity. No clear conlusion can be drawn regarding *in vitro* chromosomal aberration. Without S-9 mix there were equivocal increases in chromosomal aberration frequencies and micronuclei in CHO and SHE cells, respectively, or a negative result in a rat liver cell line. With S-9 mix ethylbenzene did not cause chromosomal aberrations in CHO cells. An *in vitro* SCE test was clearly negative with and without S-9 mix. *In vivo,* ethylbenzene was clearly negative in two micronucleus assays and in an mouse liver UDS assay. In conclusion, on the basis of various mutagenicity tests *in vitro* and *in vivo*, there is currently no relevant indication that ethylbenzene is a germ cell mutagen.

Carcinogenicity

Ethylbenzene was carcinogenic in F344 rats and B6C3F1 mice. Kidney tumors were observed in both sexes of rats, tumors in the testis, liver, and lung occurred in one sex and one species only. Data available suggest that tumors observed could be attributed to species-specific phenomenons. Evidence was considered to be sufficient that it is unlikely that ethylbenzene poses a carcinogenic risk for humans.

Toxicity for reproduction

No specific effects on fertility, pre-/postnatal toxicity or developmental neurotoxicity were noted up to exposure concentrations of 1000, respectively 100 or 500 ppm.

Table 4.1.3.1-1 **Toxicological hazard identification**

	Inhalation	**Dermal**	**Oral**
Acute toxicity	LD50 (species) 17.6 mg/l/4 hours (rat)	LD50 (species) 15 500 mg/kg (rat)	LD50 (species) 3500 mg/kg (rat) 5460 mg/kg (male rat)
Irritation / corrosivity	Skin: irritating to the skin of rabbits Eye: irritating to the eyes of humans, rabbits, guinea pigs Respiratory tract: irritating to the respiratory tract of humans and mice		
Sensitisation	Skin: no skin sensitizaiton in a patch test in humans Respiratory tract: no data		
Repeated dose toxicity (local) Repeated dose toxicity (systemic)	NOAEC 114 ppm (0.5 mg/l)	No data	NOAEL 75 mg/kg bw/d (rat, 90 day study)
Mutagenicity	no relevant evidence for mutagenicity in vitro and in vivo		
Carcinogenicity	No sufficient evidence to be considered as having a carcinogenic potential relevant for humans	No data	No sufficient database for conclusion on carcinogenic potential
Fertility impairment	$NOAEC_{fertility}$ ≥ 1000 ppm	No data	$NOAEL_{fertility}$ 750 mg/kg bw/d
Developmental toxicity	NOAEC 100 ppm LOAEC 500 ppm	No data	No data

4.1.3.2 Workers

4.1.3.2.1 Introductory remarks

For occupational risk assessment of ethylbenzene the MOS approach as outlined in the TGD (Human Health Risk Characterisation, Final Draft) is applied. This occupational risk assessment is based upon the toxicological profile of ethylbenzene (chapter 4.1.2) and the occupational exposure assessment (chapter 4.1.1.2). The threshold levels identified in the hazard assessment are taken forward to this occupational risk assessment.

Systemic availability for different routes of exposure

Experimental data from humans and animals for ethylbenzene show different absorption percentages for the different routes of exposure: According to the chapter 4.1.2.1 on toxicokinetics, metabolism and distribution an adsorption percentage up to 100% is taken for the oral route. 65% is assumed for the inhalation route in humans, 45% absorption percentage after inhalation in animals. Concerning dermal absorption, percentages of 50% for humans and 70% for animals is taken for the risk characterisation.

Occupational exposure and internal body burden

Table 4.1.3.2.A: Ethylbenzene exposure levels which are relevant for occupational risk assessment and internal body burden

Exposure scenario		Inhalation shift average	Dermal contact shift average		Internal body burden of workers after repeated exposure		
					Inhalation(1)	Dermal(2)	Combined
		mg/m^3	mg/p/day	mg/kg/day	mg/kg/day		
1.	Production and further processing	1.3	4.2(3)	0.06	0.12	0.03	0.15
2.	Use of paints, lacquers, inks (containing 20% ethylbenzene)	7	2000(4)	28.5	0.65	14.3	14.95

(1) based on the assumption of 65% inhalation absorption; breathing volume of 10 m^3 per shift
(2) based on the assumption of 50 % systemic availability of ethylbenzene after dermal contact
(3) EASE (90 % protection by suitable gloves)
(4) Analogous data (TGD)

MOS Approach

The MOS approach for human risk characterisation is described in detail in the TGD (Human Health Risk Characterisation, Final Draft). The following chapter contains a short introduction to the MOS approach used. The basic principle of the MOS approach is a comparison of scenario-specific MOS values (the relationship between the experimental NOAEL respectively the adjusted starting point and the exposure level) with a reference MOS (product of various assessment factors).

MOS calculation and the adequate starting point

Basically, MOS values are calculated as quotient of a relevant NOAEL from experimental animal testing or human studies and actual workplace exposure levels. In specific situations, the MOS approach requires a conversion of the original NOAEL into an adequate starting point or corrected NOAEL previously to MOS calculation in order to be directly comparable to the exposure assessment. If the route of application in animal or human studies is different from the actual occupational exposure, the dose units of the experimental data should be converted to the dose unit of the exposure data. Additionally, possible differences in bioavailability between routes, as well as possible differences in bioavailability between animals and hu-

mans should be accounted for the calculation of the corrected NOAEL. If route-specific information on oral and inhalation absorption is not available, the TGD recomments to assume 50% oral absorption and 100% inhalation absorption. For ethylbenzene 65% absorption after inhalation, 50% absorption after dermal contact and 100% absorption after oral exposure are assumed (experimental values).
For occupational risk assessment, the corrected inhalation NOAEC accounts for the difference of the standard respiratory volume (6.7 m^3) and the respiratory volume for light activity (10 m^3).
MOS values are calculated for different routes of exposure and for different toxicological endpoints. The routes of exposure specifically considered in occupational risk assessment are exposure by inhalation and dermal contact.
In addition, for risk assessment of combined exposure (exposure by inhalation and dermal contact) an adequate NOAEL is derived from external NOAELs and specific information on route-specific absorption. For MOS calculation, the adjusted internal starting point is divided by the internal body burden. Depending on route-specific exposure and absorption, inhalation exposure and/or dermal exposure may contribute to the internal body burden. With respect to the possible outcome of an assessment for combined risks, interest focuses on scenarios with conclusion ii at both exposure routes. Based on theoretical considerations, combined exposure will not increase the most critical route-specific risk component more than twice.

Reference MOS

The MOS values calculated have to be compared with a reference MOS. The reference MOS is an overall assessment factor, which is obtained by multiplication of individual assessment factors. The Technical Guidance Document emphasises several aspects which are involved in the extrapolation of experimental data to the human situation. For these assessment factors, default values are recommended. It is important to point out that any relevant substance-specific data and information may overrule the defined default values.
Interspecies extrapolation on the one hand is based on allometric scaling (factor 4 for rats, factor 7 for mice, and factor 2 for rabbits). For remaining interspecies differences the TGD proposes an additional factor of 2.5.
For workers, an adjustment factor for intraspecies differences of 5 is recommended. Based on an evaluation of empirical data by Schneider et al. (2004) it is anticipated that a factor of 5 will be sufficient to protect the major part of the worker population (about 95%).
For chemical substances it is usually expected that the experimental NOAEL will decrease with increasing duration of application. Furthermore, other and more serious adverse effects may appear with prolonged exposure duration. For duration adjustment, a default factor of 6 is proposed for extrapolation from a subacute to chronic exposure. The duration adjustment factor is lower (a factor of 2) for the transition from subchronic experimental exposure to chronic exposure. For ethylbenzene the factor of 2 for an adaptation from subchronic to chronic exposure is used.
The TGD defines two further adjustment factors (uncertainty in route-to-route extrapolation and dose-response relationship including severity of effect). In specific cases these factors may be different from one. For ethylbenzene

Comparison of MOS and reference MOS

The MOS values for different toxicological endpoints and different exposure scenarios are compared with the substance- and endpoint-specific reference MOS. MOS values clearly above the reference MOS do not lead to concern, whereas MOS values that are clearly below

the reference MOS are cause for concern. There may be various risk-related aspects which are not covered by default assessment factors. These additional qualitative aspects should be carefully considered when performing a risk assessment and should have an adequate influence on finding of conclusions.

Critical Exposure Levels

In a parallel procedure, which gives identical but more direct results, the adjusted toxicological starting point is directly divided by the reference MOS. As a result, an exposure level (in mg/m^3 or mg/kg/d) is identified, which may serve as a direct trigger for decisions when compared with the occupational exposure levels. In the context of this risk assessment report this trigger value is called "critical exposure level". Concern will be expressed for scenarios with occupational exposure levels higher than the relevant "critical exposure level".

4.1.3.2.2 Occupational risk assessment

Acute toxicity

Inhalation

Human data on the acute toxicity of ethylbenzene are not available. Animal data show, that high concentrations of ethylbenzene result in deaths of experimental animals. An LC_{50} of 17 600 mg/m^3 after 4 hours of ethylbenzene inhalation in rats is reported. The concentration of 8 800 mg/m^3 resulted in 2 of 6 dead rats within 14 days after inhalation period.

In another, shortly reported subacute study F344 rats, B6C3F1 mice and New Zealand rabbits were exposed for 6 hours/day on 4 consecutive days at vapour concentrations of 0, 1 700, 5 300, and 10 6000 mg/m^3 (Biodynamics, 1986). The NOAEC in rats and mice was 1 700 mg/m^3 with increased kidney and liver weights without histopathological changes. For rabbits the NOAEC was 10 600 mg/m^3.

Comparing the LC_{50}-value of ca. 17 600 mg/m^3 and the concentration of 1 700 mg/m^3 without histopathological changes with the highest exposure concentration of 7 mg/m^3 (scenario 2) a relevant risk concerning acute toxicity is not expected under normal workplace conditions.

Conclusion: ii

Dermal contact

Oral and dermal toxicity of ethylbenzene is low with LD_{50} values above 2 000 mg/kg: an oral LD_{50} of 3 500 mg/kg was determined for rats in general, and an oral LD_{50} of 5 460 mg/kg specifically for male rats; the acute dermal toxicity was tested with rabbits and revealed a dermal L_{50} of 15 500 mg/kg.

Comparing the LD_{50} of above 2 000 mg/kg with the highest dermal exposure of 28.5 mg/kg (scenario 2, use of paints, laquers and inks) a relevant risk concerning acute toxicity is not expected under normal workplace conditions.

Conclusion: ii

Irritation/Corrosivity

Acute Inhalation

In humans, high concentrations of ethylbenzene vapours are irritating to mucous membranes of the eyes, nose and respiratory tract.
Chemical burns of the eyes, mouth, face, and trunk after a leakage of a pipeline with ethylbenzene are reported.
Acute exposure to vapours of ethylbenzene in air concentrations of 0.5% and 1% (equivalent to 5000 and 10 000 ppm) produced immediate intense irritation to the conjunctiva and nasal mucous membranes in guinea pigs. A concentration of 0.2% (2 000 ppm) produced moderate eye and nasal irritation within one minute and a concentration of 0.1% (1 000 ppm) caused slight nasal irritation.
Comparing the value of 1 000 ppm (corresponding to 4 340 mg/m^3) with the exposure value of 7 mg/m^3 a relevant risk concerning to irritation is not expected under normal workplace conditions.

Conclusion: ii

Sensory irritation

Sensory irritation by airborne ethylbenzene was reported from animal data. A test, made with male Swiss-Webster mice showed a RD50-value of 4 060 ppm. Alarie introduced the air concentration of 0.03 x RD50 as prediction of an exposure level with a minimal or low degree of sensory irritation in humans. The according air concentration for ethylbenzol calculates to 122 ppm or 530 mg/m^3 (4 060 ppm x 0.03). Analysis of experimental and human data on sensory irritation mainly is based on the relationship between RD50 values in animals and human thresholds for sensory irritation (and not on the corresponding relationship for minimal experimental effects). For that reason it is preferred to start risk assessment with the general approach (0.03 x RD50) instead of using lower experimental effect levels for which there is no specific experience as to adequate adjustment factors.

In workers the stinging and burning sensation caused by stimulation of the trigeminus nerve which is closely connected to respiratory depression is generally perceived within few minutes after exposure. Thus stimulation of the trigeminus nerve, unlike other effects, does not depend significantly on exposure duration. The main trigger for effects seems to be the air concentration of the substance. Risk assessment therefore does not correct for exposure duration and short term values are also included in MOS calculation.

The exposure level of about 530 mg/m^3 is chosen as starting point concerning respiratory depression. In this range of exposure a relevant effect is not anticipated to occur in humans. For evaluation of the resulting MOS values no further aspects have to be taken into account. The corresponding reference MOS is considered to be 1.

The highest identified inhalative exposure values are described for scenario 2 with an exposure value of 7 mg/m^3 . Based on the combined interpretation of the RD50 data and human experience conclusion ii is applied for these occupational exposure scenarios with respect to sensory irritation of ethylbenzene.

Conclusion: ii

Table 4.1.3.2.B: MOS values for sensory irritation of ethylbenzene

	Inhalation		
Starting point for MOS calculation	530 mg/m^3		
Reference MOS	1		
Critical exposure level	530 mg/m^3		
	Exposure (mg/m^3)	MOS	Conclusions
1 Production and further processing	1.3	407	ii
2 Use of paints, lacquers, inks (containing 20% ethylbenzene)	7	76	ii

Dermal/Eyes

Data on skin irritation tests according to international test guidelines are not available. On the basis of two available tests with rabbits a moderate skin irritation potential after single application of the substance and a high defatting potential leading to severe effects after repeated skin contact can be concluded.
Ethylbenzene caused grade 2-3 injury of the eyes of rabbits out of a scale of 10, based on the degree of corneal necrosis after instillation of various amounts and concentration of the chemical.
A classification and labelling as Xi, Irritant, R 36/38 irritating to the eys and skin is warranted.
On the grounds that control measures exist which can minimise dermal exposure and corresponding risk of irritation, conclusion ii is proposed. However, these controls must be implemented and complied with to reduce the risk of damage to skin and the eyes.

Conclusion: ii

Sensitization

Dermal contact

Animal data on skin sensitisation tests are not available.

Kligman conducted a maximisation test with 10% ethylbenzene (no data on purity) in petrolatum on 25 volunteers. Ethylbenzene produced no sensitisation reactions. No concern is expressed.

Conclusion: ii

Inhalation

No information on respiratory sensitisation is available. However, in view of the fact that during all the years of use specific case reports have not been reported, ethylbenzene seems at least not to be a strong respiratory sensitizer in humans. For the time being no animal model is available which would be able to verify the question of respiratory sensitisation. In summary concern is not expressed.

Conclusion: ii

Repeated dose toxicity

Local effects

Inhalation and dermal contact

See under chapter of Irritation. No further realizable information concerning local effects are available.

Conclusion: ii

Systemic effects

Repeated exposure of ethylbenzene (oral and inhalation route) affects the nervous system and leads to effects at the liver and kidney in experimental animals.

Inhalation exposure

An increase in liver and kidney weight of rats and mice without histopathological alterations has been found in several studies. According to chapter 4.1.2.5 these changes are most probably related to enzyme induction (see also chapter 4.1.2.5). The NOAEL in a guideline oral 90 day study with rats was 75 mg/kg bw/d (LOAEL 250 mg/kg bw/d) based on indications for a mild regenerative anemia and liver changes indicative of microsomal enzyme induction.
Repeated inhalation exposure to ethylbenzene vapor was irreversibly ototoxic in rats (Gagnaire et al., 2007). Auditory dysfunction was localised in the mid frequencies and corresponded to the loss of cochlear outer hair cells, the sensory cells in the inner ear. Hearing loss and cell damage increased with concentrations exposed. In a 90 day rat inhalation study (6 hours/day, 6 day/week) the NOAEC for ototoxicity was extrapolated to be 114 ppm (500 mg/m^3). According to several case reports, where hearing deficits in humans occupational ex-

posed to organic solvents or from people after solvent abuse is described (for review cf Risk Assessment Reports on toluene and styrene) this rat data are taken to be relevant for humans. Thus, this extrapolated NOAEC of 114 ppm (500 mg/m^3) is taken for the risk assessment of repeated dose toxicity.

The extrapolated NOAEC of 114 ppm (500 mg/m^3) from the rat is (1) multiplied with a factor of 0.45 (for rat absorption percentage of 45%), divided by a divisor of 0.65 (for human absorption percentage after inhalation of 65%) and (2) multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. Further differences regarding the the experimental inhalation duration (6 hours/day, 6 days/week) and the working conditions (8 hours/day, 5 days/week) are not considered, because they roughly balance each other. The calculation results in an adjusted inhalation starting point of 232 mg/m^3 (500 • 0.45 / 0.65 • 6.7/10).

The following adjustment factors are applied for the identification of the reference MOS. For (1) interspecies differences the default factor is 2.5 (the factor for allometric scaling is already implicitly applied), for (2) intraspecies differences (workers) the default factor is 5, and for (3) duration adjustment a factor of 2 is used. Thus the reference MOS calculates to 25 (2.5 • 5 • 2). The critical inhalation exposure level at the workplace is identified as 9.3 mg/m^3 (232 / 25).

The highest shift average value for inhalation is reported in scenario 2 (use of paints, lacquers, inks containing 20% ethylbenzene) with a value of 7 mg/m^3. With a critical exposure level of 9.3 mg/m^3 there results no concern for this endpoint. For corresponding MOS values see table 4.1.3.2.C.

Conclusion: ii

Dermal contact and combined exposure

No information is available for systemic toxicity after repeated dermal exposure. Therefore the extrapolated NOAEC of 114 ppm (500 mg/m^3) from the 90 day inhalation rat study is taken for dermal risk assessment.

Expressed as (external) dose the value of 500 mg/m^3 corresponds to 190 mg/kg/day (500 mg/m^3 • default respiratory volume for the rat for 8 hours of 0.38 m^3/kg). With a rat adsorption percentage of 45% after inhalation the internal starting point corresponds to 86 mg/kg/day (190 mg/kg/day • 0.45). To get the (external) value for dermal contact the dermal absoption percentage of 50% for humans has to be included. Thus the internal value has to be multiplied with a factor of 2. This results in an adjusted external starting point of 172 mg/kg/day (86 mg/kg/day • 2).
The following adjustment factors are applied for the identification of the reference MOS. For (1) interspecies differences the adjustment factor is 4 • 2.5 (factor 4 for allometric scaling and factor 2.5 for remaining interspacies differences), for (2) intraspecies differences (workers) the default factor is 5, and for (3) duration adjustment a factor of 2 is used. Thus the reference MOS calculates to 100 (4 • 2.5 • 5 • 2). The critical external dermal exposure level at the workplace is identified as 1.7 mg/kg/day (172 / 100). The internal critical exposure level is 0.86 mg/kg/day (86 / 100).

The exposure scenario 2 (use of paints, lacquers, inks containing 20% ethylebnzene) for dermal contact is reported as 28.5 mg/kg/day. The exposure level in this occupational scenario is nearly 15 fold higher than the critical dermal exposure value of 1.7 mg/kg/day. Concern is expressed for dermal exposure of this scenario 2. Because of the concern for dermal exposure also for combined exposure of scenario 2 concern is expressed as well. Scenario 1 does not reach concern. For corresponding MOS values see table 4.1.3.2.C.

Conclusion: iii

Table 4.1.3.2.C: MOS values for repeated dose toxicity of ethylbenzene, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	233 mg/m^3			172 mg/kg/day			86 mg/kg/day		
Reference MOS	25			100			100		
Critical exposure level	9.3 mg/m^3			1.7 mg/kg/day			0.86 mg/kg/day		
	Exposure (mg/m^3)	MOS	Conclusions	Exposure (mg/kg/d)	MOS	Conclusions	Internal body burden (mg/kg/d)	MOS	Conclusions
1. Production and further processing	1.3	180	ii	0.06	2 870	ii	0.15	573	ii
2. Use of paints, lacquers, inks (containing 20% ethylbenzene)	7	33	ii	28.5	6	iii	14.95	5.7	iii

Mutagenicity

Ethylbenzene produced consistently negative results in bacterial gene mutation tests and in the yeast assay on mitotic recombination. In mouse lymphoma mammalian mutation assays a weak positive response was reported but only at doses with strong cytotoxicity. No clear conclusion can be drawn regarding *in vitro* chromosomal aberration. Without S-9 mix there were equivocal increases in chromosomal aberration frequencies and micronuclei in CHO and SHE cells, respectively, or a negative result in a rat liver cell line. With S-9 mix ethylbenzene did not cause chromosomal aberrations in CHO cells. An *in vitro* SCE test was clearly negative with and without S-9 mix. *In vivo*, ethylenzene was clearly negative in two micronucleus assays and in a mouse liver UDS assay. In conclusion, on the basis of various mutagenicity tests *in vitro* and *in vivo*, there is currently no relevant indication that ethylbenzene is a germ cell mutagen.

Conclusion: ii

Carcinogenicity

Long-term inhalation exposure on rats and mice (0, 75, 250 and 750 ppm ethylbenzene for 104 weeks, 6 hours/day, 5 days/week) was carcinogenic in F344 rats and B6C3F1 mice. A significant increase of tumor incidences has been observed in the kidneys (renal tubule adenoma and carcinoma), testis (interstitial cell adenoma), liver (adenoma and carcinoma) and lung (alveolar/bronchiolar adenoma and carcinoma).

There was no concordance in carcinogenic response between rats and mice. Elevated rates of kidney tumors were seen in male and female rats. Each of other tumors occurred in one sex and in one species only. Genotoxicity data did not indicate a direct DNA damaging effect.

With reference to chapter 4.1.2.7 there is sufficient evidence that kidney tumors in male and female rats are associated with the high strain-specific incidence of chronic progressive nephropathy (CPN) that is unknown for humans. For tumors in the testis, liver and lung high or very high spontaneous rates occur in the mouse and rat strains used. Ethylbenzene may exert its carcinogenic action by enhancement of tumor development in genetically disposed animals or by reduction in latency periods in tumor development.

Although the detailed mechanisms underlying the increases in tumor rates are presently not clarified, it appears likely that the mode of carcinogenic action of ethylbenzene possesses species and strain specificity. Therefore the toxicological significance and relevance to human health of these findings is uncertain. It appears unlikely from the data available that ethylbenzene poses a carcinogenic risk for humans exposed.

Conclusion: ii

Reproductive toxicity

Fertility impairment

No human data are available. In a guideline 2-generation study in rats by inhalation, no effects on reproduction were noted at exposure levels up to and including 500 ppm with minimal parental toxicity at this exposure level (decreased body weight, increased liver weight). In the preceding 1-generation study no effects on reproduction were found up to and including 1 000 ppm. The findings from the functional tests on fertility with the 1- and 2-generation studies are supported with the results from repeated dose toxicity studies without adverse findings by weight and histopathology of reproductive organs, sperm parameters and estrous cyclicity. Thereby no such adverse effects were found in guideline studies after 13 weeks of inhalation at 1 000 ppm, after 2 years of inhalation at 750 ppm (apart from possibly neoplastic related testicular effects) and after 13 weeks of oral gavage application at 750 mg/kg.

Based on the available data, there seems to be no specific risk for fertility effects.

Conclusion: ii

Developmental toxicity

Data from a 2-generation study and prenatal toxicity studies and a developmental neurotoxicity study are available. From the results of these studies there is no indication for substance induced teratogenicity (up to and including 2000 ppm) or developmental toxicity (up to and including 500 ppm). In the presence of maternal toxicity there is indication for slight fetotoxicity (reduced fetal body weight and occasional increases in skeletal variations) with a NOAEC for fetotoxicity and maternal toxicity of 500 ppm.

However, a reduction of postnatal viability and pup survival, respectively weanling body weight gain was found in a 1-generation reproduction toxicity study with Sprague Dawley rats which inhaled 100, 500 and 1 000 ppm (Strump, 2003; Faber et al., 2007, see also chapter 4.1.2.9). Based on increased postnatal mortality and body weight gain depression in the offspring a NOAEC of 100 ppm (441 mg/m^3) was derived for developmental toxicity. This value is used for the quantitative risk assessment of developmental effects after inhalation and also after dermal contact.

Inhalation exposure

The NOAEC of 100 ppm (441 mg/m^3) from the rat is (1) multiplied with a factor of 0.45 (for rat absorption percentage of 45%) and divided by a divisor of 0.65 (for human absorption percentage after inhalation of 65%) and (2) multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. Further differences regarding the the experimental inhalation duration and the working conditions are not considered, because there is no detailed information about exposure conditions. The calculation gives an inhalation starting point of 205 mg/m^3 (441 • 0.45 / 0.65 • 6.7/10).

The following adjustment factors are applied for the identification of the reference MOS. For (1) interspecies differences the default factor is 2.5 (the factor for allometric scaling is already implicitly applied), for (2) intraspecies differences (workers) the default factor is 5. Thus the reference MOS calculates to 12.5 (2.5 • 5). The critical inhalation exposure level at the workplace is identified as 16.4 mg/m^3 (205 / 12.5).

The shift average value for inhalation is reported as 7 mg/m^3 for scenario 2 of ethylbenzene. The exposure level in this occupational scenario is higher than the critical inhalation exposure of 16.4 mg/m^3. No concern is derived. For corresponding MOS values see table 4.1.3.2.D.

Conclusion: ii

Dermal contact and combined exposure

The NOAEC of 100 ppm (441 mg/m^3) from the 1-generation rat study is taken for dermal risk assessment (see above).

The NOAEC of 441 mg/m^3 corresponds to an external dose of 127 mg/kg/day (441 mg/m^3 • default respiratory volume for the rat for 6 hours of 0.288 m^3/kg). With a rat adsorption percentage of 45% after inhalation the internal critical exposure level corresponds to 57 mg/kg/day (127 mg/kg/day • 0.45). To get the (external) value for dermal contact the dermal absoption percentage of 50% for humans has to be included. Thus the internal value has to be multiplied with a factor of 2. This gives an external starting point of 114 mg/kg/day (57 mg/kg/day • 2).

The following adjustment factors are applied for the identification of the reference MOS. For (1) interspecies differences the adjustment factor is 4 • 2.5 (factor 4 for allometric scaling and factor 2.5 for remaining interspacies differences), for (2) intraspecies differences (workers) the default factor is 5. Thus the reference MOS calculates to 50 (4 • 2.5 • 5). The critical dermal exposure level at the workplace is identified as 2.3 mg/kg/day (114 / 50). The internal critical exposure level is 1.1 mg/kg/day (57 / 50).

The shift average value for dermal contact is reported as 28.5 mg/kg/day for scenario 2 (use of paints, lacquers, inks containing 20% ethylbenzene). The exposure level in this occupational scenario is about 12 fold higher than the critical dermal exposure of 2.3 mg/kg/day. Concern is expressed regarding dermal and combined exposure for this scenario 2. For corresponding MOS values see table 4.1.3.2.D.

Conclusion: iii

Table 4.1.3.2.D: MOS values regarding developmental effects of ethylbenzene

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	205 mg/m^3			114 mg/kg/day			57 mg/kg/day		
Reference MOS	12.5			50			50		
Critical exposure level	16.4 mg/m^3			2.3 mg/kg/day			1.1 mg/kg/day		
	Exposure (mg/m^3)	MOS	Conclusions	Exposure (mg/kg/d)	MOS	Conclusions	Internal body burden (mg/kg/d)	MOS	Conclusions
1 Production and further processing	1.3	158	ii	0.06	1900	ii	0.15	380	ii
2 Use of paints, lacquers, inks (containing 20% ethylbenzene)	7	29	ii	28.5	4	iii	14.95	3.8	iii

4.1.3.2.3 Summary of conclusions for the occupational risk assessment

As result of occupational risk assessment for ethylbenzene, concern is expressed and risk reduction measurs have to be initiated. The most important toxicological endpoints are repeated dose toxicity and developmental toxicity. For all other endpoints no concern is expressed. Table 4.1.3.2.E indicates the toxicological endpoints of concern for ethylbenzene.

Table 4.1.3.2.E indicates the toxicological endpoints of concern for ethylbenzene

Table 4.1.3.2.E: Endpoint-specific overall conclusions

Toxicological endpoints		concern for at least one scenario
Acute toxicity	inhalation	ii
	dermal	ii
	combined	ii
Irritation/ Corrosivity	dermal	ii
	eye	ii
	acute respiratory tract	ii
Sensitisation	skin	ii
	respiratory	ii
Repeated dose toxicity	local, inhalation	ii
	local, dermal	ii
	systemic, inhalation	ii
	systemic, dermal	iii
	systemic, combined	iii(1)
Mutagenicity		ii
Carcinogenicity	inhalation	ii
	dermal	ii
	combined	ii
Fertility impairment	inhalation	ii
	dermal	ii
	combined	ii
Developmental toxicity	inhalation	ii
	dermal	iii
	combined	iii(1)

1) conclusion iii already results from dermal exposure, therefore no specific concern for the combined exposure scenario is indicated

Risk estimation is mainly based on animal inhalation studies. Based on experimental data an adsorption percentage of 45% is taken for the rat inhalation route, whereas for humans an absporption percentage of 65 % is assumed. For the dermal pathway an absorption percentage of 50% is assumed for humans.

The most important toxicological endpoints are repeated dose toxicity and developmental toxicity of ethylbenzene. On the background of the exposure assessment and the proposed critical exposure levels, the according health risks especially after dermal contact have to be reduced.

Conclusion (iii) applies to dermal and combined exposure of scenario 2 (use of paints, lacquers, inks containing 20% ethylbenzene) after repeated dose toxicity and regarding developmental toxicity. The exposure value of this scenario with a value of 28.6 mg/kg/day is about 17 fold higher than the critical exposure level of 1.7 mg/kg/day (systemic effects after repeated exposure) and about 12 fold higher than the critical exposure level, resulting from developmental toxicity.

For inhalation the critical exposure level of 9.3 mg/m^3 results from systemic effects after repeated exposure. The inhalation exposure values of scenario 1 (production and processing) with 1.3 mg/m^3 and scenario 2 (use of paints, lacquers, inks containing 20% ethylbenzene) with 7 mg/m^3 are below this value, thus reaching no concern.

4.1.3.3 Consumers

Consumer exposure

From the table in 4.1.1 it can be taken that priming of floors may lead to highest exposures from inhalation. The mean event concentration is 970 mg/m3. Measured exposure data e.g. on manual painting and metal surface coating at working environment has been provided. The 50 percentile of the exposure levels (German data) is much lower than the calculated value with CONSEXPO for consumer. This shows an unequivocal overestimation of the inhalation exposure level for the consumer. The above information will be used for risk characterisation in acute toxicity.

The chronic exposure due to daily use of nail hardener would lead to an average yearly exposure of 4 µg/m^3 . Measured exposure data on indoor air are available from two studies. The reported 95 percentiles from indoor air levels of ethylbenzene account about 20 µg/m^3. In a more recent investigation (involved 11 laboratories) an average indoor air level in an office room of 1.3 µg/m^3 was determined (2004).
Due to the selected inhalation exposure scenarios only the route by inhalation will be used forward to risk characterisation.

Acute toxicity

The LC_{50} value after inhalation was determined as 17600 mg/m^3 in rats. The sign of toxicity were not described. The exposure duration in this study was 4 h. The highest acute exposure of consumers from inhalation is estimated to be 970 mg/m^3. The corresponding scenario (priming of floors) is an over estimation. In view of this the margin of safety is judged to be sufficient.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Irritation/Corrosivity

From human and animal data ethyl benzene has shown the potential to cause adverse effects to the skin, eyes and respiratory tract. Therefore a classification based on GHS H319 eye Irrit.2, H315 Skin Irrit.2 and H335 STOT Single3 is proposed. .

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Sensitization

Animal and data on skin or inhalation sensitisation tests with ethylbenzene are not available. Human data show that no sensitisation potential is expected for ethylbenzene. Therefore there is no concern for consumers.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Repeated dose toxicity

Inhalation exposure of the consumer:

Repeated inhalation exposure to ethylbenzene vapor was irreversibly ototoxic in rats (Gagnaire et al., 2007). Auditory dysfunction was localised in the mid frequencies and corresponded to the loss of cochlear outer hair cells, the sensory cells in the inner ear. Hearing loss and cell damage increased with concentrations exposed. In a 90 day rat inhalation study (6 hours/day, 6 day/week) the NOAEC for ototoxicity was extrapolated to be 114 ppm (500 mg/m^3). According to several case reports, where hearing deficits in humans occupational exposed to organic solvents or from people after solvent abuse is described (for review cf Risk Assessment Reports on toluene and styrene) this rat data are taken to be relevant for humans. Thus, this extrapolated NOAEC of 114 ppm (500 mg/m^3) is taken for the risk assessment of repeated dose toxicity by inhalation.

For the MOS calculation via inhalation route one inhalation rat study is available with 9 exposure days.

For the decision on the appropriateness of MOS, the following aspects have been considered and taken into account:

- overall confidence in the database

The data taken into account for performing the risk characterisation have been evaluated with regard to their reliability, relevance and completeness according to section 3.2 of the TGD. The data were published in a peer reviewed journal. There are no reasons to assume limited confidence in the data which are chosen as the basis of the risk characterisation.

- intra- and interspecies variation

Specific investigations about toxicokinetic behaviour and metabolism after inhalation are available. The risk assessment purposes inhalation absorption percentages of 65% for humans and 45 % for animals that should be taken into account for risk characterisation.

- the nature and severity of the effect

The systemic effects described are mainly effects regarding ototoxicity. These effects are considered as serious health effects.

There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

- dose-response relationship

A dose response relationship has beendescribed in the 90 day rat study..

- differences in exposure (route, duration, frequency and pattern)

The estimated chronic exposure after inhalation is compared with a $NOAEC_{sys}$ derived from the 90 day rat study. The exposure estimate is based on the use of nail hardener with 4 ug/m3 the scenario with the highest value described. There are no reasons to assume that special concern can be derived from this procedure.

- the human population to which the quantitative and/or qualitative information on exposure applies.

There are no substance-specific data which allow to quantify possible sensitivity differences among consumers. Following the exposure scenario there is no reason to assume a special risk for elderly, children or other people suffering from special diseases.

- other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for inhalation exposure of the consumer, systemic effects:

The use of nail hardener will result in exposure via inhalation of 4 μug/m^3, corrresponding to an internal exposure of 1,8 μg/m3.

The margin of safety between the

exposure estimate 1,8 μ/mg3

and the

NOAECL$_{sys}$ 441 mg/m3

is judged to be sufficient.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

MOS for inhalation exposure of the consumer, local effects:

Due to the very low exposure value for the use of nail hardener there is no need for MOS calculation.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Mutagenicity

There is no relevant concern with respect to mutagenicity. Ethylbenzene should not be classified as a germ cell mutagen.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Cancerogenicity

Long-term inhalation study in animals to ethylbenzene demonstrated carcinogenic effects. There was no concordance in carcinogenic response between rats and mice. It appears likely that the mode of carcinogenic action of ethylbenzene possesses species and strain specificity.The toxicological significance and relevance to human health of the animal data is uncertain. Therefore it appears unlikely from the data available that ethylbenzene poses a carcinogenic risk for humans exposed.

However, taking into account the negative mutagenicity data it is concluded that carcinogenicity should not be an endpoint of concern.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Reproductive Toxicity

Fertility Impairment

No human data are available. In a guideline 2-generation study in rats by inhalation, no effects on reproduction were noted at exposure levels up to and including 500 ppm with minimal parental toxicity at this exposure level (decreased body weight, increased liver weight). In the preceding 1-generation study no effects on reproduction were found up to and including 1 000 ppm. The findings from the functional tests on fertility with the 1- and 2-generation studies are supported with the results from repeated dose toxicity studies without adverse findings by weight and histopathology of reproductive organs, sperm parameters and estrous cyclicity. Thereby no such adverse effects were found in guideline studies after 13 weeks of inhalation at 1 000 ppm, after 2 years of inhalation at 750 ppm (apart from possibly neoplastic related testicular effects) and after 13 weeks of oral gavage application at 750 mg/kg.

Based on the available data, there seems to be no specific risk for fertility effects.

Therefore a risk characterisiation will not be performed under these circumstances.

Developmental toxicity

Data from a 2-generation study and prenatal toxicity studies and a developmental neurotoxicity study are available. From the results of these studies there is no indication for substance induced teratogenicity (up to and including 2000 ppm) or developmental toxicity (up to and including 500 ppm). In the presence of maternal toxicity there is indication for slight fetotoxicity (reduced fetal body weight and occasional increases in skeletal variations) with a NOAEC for fetotoxicity and maternal toxicity of 500 ppm.

However, a reduction of postnatal viability and pup survival, respectively weanling body weight gain was found in a 1-generation reproduction toxicity study with Sprague Dawley rats which inhaled 100, 500 and 1 000 ppm (Strump, 2003; Faber et al., 2007, see also chapter 4.1.2.9). Based on increased postnatal mortality and body weight gain depression in the offspring a NOAEC of 100 ppm (441 mg/m^3) was derived for developmental toxicity. This value is used for the quantitative risk assessment of developmental effects after inhalation .

For the decision on the appropriateness of MOS, the following aspects regarding the critical effect as well as exposure have been considered and taken into account:

- overall confidence in the database

The data taken into account for performing the risk characterization have been evaluated with regard to their reliability, relevance and completeness according to section 3.2 of the TGD. The data were submitted to the Competent Authority in private reports being adequately detailed and in accordance with internationally recognized guidelines and to GLP.

There are no reasons to assume limited confidence.

- uncertainty arising from the variability in the experimental data

The findings of all studies are not contradictory so that the judgement can be based on the database (cf. 4.1.2.9).

-intra- and interspecies variation.

There are no indications to limit the findings to a single species. The effects are expected to be based on the chemical structure.

- the nature and severity of the effect

The developmental effects are considered to be severe health effects per se. They were observed essentially at high exposure concentrations of 500 and 1000 ppm.. There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

- dose-response relationship

The mentioned effects were observed at maternally toxic doses.

- differences in exposure (route, duration, frequency and pattern)

Following the exposure assessment, the consumer may be exposed to ethylbenzene by inhalation. The systemic NOAEC was derived from a one generation study. The resulting internal intake can be used for the risk characterisation the absorption rate on board (cf. 4.1.3.3)

MOS for inhalation exposure of the consumer:

The use of nail hardener will result in an internal ethylbenzene exposure concentration of 1,8 µg/m³. The margin of safety between the

exposure estimate 1,8 µg/m³

and the

____

NOAEC/dev.tox. of 441 mg/m³

is judged to be sufficient, taking into account the nature and severity of the effect and the overestimated daily exposure figures.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Summary of risk characterisation for consumers

For all calculated toxicological endpoint for MOS values there is no need for further information or testing and for a follow up for risk reduction measures.

4.1.3.4 Man exposed indirectly via the environment

When considering possible risks to human health arising from indirect exposure to ethylbenzene via the environment the key areas of possible concerns are for repeated dose toxicity, mutagenicity, carcinogenicity, and reproductive toxicity.

As written in 4.1.1.4 Indirect exposure via the environment the applied model calculations are of preliminary nature and therefore in addition due to the very low seleczted exposure values there is no need for performing MOS calculation.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

4.1.3.5 (Combined exposure)

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Occupational exposure

4.2.1.2 Consumer exposure

4.2.1.3 Indirect exposure via the environment

4.2.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.2.2.1 Explosivity

4.2.2.2 Flammability

4.2.2.3 Oxidising potential

4.2.3 Risk characterisation

4.2.3.1 Workers

4.2.3.2 Consumers

4.2.3.3 Man exposed indirectly via the environment

5 CONCLUSIONS / RESULTS

Overall results of the risk assessment:

( ) i) There is need for further information and/or testing

( ) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(x) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (ii) applies to

all production sites as well as all production and processing sites to *Surface water*, *Waste water treatment plants* and the *Terrestrial compartment*. It also applies to biotic effects of ethylbenzene in the *Atmosphere*.

Conclusion (iii) applies to

Atmosphere (indirect effects of ethylbenzene)

Conclusion (iii) applies to the contribution of the commercial product ethylbenzene to the formation of ozone. In the context of the consideration of which risk reduction measures that would the most appropriate, it is recommended that under the relevant Air Quality Directives a specific in-depth evaluation be performed. Such an evaluation should focus on the contribution of isolated as well as non-isolated ethylbenzene to the complex issue of ozone and smog formation and the resulting impact on air quality.

Human Health

Workers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Conclusion (iii) applies to dermal and combined exposure of scenario 2 (use of paints, lacquers, inks containing 20% ethylbenzene) after repeated dose toxicity and regarding

developmental toxicity. On the background of systemic effects after repeated exposure, dermal exposure should be controlled to levels in the range of 1.7 mg/kg/day or 120 mg/person/day (critical exposure level for systemic effects of repeated dose toxicity). If the exposure is reduced to this level, dermal risks from other endpoints, as developmental toxicity would similary and effectively be mitigated too.
Concerning inhalation exposure, the critical exposure level is 9.3 mg/m^3. The exposure values of scenario 1 (production and processing) and scenario 2 (use of paints, lacquers, inks containing 20% ethylbenzene) are below this value, thus reaching no concern.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

consumers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

For all calculated toxicological endpoint for MOS values there is no need for further information or testing and for a follow up for risk reduction measures.

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Appendix III

Continental and regional exposure

SimpleBox2.0a - Berechnung regionaler + kontinentaler PEC's

- Anpassung an TGD (1996) / EUSES 1.00: Michael Feibicke (06/98)

INPUT - Ethylbenzene

Parameter names acc. SimpleBox20	Unit	Input	Parameter names according Euses
Physicochemical properties			
COMPOUND NAME	[-]	Ethylbenzene	Substance
MOL WEIGHT	[$g.mol^{-1}$]	106,17	Molecular weight
MELTING POINT	[° C]	-95	Melting Point
VAPOR PRESSURE(25)	[Pa]	930	Vapour pressure at 25°C
log Kow	[log10]	3,13	Octanol-water partition coefficient
SOLUBILITY(25)	[$mg.l^{-1}$]	160	Water solubility
Distribution - Partition coefficients			
- Solids water partitioning (derived from K_{oc})			
Kp(soil)	[$l.kg_d^{-1}$]	8,636	Solids-water partitioning in soil
Kp(sed)	[$l.kg_d^{-1}$]	21,59	Solids-water partitioning in sediment
Kp(susp)	[$l.kg_d^{-1}$]	43,182	Solids-water partitioning in sudpended matter
- Biota-water			
BCF(fish)	[$l.kg_w^{-1}$]	91,3	Biocentration factor for aquatic biota
Degradation and Transfromation rates			
- Characterisation and STP			
PASSreadytest	[y / n]	y	Characterization of biodegradability
- Environmental Total Degradation			
kdeg(air)	[d^{-1}]	3,24E-01	Rate constant for degradation in air
kdeg(water)	[d^{-1}]	4,70E-02	Rate constant for degradation in bulk surface water
kdeg(soil)	[d^{-1}]	2,31E-02	Rate constant for degradation in bulk soil
kdeg(sed)	[d^{-1}]	2,31E-03	Rate constant for degradation in bulk sediment
Sewage treatment (e.g. calculated by SimpleTreat)			
- Continental			
FR(volatstp) [C]	[-]	4,67E-01	Fraction of emission directed to air (STPcont)
FR(effstp) [C]	[-]	5,40E-02	Fraction of emission directed to water (STPcont)
FR(sludgestp) [C]	[-]	3,70E-02	Fraction of emission directed to sludge (STPcont)
- Regional			
FR(volatstp) [R]	[-]	4,67E-01	Fraction of emission directed to air (STPreg)
FR(effstp) [R]	[-]	5,40E-02	Fraction of emission directed to water (STPreg)
FR(sludgestp) [R]	[-]	3,70E-02	Fraction of emission directed to sludge (STPreg)
Release estimation			
- Continental			
Edirect(air) [C]	[$t.y^{-1}$]	79156	Total continental emission to air
STPload [C]	[$t.y^{-1}$]	1495	Total continental emission to wastewater
Edirect(water1) [C]	[$t.y^{-1}$]	256	Total continental emission to surface water
Edirect(soil3) [C]	[$t.y^{-1}$]	1012	Total continental emission to industrial soil
Edirect(soil2) [C]	[$t.y^{-1}$]	0	Total continental emission to agricultural soil
- Regional			
Edirect(air) [R]	[$t.y^{-1}$]	8795	Total regional emission to air
STPload [R]	[$t.y^{-1}$]	166	Total regional emission to wastewater
Edirect(water1) [R]	[$t.y^{-1}$]	28	Total regional emission to surface water
Edirect(soil3) [R]	[$t.y^{-1}$]	112	Total regional emission to industrial soil
Edirect(soil2) [R]	[$t.y^{-1}$]	0	Total regional emission to agricultural soil

OUTPUT - Ethylbenzene

Zur **Neuberechnung der Daten**: ->Extras ->Optionen ->Berechnen -> Datei_berechnen -> F9 drücken, sonst keine komplette Neuberechnung aller Bezüge!!

Parameter names acc. SimpleBox20	Unit	Output	Parameter names according Euses
Physicochemical properties			
COMPOUND NAME	[-]	Ethylbenzene	Substance
Output			
- Continental			
PECsurfacewater (total)	$[mg.l^{-1}]$	7,20E-06	Continental PEC in surface water (total)
PECsurfacewater (dissolved)	$[mg.l^{-1}]$	7,19E-06	Continental PEC in surface water (dissolved)
PECair	$[mg.m^{-3}]$	1,43E-04	Continental PEC in air (total)
PECagr.soil	$[mg.kg_{wwt}^{-1}]$	9,50E-06	Continental PEC in agricultural soil (total)
PECporewater agr.soil	$[mg.l^{-1}]$	1,23E-06	Continental PEC in pore water of agricultural soils
PECnat.soil	$[mg.kg_{wwt}^{-1}]$	3,81E-06	Continental PEC in natural soil (total)
PECind.soil	$[mg.kg_{wwt}^{-1}]$	4,37E-04	Continental PEC in industrial soil (total)
PECsediment	$[mg.kg_{wwt}^{-1}]$	7,03E-05	Continental PEC in sediment (total)
- Regional			
PECsurfacewater (total)	$[mg.l^{-1}]$	6,40E-05	Regional PEC in surface water (total)
PECsurfacewater (dissolved)	$[mg.l^{-1}]$	6,39E-05	Regional PEC in surface water (dissolved)
PECair	$[mg.m^{-3}]$	4,62E-04	Regional PEC in air (total)
PECagr.soil	$[mg.kg_{wwt}^{-1}]$	7,40E-05	Regional PEC in agricultural soil (total)
PECporewater agr.soil	$[mg.l^{-1}]$	9,56E-06	Regional PEC in pore water of agricultural soils
PECnat.soil	$[mg.kg_{wwt}^{-1}]$	1,23E-05	Regional PEC in natural soil (total)
PECind.soil	$[mg.kg_{wwt}^{-1}]$	4,23E-03	Regional PEC in industrial soil (total)
PECsediment	$[mg.kg_{wwt}^{-1}]$	5,62E-04	Regional PEC in sediment (total)

Appendix IV

Indirect exposure via the environment

INDIRECT EXPOSURE VIA THE ENVIRONMENT

(TGD On New and Existing Chemicals, chapter 2)

Parameter [Unit]	*Symbol*
Definitions (for the use in this document)	
definition of the unit 'kg$_{bw}$' for body weight	$kg_{bw} := 1 \cdot kg$
definition of the unit 'd' for day	$d := 1 \cdot Tag$
	$scenario := 1 .. 2$
	$local := 1$ $regional := 2$
Constants	
gas - constant R	$R := 8.314 J \cdot K^{-1} \cdot mol^{-1}$
Defaults	
volumefraction air in plant tissue [-]	$Fair_{plant} := 0.3$
volumefraction water in plant tissue [-]	$Fwater_{plant} := 0.65$
volumefraction lipids in plant tissue [-]	$Flipid_{plant} := 0.01$
bulk density of plant tissue [$kg_{wet\ plant} * m_{plant}^{-3}$]	$RHO_{plant} := 700 \cdot kg \cdot m^{-3}$
leaf surface area [m^2]	$AREA_{plant} := 5 \cdot m^2$
conductance (0.001 $m*s^{-1}$) [$m*d^{-1}$]	$g_{plant} := 0.001 \cdot m \cdot s^{-1}$
shoot volume [m^3]	$V_{leaf} := 0.002 \cdot m^3$
transpiration stream [m^3*d^{-1}]	$Q_{transp} := 1 \cdot 10^{-3} \cdot m^3 \cdot d^{-1}$
correction exponent for differences between plant lipids and octanol [-]	$b := 0.95$
growth rate constant for dilution by growth [d^{-1}]	$kgrowth_{plant} := 0.035 \cdot d^{-1}$
pseudo-first order rate constant for metabolism in plants [d^{-1}]	$kmetab_{plant} := 0 \cdot d^{-1}$
pseudo-first order rate constant for photolysis in plants [d^{-1}]	$kphoto_{plant} := 0 \cdot d^{-1}$

concentration in meat and milk

daily intake of grass
[$kg_{wetgrass}$*d^{-1}]
$IC_{grass} := 67.6 \cdot kg \cdot d^{-1}$

daily intake of soil
[$kg_{wet\ soil}$*d^{-1}]
$IC_{soil} := 0.46 \cdot kg \cdot d^{-1}$

daily intake of air
[m_{air}^{3}*d^{-1}]
$IC_{air} := 122 \cdot m^{3} \cdot d^{-1}$

daily intake of drinkingwater
[l*d^{-1}]
$IC_{drw} := 55 \cdot l \cdot d^{-1}$

daily intake for human

daily intake for the several pathways
[kg_{chem}*d^{-1}] or [m^{3}*d^{-1}]

$IH_{drw} := 2 \cdot l \cdot d^{-1}$

$IH_{fish} := 0.115 \cdot kg \cdot d^{-1}$

$IH_{stem} := 1.2 \cdot kg \cdot d^{-1}$

$IH_{root} := 0.384 \cdot kg \cdot d^{-1}$

$IH_{meat} := 0.301 \cdot kg \cdot d^{-1}$

$IH_{milk} := 0.561 \cdot kg \cdot d^{-1}$

$IH_{air} := 20 \cdot m^{3} \cdot d^{-1}$

bioavailability through route of intake
[-]

$BIO_{inh} := 0.75$

$BIO_{oral} := 1.0$

average body weight of human
[kg]

$BW := 70 \cdot kg_{bw}$

Name: Ethyl benzene CAS - No.: 100-41-4

Input

chemical properties

	$logK_{OW} := 3.13$
octanol-water partitioning coefficient [-]	$K_{OW} := 10^{logK_{OW}}$
Henry - partitioning coefficient [Pa*m³*mol⁻¹]	$HENRY := 617.113 \cdot Pa \cdot m^3 \cdot mol^{-1}$
air-water partitioning coefficient [-]	$K_{air_water} := 0.2604$
fraction of the chemical associated with aerosol particles [-]	$F_{ass_aer} := 1.075 \cdot 10^{-7}$
half-life for biodegration in surface water [d]	$DT_{50_bio_water} := 15 \cdot d$

environmental concentrations

annual average local PEC in surface water (dissolved) [mg_{chem} * l_{water}^{-1}]	$PEClocal_{water_ann} := 0.0116 \cdot mg \cdot l^{-1}$
annual average local PEC in air (total) [mg_{chem} * m_{air}^{-3}]	$PEClocal_{air_ann} := 0.034 \cdot mg \cdot m^{-3}$
local PEC in grassland (total), averaged over 180 days [mg_{chem} * kg_{soil}^{-1}]	$PEClocal_{grassland} := 0.00261 \cdot mg \cdot kg^{-1}$
local PEC in porewater of agriculture soil [mg_{chem} * $l_{porewater}^{-1}$]	$PEClocal_{agr_soil_porew} := 2.811 \cdot 10^{-4} \cdot mg \cdot l^{-1}$
local PEC in porewater of grassland [mg_{chem} * $l_{porewater}^{-1}$]	$PEClocal_{grassland_porew} := 3.359 \cdot 10^{-4} \cdot mg \cdot l^{-1}$
local PEC in groundwater under agriculture soil [mg_{chem} * l_{water}^{-1}]	$PEClocal_{grw} := 2.811 \cdot 10^{-4} \cdot mg \cdot l^{-1}$
regional PEC in surface water (dissolved) [mg_{chem} * l_{water}^{-1}]	$PECregional_{water} := 6.40 \cdot 10^{-5} \cdot mg \cdot l^{-1}$
regional PEC in air (total) [mg_{chem} * m_{air}^{-3}]	$PECregional_{air} := 4.62 \cdot 10^{-4} \cdot mg \cdot m^{-3}$
regional PEC in agriculture soil (total) [mg_{chem}*kg_{soil}^{-1}	$PECregional_{agr_soil} := 7.40 \cdot 10^{-5} \cdot mg \cdot kg^{-1}$
regional PEC in porewater of agriculture soils [mg_{chem}*l_{water}^{-1}	$PECregional_{agr_soil_porew} := 9.56 \cdot 10^{-6} \cdot mg \cdot l^{-1}$

Definition of the concentrations used for indirect exposure

$C_{water_{local}} := PEClocal_{water_ann}$

$C_{air_{local}} := PEClocal_{air_ann}$

$C_{grassland_{local}} := PEClocal_{grassland}$

$C_{agr_porew_{local}} := PEClocal_{agr_soil_porew}$

$C_{grass_porew_{local}} := PEClocal_{grassland_porew}$

$C_{grw_{local}} := PEClocal_{grw}$

$C_{water_{regional}} := PECregional_{water}$

$C_{air_{regional}} := PECregional_{air}$

$C_{grassland_{regional}} := PECregional_{agr_soil}$

$C_{agr_porew_{regional}} := PECregional_{agr_soil_porew}$

$C_{grass_porew_{regional}} := PECregional_{agr_soil_porew}$

$C_{grw_{regional}} := PECregional_{agr_soil_porew}$

<u>bioconcentration in fish</u>

bioconcentration factor for fish
$[m_{water}^{3} * kg_{chem}^{-1}]$

$BCF_{fish} := 10^{0.85 \cdot logK_{OW} - 0.7} \cdot l \cdot kg^{-1}$

modified equation for logKow > 6

$BCF_{fish} := wenn\left[logK_{OW} > 6, \left[-0.278 \cdot \left(logK_{OW}\right)^{2} + 3.38 \cdot logK_{OW} - 5.94\right] \cdot l \cdot kg^{-1}, BCF_{fish}\right]$

$C_{fish_{scenario}} := BCF_{fish} \cdot C_{water_{scenario}}$

<u>bioconcentration in plants</u>

$K_{plant_water} := Fwater_{plant} + Flipid_{plant} \cdot K_{OW}^{b}$

$Croot_{agr_plant_{scenario}} := \frac{K_{plant_water} \cdot C_{agr_porew_{scenario}}}{RHO_{plant}}$

$TSCF := 0.784 \cdot e^{\frac{-\left(logK_{OW} - 1.78\right)^{2}}{2.44}}$

remark: for $logK_{OW}$ out of the range from -0.5 to 4.5
the TSCF is limited by the values for $logK_{OW}$ = -0.5 resp. 4.5

$TSCF := wenn\left(logK_{OW} < -0.5, 0.903, TSCF\right)$

$TSCF := wenn\left(logK_{OW} > 4.5, 0.832, TSCF\right)$

$K_{leaf_air} := Fair_{plant} + \frac{K_{plant_water}}{K_{air_water}}$

$kelim_{plant} := kmetab_{plant} + kphoto_{plant}$

$\alpha := \frac{AREA_{plant} \cdot g_{plant}}{K_{leaf_air} \cdot V_{leaf}} + kelim_{plant} + kgrowth_{plant}$

$$\beta_{agr_plant_{scenario}} := C_{agr_porew_{scenario}} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + \left(1 - F_{ass_aer}\right) \cdot C_{air_{scenario}} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C_{leaf_crops_{scenario}} := \frac{\beta_{agr_plant_{scenario}}}{\alpha \cdot RHO_{plant}}$$

$$\beta_{grass_plant_{scenario}} := C_{grass_porew_{scenario}} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + \left(1 - F_{ass_aer}\right) \cdot C_{air_{scenario}} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C_{leaf_grass_{scenario}} := \frac{\beta_{grass_plant_{scenario}}}{\alpha \cdot RHO_{plant}}$$

<u>purification of drinking water</u>

system may defined dependent from the aerobic biodegradation

$$system := wenn\left(DT_{50_bio_water} < 10 \cdot d, 0, 1\right)$$

select a column on dependence from log K_{OW}

$$FIndex := wenn\left(logK_{OW} < 4, 0, wenn\left(logK_{OW} > 5, 2, 1\right)\right)$$

$$Fpur_{logKow} := \begin{bmatrix} 1 & \frac{1}{4} & \frac{1}{16} \\ 1 & \frac{1}{2} & \frac{1}{4} \end{bmatrix}$$

$$Fpur := \frac{Fpur_{logKow_{system, FIndex}}}{wenn\left(HENRY > 100 \cdot Pa \cdot m^3 \cdot mol^{-1}, 2, 1\right)}$$

$$C_{drw_{scenario}} := wenn\left[C_{grw_{scenario}} > \left(C_{water_{scenario}} \cdot Fpur\right), C_{grw_{scenario}}, C_{water_{scenario}} \cdot Fpur\right]$$

<u>Biotransfer to meat and milk</u>

$$BTF_{meat} := 10^{-7.6 + logK_{OW}} \cdot kg^{-1} \cdot d$$

remark: for $logK_{OW}$ out of the range from 1.5 to 6.5
the BTF_{meat} is limited by the values for $logK_{OW}$ = 1.5 resp. 6.5

$$BTF_{meat} := wenn\left(logK_{OW} < 1.5, 7.943 \cdot 10^{-7} \cdot kg^{-1} \cdot d, BTF_{meat}\right)$$

$$BTF_{meat} := wenn\left(logK_{OW} > 6.5, 0.07943 kg^{-1} \cdot d, BTF_{meat}\right)$$

$$C_{meat_{scenario}} := BTF_{meat} \cdot \left(C_{leaf_grass_{scenario}} \cdot IC_{grass} + C_{grassland_{scenario}} \cdot IC_{soil} \cdots + C_{air_{scenario}} \cdot IC_{air} + C_{drw_{scenario}} \cdot IC_{drw}\right)$$

$$BTF_{milk} := 10^{-8.1 + logK_{OW}} \cdot kg^{-1} \cdot d$$

remark: for $logK_{OW}$ out of the range from 3 to 6.5
the BTF_{milk} is limited by the values for $logK_{OW}$ = 1.5 resp. 6.5

$$BTF_{milk} := wenn\left(logK_{OW} < 3, 7.943 \cdot 10^{-6} \cdot kg^{-1} \cdot d, BTF_{milk}\right)$$

$$BTF_{milk} := wenn\left(logK_{OW} > 6.5, 0.02512 kg^{-1} \cdot d, BTF_{milk}\right)$$

$$C_{milk_{scenario}} := BTF_{milk} \cdot \left(C_{leaf_grass_{scenario}} \cdot IC_{grass} + C_{grassland_{scenario}} \cdot IC_{soil} \ldots + C_{air_{scenario}} \cdot IC_{air} + C_{drw_{scenario}} \cdot IC_{drw}\right)$$

total daily intake for human

daily dose through intake of several pathways
$[kg_{chem}*kg_{bw}^{-1}*d^{-1}]$

$$DOSE_{drw_{scenario}} := \frac{C_{drw_{scenario}} \cdot IH_{drw}}{BW}$$

$$DOSE_{air_{scenario}} := \frac{C_{air_{scenario}} \cdot IH_{air} \cdot BIO_{inh}}{BW \cdot BIO_{oral}}$$

$$DOSE_{stem_{scenario}} := \frac{C_{leaf_crops_{scenario}} \cdot IH_{stem}}{BW}$$

$$DOSE_{root_{scenario}} := \frac{Croot_{agr_plant_{scenario}} \cdot IH_{root}}{BW}$$

$$DOSE_{meat_{scenario}} := \frac{C_{meat_{scenario}} \cdot IH_{meat}}{BW}$$

$$DOSE_{milk_{scenario}} := \frac{C_{milk_{scenario}} \cdot IH_{milk}}{BW}$$

$$DOSE_{fish_{scenario}} := \frac{C_{fish_{scenario}} \cdot IH_{fish}}{BW}$$

total daily intake for human

total daily intake for human as sum of each pathway
$[kg_{chem}*kg_{bw}^{-1}*d^{-1}]$

$$DOSE_{tot_{scenario}} := DOSE_{drw_{scenario}} + DOSE_{fish_{scenario}} + DOSE_{stem_{scenario}} + DOSE_{root_{scenario}} \ldots + DOSE_{meat_{scenario}} + DOSE_{milk_{scenario}} + DOSE_{air_{scenario}}$$

relative doses of specific different pathway (%)

$$RDOSE_{drw_{scenario}} := \frac{DOSE_{drw_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{air_{scenario}} := \frac{DOSE_{air_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{stem_{scenario}} := \frac{DOSE_{stem_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{root_{scenario}} := \frac{DOSE_{root_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{meat_{scenario}} := \frac{DOSE_{meat_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{milk_{scenario}} := \frac{DOSE_{milk_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{fish_{scenario}} := \frac{DOSE_{fish_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

Results of calculation

$DOSE_{tot_{local}} = 9.247095 \cdot 10^{-3} \cdot \frac{mg}{kg_{bw} \cdot d}$

$DOSE_{tot_{regional}} = 1.107223 \cdot 10^{-4} \cdot \frac{mg}{kg_{bw} \cdot d}$

$RDOSE_{drw_{local}} = 1.792069 \cdot \%$

$RDOSE_{drw_{regional}} = 0.825747 \cdot \%$

$RDOSE_{air_{local}} = 78.789219 \cdot \%$

$RDOSE_{air_{regional}} = 89.412879 \cdot \%$

$RDOSE_{stem_{local}} = 0.350503 \cdot \%$

$RDOSE_{stem_{regional}} = 0.397768 \cdot \%$

$RDOSE_{root_{local}} = 0.23961 \cdot \%$

$RDOSE_{root_{regional}} = 0.680569 \cdot \%$

$RDOSE_{meat_{local}} = 7.241759 \cdot 10^{-3} \cdot \%$

$RDOSE_{meat_{regional}} = 7.881741 \cdot 10^{-3} \cdot \%$

$RDOSE_{milk_{local}} = 4.268157 \cdot 10^{-3} \cdot \%$

$RDOSE_{milk_{regional}} = 4.645351 \cdot 10^{-3} \cdot \%$

$RDOSE_{fish_{local}} = 18.817089 \cdot \%$

$RDOSE_{fish_{regional}} = 8.67051 \cdot \%$