

**Annex XV dossier****PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING****Substance Name: Ethylbenzene****EC Number: 202-849-4****CAS Number: 100-41-4****Submitted by: Germany****Date: 15 Nov. 2010**

## CONTENTS

1	IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES .....	6
1.1	Name and other identifiers of the substance .....	6
1.2	Composition of the substance .....	6
1.3	Physico-chemical properties .....	6
2	MANUFACTURE AND USES .....	8
3	CLASSIFICATION AND LABELLING .....	8
3.1	Classification in Annex VI of Regulation (EC) 1272/2008 .....	8
3.2	Self classification(s) .....	9
4	ENVIRONMENTAL FATE PROPERTIES .....	9
5	HUMAN HEALTH HAZARD ASSESSMENT .....	9
5.1	Toxicokinetics (absorption, metabolism, distribution and elimination) .....	9
5.2	Acute toxicity .....	9
5.3	Irritation .....	9
5.4	Corrosivity .....	9
5.5	Sensitisation .....	9
5.6	Repeated dose toxicity .....	9
5.6.1	Repeated dose toxicity: oral .....	10
5.6.2	Repeated dose toxicity: inhalation .....	12
5.6.3	Repeated dose toxicity: dermal .....	24
5.6.4	Other relevant information .....	24
5.6.5	Summary and discussion of repeated dose toxicity: .....	24
5.7	Mutagenicity .....	25
5.8	Carcinogenicity .....	26
5.9	Toxicity for reproduction .....	26
5.10	Other effects .....	26
5.11	Derivation of DNEL(s) or other quantitative or qualitative measure for dose response .....	26
6	HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES .....	26
7	ENVIRONMENTAL HAZARD ASSESSMENT .....	27

## TABLES

Table 1.3-1: Summary of physico- chemical properties .....6  
Table 5.6-1: Animal studies with repeated oral exposure to ethylbenzene (EB) ..... 10  
Table 5.6-2: Animal studies with repeated inhalation exposure to ethylenbenzene (EB): General toxicity ..... 13  
Table 5.6-3: Animal studies with repeated inhalation exposure: Ototoxicity ..... 19

## PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

**Substance Name:** Ethylbenzene

**EC Number:** 202-849-4

CAS number: 100-41-4

Registration number (s):

Purity: > 99.5 %

Impurities: This information is confidential and then provided in the confidential part of the dossier provided in appendix 1.

*[Note: In case where the proposed classification is based on the presence of an impurity, this should clearly be stated, along with the identity of the impurity and the composition of impurity in the substance leading to the classification].*

### **Proposed classification based on Directive 67/548/EEC criteria:**

F, R11            highly flammable

Xn; R20           Harmful: Harmful by inhalation.

Xn; R65           Harmful: May cause lung damage if swallowed

### **Proposed classification based on GHS criteria:**

Flam. Liq. 2 - H225            Highly flammable liquid and vapour

Acute Tox. 4 \* - H 332           Harmful if inhaled.

Asp.Tox.1 - H304            May be fatal if swallowed and enters airways.

STOT Rep.2 - H373            May cause damage to organs through prolonged or repeated exposure

### **Proposed specific concentration limits (if any):**

None

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\* Minimum classification

**Proposed notes (if any):**

Ethylbenzene was a priority substance in the Existing Chemicals program (EEC) 793/93. In the transitional Annex XV Dossier on ethylbenzene it is noted that the discussion on the risk assessment report was not concluded at the Technical Committee for New and Existing Substances (TC NES).

The current classification for ethylbenzene with regard to human health is: Xn, R 20.

In the draft Risk Assessment Report (November 2008) on ethylbenzene it was noted that the substance should be classified and labelled additionally with:

R36/37/38 - Irritating to eyes, respiratory tract and to skin

R48/20 - Harmful: Danger of serious damage to health by prolonged exposure through inhalation

R65 - Harmful: May cause lung damage if swallowed

After re-evaluation of the toxicity of ethylbenzene, classification and labelling as 'R 36/37/38 Irritating to eyes, respiratory tract and to skin' is no longer supported.

Furthermore, the proposal for 'R48/20 Harmful: Danger of serious damage to health by prolonged exposure through inhalation' is also withdrawn. Nevertheless, STOT Rep.2 – H373 is proposed. This is due to the guidance value range of  $0.2 < C \leq 1.0$  mg/L/6 h/d according to Regulation (EC) No. 1272/2008 (Annex I, Table 3.9.2), whereas according to Directive 67/548/EEC the R48/20 is foreseen for a guidance value of  $\leq 0.25$  mg/L air/6 h/d.

## JUSTIFICATION

### 1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

#### 1.1 Name and other identifiers of the substance

Chemical Name: Ethylbenzene

EC Name: Ethylbenzene

CAS Number: 100-41-4

IUPAC Name: Ethylbenzene

#### 1.2 Composition of the substance

Chemical Name: Ethylbenzene

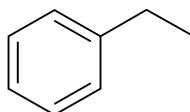
EC Number: 202-849-4

CAS Number: 100-41-4

IUPAC Name: Ethylbenzene

Molecular Formula: C<sub>8</sub>H<sub>10</sub>

Structural Formula:



Molecular Weight: 106.165 g/mol

Typical concentration (% w/w): > 99,5 %

Concentration range (% w/w): 99,5 – 100 %

#### 1.3 Physico-chemical properties

**Table 1.3-1: Summary of physico- chemical properties**

REACH ref Annex, §	Property	IUCLID section	Value	[enter comment/reference or delete column]
VII, 7.1	Physical state at 20°C and 101.3 KPa	3.1	liquid at 25 °C	
VII, 7.2	Melting/freezing point	3.2	- 94.949 °C	Gerhartz (1987)
VII, 7.3	Boiling point	3.3	136.186 °C	Gerhartz (1987)



REACH ref Annex, §	Property	IUCLID section	Value	[enter comment/reference or delete column]
			(DIN 51794)	
VII, 7.13	Oxidising properties	4.15	The classification procedure needs not to be applied because the organic substance does not contain oxygen, fluorine or chlorine.	BAM-II.2 (2010)
VII, 7.14	Granulometry	3.5	Not applicable (liquid)	
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17		
XI, 7.16	Dissociation constant	3.21		
XI, 7.17	Viscosity	3.22	0.63 mm <sup>2</sup> /s at 40 °C (Kinematic viscosity)	Knothe & Steidley (2005)
	Reactivity towards container material	3.18		
	Thermal stability	3.19		
	Index of refraction (nd)		1.49588 at 20 °C 1.49320 at 25 °C	Gerhartz (1987)

- 1) Typically impurities may **cause a decrease** of the flashpoint.
- 2) The amount of impurities has an effect on the auto flammability.

## 2 MANUFACTURE AND USES

Not relevant for this dossier.

## 3 CLASSIFICATION AND LABELLING

### 3.1 Classification in Annex VI of Regulation (EC) 1272/2008

Acc. to the CLP criteria (Table 3.1):

Flam. Liq. 2 - H225                      Highly flammable liquid and vapour

Acute Tox. 4 \* – H 332      Harmful if inhaled.

Acc. to the criteria of Dir. 67/548/EEC (Table 3.2):

F; R11

Xn; R20

### **3.2 Self classification(s)**

Not applicable

## **4 ENVIRONMENTAL FATE PROPERTIES**

Not relevant for this dossier.

## **5 HUMAN HEALTH HAZARD ASSESSMENT**

### **5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)**

Toxicokinetics have not been considered as part of this dossier.

### **5.2 Acute toxicity**

Acute toxicity has not been considered as part of this dossier.

### **5.3 Irritation**

Irritation has not been considered as part of this dossier.

### **5.4 Corrosivity**

Corrosivity has not been considered as part of this dossier.

### **5.5 Sensitisation**

Sensitisation has not been considered as part of this dossier.

### **5.6 Repeated dose toxicity**

The proposed classification of ethylbenzene refers to ototoxicity and was observed after inhalation exposure.

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\* Minimum classification

Specific human data for ethylbenzene are not available. No clear conclusion can be drawn from human data after exposure to other aromatic solvents or a combination of ethylbenzene with other solvents.

Animal data after oral administration mainly refer to general toxicity; one investigation is available on ototoxicity; data is presented in a tabular form (5.6.1). Data on general toxicity after inhalation exposure is also given in tabular form; data referring to ototoxicity is presented both, in tabular form and in detailed text (5.6.2). Concerning dermal administration, no relevant data is available (5.6.3).

### 5.6.1 Repeated dose toxicity: oral

Studies on oral repeated dose toxicity are summarised in Table 5.6-1.

**Table 5.6-1: Animal studies with repeated oral exposure to ethylbenzene (EB)**

Species (strain, animal no.)	Study design/test material (purity)	Compliance to test guidelines	Results	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Reference
<u>Rat</u> (Sprague–Dawley, 6-8 m)	8.47 mmol/kg bw/d or 900 mg/kg bw/d, gavage  5 d/wk for <b>2 wk</b>  Prep. of cochleae 10 days after last treatment  EB (99 %)	Specifically designed for ototoxicity	No effect on clinical signs or body weight gain  Nearly complete loss of outer hair cells in all 3 rows, minute loss of inner hair cells  EB blood levels: Not reported	-	900	Gagnaire and Langlais (2005)
<u>Rat</u> (Wistar, 5/sex/grp)	75-250-750 mg/kg bw/d, gavage  for <b>4 wk</b> EB (99.7 %)	Range finding study similar to OECD TG 407	≥ 250 mg/kg bw/d: Increased liver weight with centrilobular hypertrophy (m in high and mid dose, f in high dose). 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  EB blood levels: Not reported	75	250	Mellert et al. (2003; 2007)

Species (strain, animal no.)	Study design/test material (purity)	Compliance to test guidelines	Results	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Reference
<u>Rat</u> (Wistar 10/sex/grp)	75-250-750 mg/kg bw/d, gavage for <b>3 mo</b> EB (99.7 %)	OECD TG 408	<p>≥ 250 mg/kg: Changes in haematology indicative of mild regenerative anaemia, elevated activity of ALAT, TBil and Chol, increased liver weight with centrilobular hypertrophy, increased kidney weight (males)</p> <p>Changes in male rat kidney indicative of male specific alpha-2<math>\mu</math>-globulin nephropathy</p> <p>EB blood levels: Not reported</p>	75	250	Mellert et al. (2004; 2007)
<u>Rat</u> (Sprague-Dawley, 10-16/sex/grp)	50-250-500 mg/kg bw/d, gavage for <b>3 mo</b> EB (99.96 %)	Similar to OECD TG 424; specifically designed for neurotoxicity	<p>≥ 250 mg/kg: Increased liver and kidney weight (m+f) without histopathological correlates;</p> <p>500 mg/kg: Increased incidence of marginal clinical signs (e.g., excess salivation).</p> <p>No effects in a functional observational battery, motor activity or histopathology of the central and peripheral nervous system</p> <p>EB blood levels: Not reported</p>	500	-	Barnett et al. (2006)/Li et al. (2010)
<u>Rat</u> (Wistar, 10 f/grp)	13.6-136-408-680 mg/kg bw/d, gavage 5 d/wk; 130 applications in <b>6 mo</b> EB (> 98 %)	Not given	<p>≥ 408 mg/kg bw/d:</p> <p>Weight<math>\uparrow</math> in liver and kidneys, slight cloudy swelling of hepatocytes and renal tubular epithelium</p> <p>EB blood levels: Not reported</p>	136	408	Wolf et al., 1956

Ototoxicity was investigated only in one study (Gagnaire and Langlais, 2005) with oral administration of 900 mg/kg bw/d to rats. This high dose caused strong hair cell death in cochleae. By quantitative comparison with other aromatic solvents, ethylbenzene belonged to those with the highest ototoxic potency.

## **5.6.2 Repeated dose toxicity: inhalation**

When using a molecular weight of ethylbenzene of 106.17 g/mol and a molar volume of 24.1 L at 20 °C and 101.3 kPa, a concentration of 1 ppm ethylbenzene is equivalent to 0.0044 mg ethylbenzene/L air.

### **5.6.2.1 Human data**

There are no specific data on neurotoxicity in humans with mono-exposure to ethylbenzene. However, for other aromatic solvents there is evidence for neurotoxicity in humans, e.g. for toluene, xylenes or styrene. Depressive and narcotic effects are described, and there is strong experimental evidence for ototoxicity. 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. However, exposure data for ethylbenzene were vague. Altogether no conclusion can be drawn concerning ethylbenzene.

### **5.6.2.2 Animal data - general toxicity**

**Studies on general toxicity after repeated inhalation exposure are summarised in Table 5.6-2.**

**Table 5.6-2: Animal studies with repeated inhalation exposure to ethylenbenzene (EB): General toxicity**

Species (strain, animal no.)	Study design/test material	Compliance to test guidelines	Results	NOAEC	LOAEC	Reference
<u>Rat</u> (Sprague-Dawley, 6 m/grp)	2000 ppm or 8.8 mg/L air 6 h/d for <b>3 d</b>  EB (> 99 %)	Specifically designed for neurotoxic effects	Decrease in nor-adrenaline levels, increase in turnover of nor-adrenaline and dopamine in the forebrain and hypothalamus  EB blood levels: Not reported	-	2000 ppm (8.8 mg/L air)	Andersson et al. (1981)
<u>Rat</u> (Sprague-Dawley, 4m)	2000 ppm or 8.8 mg/L air 6 h/d for <b>3 d</b> Specific study on enzyme activities  EB (> 99 %)	Not given	Increase in relative liver and kidney weight; enzyme induction in liver and kidney  EB blood levels: Not reported	-	-	Toftgard and Nilsen (1982)
<u>Rat</u> (F344, 5 m/grp)	400-1200-2400 ppm or 1.8-5.3-10.6 mg/L air  6 h/d for <b>4 d</b>  EB (99.7 %)	Not given	≥ 400 ppm: Increase in liver weight  1200 ppm: Decrease in body weight, increase in kidney weight without histopathological changes  ≥ 1200 ppm: Lacrimation, shallow breathing, prostration  2400 ppm: Death of all males before day 4 with red discoloration of lungs, congestion in lungs, nasal mucosa, liver, kidneys  EB blood levels: Not reported	400 ppm (1.8 mg/L air)	1200 ppm (5.3 mg/L air)	Biodynamics (1986)

Species (strain, animal no.)	Study design/test material	Compliance to test guidelines	Results	NOAEC	LOAEC	Reference
<u>Mouse</u> (B6C3F1, 5 m/grp)	400-1200-2400 ppm or 1.8-5.3-10.6 mg/L air  6 h/d for <b>4 d</b>  EB (99.7 %)		400 ppm: Excess lacrimation  1200 ppm: death of 4/5 males, lacrimation, shallow breathing, prostration, closed eyes, red discoloration of lungs, congestion of lungs, nasal mucosa, liver, kidneys  2400 ppm: Death of all males (symptoms as with dead males at 1200 ppm)  EB blood levels: Not reported	400 ppm (1.8 mg/L air)	1200 ppm (5.3 mg/L air)	Biodynamics (1986), ctd.
<u>Rabbit</u> (NZW, 4 m/grp)	400-1200-2400 ppm or 1.8-5.3-10.6 mg/L air  6 h/d for <b>4 d</b>  EB (99.7 %)		No toxic signs, deaths, treatment related findings  EB blood levels: Not reported	2400 ppm (10.6 mg/L air)	-	
<u>Rat</u> (F344, 6-8/sex/grp)	75-750 ppm or 0.3-3.3 mg/L air 6 h/d for <b>5 d</b>  750 ppm or 3.3 mg/L air 5 d/w for <b>4 wk</b> Investigation of kidneys in rats  EB ( $\geq 99.9$ %)	Not a guideline study	750 ppm: Slight increase in kidney weight (m + f), hyaline droplets (after 1 wk only), nephropathy, DNA synthesis (males)  EB blood levels: Not reported	75 ppm (0.3 mg/L air)	750 ppm (3.3 mg/L air)	Stott et al. (1999; 2003)

Species (strain, animal no.)	Study design/test material	Compliance to test guidelines	Results	NOAEC	LOAEC	Reference
<u>Mouse</u> (B6C3F1, 6-8/sex/grp)	75-750 ppm or 0.3-3.3 mg/L air 6 h/d for <b>5 d</b>  750 ppm or 3.3 mg/L air 5 d/w for <b>4 wk</b> Investigation of liver and lung in mice  EB (≥ 99.9 %)		Increased liver weight at 750 ppm (m + f) and DNA synthesis (m > f). Increased DNA synthesis in lung (m + f). Some changes in enzymes in liver and lung  EB blood levels: Not reported	75 ppm (0.3 mg/L air)	750 ppm (3.3 mg/L air)	Stott et al. (1999; 2003), ctd.
<u>Rabbit</u> (NZ, 8m/grp)	750 ppm or 3.3 mg/L air 12 h/d for <b>7 d</b>  EB (p.a.)	Specifically designed for neurotoxic effects	Significant depletion of dopamine and increase of homovanillic acid levels in the striatum and tubero-infundibular region of the hypothalamus  EB blood levels: Not reported	-	750 ppm (3.3 mg/L air)	Romanelli et al. (1986); Mutti and Franchini (1987); Mutti et al. (1988)
<u>Rat</u> (Wistar 5m/grp)	50-300-600 ppm or 0.2-1.32-2.64 mg/L air 6 h/d, 5 d/wk for <b>2, 5, 9, 16 wk</b>  EB (99 %)	Not given	≥ 50 ppm: Proliferation of SER in hepatocytes and induction of some liver enzymes,  600 ppm: Induction of some kidney enzymes, kidney/body weight ratio↑  EB blood levels: Not reported	-	-	Elovaara et al. (1985)
<u>Rat</u> (F344)	99-382-782 ppm or 0.4-1.7-3.4 mg/L air 6 h/d, 5 d/wk for <b>4 wk</b> (all species)  EB (99.7 %)	Similar to OECD 407 (version 1981)	≥ 382 ppm: Sporadic lacrimation, salivation, liver weight↑ without corresponding changes in histopathology and clinical chemistry  782 ppm: Total WBC counts↑  EB blood levels: Not reported	782 ppm (3.4 mg/L air)	-	Cragg et al. (1989)

Species (strain, animal no.)	Study design/test material	Compliance to test guidelines	Results	NOAEC	LOAEC	Reference
<u>Mouse</u> (B6C3F1)	99-382-782 ppm or 0.4-1.7-3.4 mg/L air  6 h/d, 5 d/wk for <b>4 wk</b> (all species)  EB (99.7 %)		782 ppm: Liver weight↑ without corresponding changes in histopathology and clinical chemistry  EB blood levels: Not reported	782 ppm (3.4 mg/L air)	-	Cragg et al. (1989), ctd.
<u>Rabbit</u> (NZW) (5/sex/grp)	382-782-1610 ppm or 1.7-3.4-7.1 mg/L air  6 h/d, 5 d/wk for <b>4 wk</b> (all species)  EB (99.7 %)		No adverse effects observed up to the highest dose level  EB blood levels: Not reported	1610 ppm (7.1 mg/L air)	-	
<u>Rat</u> (Sprague-Dawley, 10 f/grp)	25-100-500 ppm or 0.11-0.4-2.2 mg/L air 6 h/d for <b>28 d</b>  EB (99 %)	OPPTS 870.7800	500 ppm: Increased liver and kidney weight  No effect on humoral immunological response  EB blood levels: Not reported	100 ppm (0.4 mg/L air)	500 ppm (2.2 mg/L air)	Stump (2004)
<u>Rat</u> (Wistar, 18/sex/ grp)	100 ppm or 0.4 mg/L air 6h/d, 5 d/wk for <b>12 wk</b>  EB (99 %)	Not given	No adverse effect  EB blood levels: Not reported	100 ppm (0.4 mg/L air)	--	Clark (1983)
<u>Rat</u> (F344, 10/sex/grp)	100-250-500-750-1000 ppm or 0.4-1.1-2.2-3.3-4.4 mg/L air  6h/d, 5 d/wk for <b>92-98 d</b>  EB (> 99 %)	Close to B.29	≥ 250 ppm: Liver weight↑  ≥ 500 ppm: Kidney weight↑  ≥ 750 ppm: Histopathol. reevaluation of kidneys (Hard, 2002): increased incidence of CPN and hyaline droplets (male rats)  EB blood levels: Not reported	1000 ppm (4.4 mg/L air)  Males (kidney): 500 ppm (2.2 mg/L air)	-	NTP (1992) Hard (2002)

Species (strain, animal no.)	Study design/test material	Compliance to test guidelines	Results	NOAEC	LOAEC	Reference
<u>Mouse</u> (B6C3F1, 10/sex/grp)	100-250-500-750-1000 ppm or 0.4-1.1-2.2-3.3-4.4 mg/L air  6h/d, 5 d/wk for <b>92-98 d</b>  EB (> 99 %)		≥ 750 ppm: Liver weight ↑, not relevant  1000 ppm: Kidney weight ↑, not relevant  EB blood levels: Not reported (both species)	1000 ppm (4.4 mg/L air)	-	NTP (1992) Hard (2002), ctd.
<u>Rat</u> (Wistar, 10-25/sex/grp)	400-600-1250-2200 ppm or 1.8-2.6-5.5-9.68 mg/L air  7-8 h/d, 5 d/wk for <b>103-138 exposure days</b> within a <b>period of 144-214 days</b>  EB (> 98 %)	Not given	≥ 400 ppm: Liver + kidney weight ↑  ≥ 1250 ppm: Growth ↓, cloudy swelling of liver cells and renal tubular epithelium  EB blood levels: Not reported	-	400 ppm (1.8 mg/L air)	Wolf et al. (1956)
<u>Guinea pig</u> (5-10)	400 – 600 – 1250 ppm or 1.8-2.6-5.5 mg/L air  7-8 h/d, 5 d/wk for <b>103-138 exposure days</b> within a <b>period of 144-214 days</b>  EB (> 98 %)		600 ppm: Liver weight ↑  1250 ppm: Growth ↓  EB blood levels: Not reported	400 ppm (1.8 mg/L air)	<u>600 ppm</u> <u>(2.6 mg/L air)</u>	
<u>Rabbit</u> (1-2)	400 – 600 – 1250 ppm or 1.8-2.6-5.5 mg/L air  7-8 h/d, 5 d/wk for <b>103-138 exposure days</b> within a <b>period of 144-214 days</b>  EB (> 98 %)		600 ppm: Degeneration of testicular germinal epithelium  EB blood levels: Not reported	400 ppm (1.8 mg/L air)	<u>600 ppm</u> <u>(2.6 mg/L air)</u>	

Species (strain, animal no.)	Study design/test material	Compliance to test guidelines	Results	NOAEC	LOAEC	Reference
<u>Rhesus monkey</u>  (1-2f)	400–600 ppm or 1.8-2.6 mg/L air  7-8 h/d, 5 d/wk for <b>103-138 exposure days</b> within a <b>period of 144-214 days</b>  EB (> 98 %)		600 ppm: Liver weight <sup>↑</sup> , degeneration of testicular germinal epithelium  EB blood levels: Not reported	400 ppm (1.8 mg/L air)	600 ppm (2.6 mg/L air)	Wolf et al. (1956), ctd.
<u>Rat</u>  (F344, 50/sex/grp)	75-250-750 ppm or 0.3-1.1-3.3 mg/L air 6 h/d, 5 d/wk for <b>104 wk</b> (both species)  EB (> 99 %.)	NTP carcinogenicity bioassay	Slightly decr. bw in f at all dose levels  ≥ 75 ppm: Chronic nephropathy in f (not relevant for humans)  ≥ 250 ppm Chronic nephropathy in m (not relevant for humans)  750 ppm: Decr. survival and decr. bw (m)  EB blood levels: Not reported	Males: 250 ppm, (1.1 mg/L air)  Females: 75 ppm (0.3 mg/L air)	Males: 750 ppm (3.3 mg/L air)  Females: 250 ppm, (1.1 mg/L air)	NTP (1999) Hard (2002) Brown (2000)
<u>Mouse</u>  (B6C3F1, 50/sex/ grp)	75-250-750 ppm or 0.3-1.1-3.3 mg/L air 6 h/d, 5 d/wk for <b>104 wk</b> (both species)  EB (> 99 %)		≥ 250ppm: Liver effects in males (syncytial alteration of hepatocytes), pituitary effects in females (hyperplasia pars distalis)  750 ppm: Histopathological effects in male lungs (alveolar epithelial metaplasia), liver effects in females (eosinophilic foci, syncytial alteration, hypertrophy, necrosis), thyroid effects in males and females (thyroid cell hyperplasia)  EB blood levels: Not reported	75 ppm (0.3 mg/L air)	250 ppm (1.1 mg/L air)	

### 5.6.2.3 Animal data – ototoxicity

A number of studies was specifically designed for investigation of ototoxicity after repeated inhalation exposure. These are summarised in Table 5.6-3. More detailed information is given in the text following the table.

**Table 5.6-3: Animal studies with repeated inhalation exposure: Ototoxicity**

Species (strain, animal no.)	Study design/test material	Results	NOAEC	LOAEC	Reference
<u>Rat</u> (Wag/Rij/Cpb/Hsd, 16 m/grp)	800 ppm or 3.5 mg/L air 8 h/d for <b>5 d</b> Reflex modification for audiometry EB (99 %)	Persistently increased auditory thresholds of a noise-evoked startle response; significant loss of outer hair cells of the cochlea  EB blood levels: Not reported	-	800 ppm (3.5 mg/L air)	Cappaert et al. (1999)
<u>Rat</u> (Wag/Rij/Cpb/Hsd, 8 /grp)	300-400-550 ppm or 1.3-1.8-2.4 mg/L air 5 h/d for <b>5 d</b> EB (99 %)	Increased auditory thresholds, significant loss of outer hair cells of the cochlea  EB blood levels: Not reported	300 ppm (1.3 mg/L air)	400 ppm (1.8 mg/L air)	Cappaert et al. (2000)
<u>Rat</u> (Wag/Rij/Cpb/Hsd, 8 /grp)	300-400 ppm or 1.3-1.8 mg/L air 8 h/d for <b>5 d</b> EB (99 %)	Significant loss of outer hair cells after exposure to 400 ppm ethylbenzene alone  Synergistic effects of noise and ethylbenzene on the loss of outer hair cells of the cochlea, especially of the 3rd row  EB blood levels: Not reported	-	EB: 400 ppm (1.8 mg/L air)  EB + noise: 300 ppm (1.3 mg/L air)	Cappaert et al. (2001)
<u>Rat</u> (Wag/Rij, 4-8, m+f)	550 ppm or 2.4 mg/L air 8 h/d for <b>5 d</b> EB (99 %)	Mid-frequency hearing; significant loss of outer hair cells of the cochlea  EB blood levels: Average concentration ca. 23 and 6 µg EB/mL blood on days 1 and 3	-	550 ppm (2.4 mg/L air)	Cappaert et al. (2002)
<u>Guinea pig</u> (4-8 f/grp)	2500 ppm or 11.0 mg/L air 6-8 h/d for <b>5 d</b> EB (99 %)	Only little outer hair cell loss.  EB blood levels: Average concentration ca. 3 and 1 µg EB/mL blood on days 1 and 3	-	2500 ppm (11.0 mg/L air)	

Species (strain, animal no.)	Study design/test material	Results	NOAEC	LOAEC	Reference
<u>Rats</u> (Long-Evans, 6 m/grp)	Exposure to 400 ppm toluene and 660 ppm (or 2.9 mg/L air) EB 6 h/d, 5 d/wk for <b>1 or 2 wk</b> No data on test material purity	No adverse hearing effects (DPOAE amplitude, auditory threshold; loss of outer hair cells) Co-exposure with noise resulted in a potentiation of permanent auditory damage EB blood levels: ca. 0.28 and 0.12 µg/mL at the end of exposure and after a 1 hr recovery period Toluene blood levels: ca. 0.11 µg/mL and < LOQ at the end of exposure and after a 1 hr recovery period	660 ppm (2.9 mg/L air)  2 wk, in co-exposure with 400 ppm toluene	-	Fechter et al. (2007)
<u>Rat</u> (Sprague-Dawley, 14 m/grp)	200-400-600-800 ppm or 0.9-1.8-2.6-3.5 mg/L air 6 h/d, 6 d/wk for <b>4, 8 and 13 wk</b> EB (99 %)	Dose-dependent slight to severe ototoxicity Electrophysiological measurements: effect on audiometric thresholds at 400-800 ppm, no recovery within 8 weeks Morphological examination: loss of outer hair cells at all concentrations EB blood levels: Not reported	-	200 ppm (0.9 mg/L air)	Gagnaire et al. (2007)

*Cappaert et al., 1999*

After exposure to 800 ppm ethylbenzene for 5 days (8 h per day) rats had persistently increased auditory thresholds of a noise-evoked startle response. This was demonstrated by a reflex modification for audiometry 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 post-exposure. The RMA thresholds (reflex modification for audiometry) 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 weeks 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) 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 (required for normal hearing sensitivity, hearing loss of 40-50 dB can occur due to their absence) ( $52.1 \% \pm 9.7$ ) in the upper part of the basal turn and in the lower part of the middle turn ( $65.6 \% \pm 7.3$ ). 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 reflex modification for audiometry testing and compound action potentials, the authors concluded that ethylbenzene primarily exerts its effects on the peripheral part of the auditory system.

*Cappaert et al., 2000*

In a second study on lower ethylbenzene concentrations, auditory function in rats was tested by measuring compound action potentials in the frequency range of 1-24 kHz and distortion product otoacoustic emissions (DPOAEs, a very sensitive, noninvasive test of cochlear function, detecting impairment of outer hair cells; measurement of emitted sounds produced as stimulus-induced active outer hair cells response) in the frequency range of 4-22.6 kHz. The effects were analysed three to six weeks after the end of exposure. 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 compound action potentials 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. Outer hair cell loss was found in two of five examined localisations in the cochlea. At 400 ppm, 25 % outer hair cell loss was found at the 11- and 21-kHz region. The 550 ppm concentration evoked 40 % and 75 % outer hair cell 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.

*Cappaert et al., 2001*

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. No synergistic effect was found by using distortion product otoacoustic emissions (DPOAE) and compound action potential measurements.

The effects of 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). Ambient noise was below 50 dB 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 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 cytochleograms) 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, but not significant outer hair cell loss was found after exposure to 300 ppm ethylbenzene alone; it was located in the third row of outer hair cells. Significant hair cell loss was observed after exposure to 400 ppm ethylbenzene alone, spreading to the second and first outer hair cell rows. Noise alone hardly affected the outer hair cell counts except for a minor loss in the first row of outer hair cells after 105 dB. Noise at 105 dB in combination with ethylbenzene at 300 and 400 ppm, however, showed outer hair cell 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.

*Cappaert et al., 2002*

Guinea pigs were less susceptible to the ototoxic effect of ethylbenzene than rats. This can be related to varying blood concentrations of ethylbenzene in guinea pigs and rats.

Eight female guinea pigs (200 g) were exposed to high concentrations of ethylbenzene (2500 ppm, 8 hrs on the first day, thereafter 6 hrs/day, over the following 4 days). 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) was 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 (8 hrs/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 compound action potential 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 outer hair cells was observed in rats in the 11- and 21-kHz regions of the cochlea, the average percentage of remaining outer hair cells was only 25 % in the 21-kHz region. In guinea pigs there was only little outer hair cell 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.

*Gagnaire et al. (2007)*

Exposure of ethylbenzene to rats for 13 weeks (6h/day) produced moderate to severe ototoxicity without causing important systemic toxicity. The ototoxicity is characterised by increased electrophysiological auditory thresholds and hair cell losses, where hair cell loss was the more sensitive endpoint. The LOAEL for outer hair cell loss was 200 ppm (0.9 mg/L), a NOAEL could not be derived. Calculated theoretical lowest adverse effect levels were around 120 ppm.

Rats were exposed to ethylbenzene concentrations of 200, 400, 600 and 800 ppm (corresponding to 0.9 to 3.5 mg/L). Exposure was for 6 hrs daily, 6 days/week over 13 weeks; 14 male Sprague-Dawley were used per group. The animals were maintained for a recovery period of 8 weeks before they were sacrificed. There was no significant difference in weight gain between controls and groups exposed to ethylbenzene.

Electrophysiological measurements were performed at the end of the 4<sup>th</sup>, 8<sup>th</sup> and 13<sup>th</sup> week of exposure and after a recovery for 8 weeks (at week 21). Electrodes were implanted 3 to 4 weeks before start of exposure. Auditory thresholds were investigated by brainstem auditory-evoked responses with frequencies of 2, 4, 8 and 16 kHz.

At all frequencies analysed the concentration-effect relationship was characterised by increased thresholds after exposure to 400, 600 and 800 ppm. Highest hearing losses were found in the rats exposed to 600 and 800 ppm. Concerning the time-effect relationship, these threshold shifts appeared from the fourth week of exposure onwards; there was no significant increase throughout the exposure period and no recovery 8 weeks after end of exposure.

Morphological examinations were carried out after the recovery period; 8 rats were investigated per concentration. The cochleae were prepared and cytochleograms (total cell count by histopathology) were constructed from the surface preparation. At all tested concentrations losses of outer hair cells of the organ of Corti were found. Outer cell hair loss was nearly complete in the three rows of the organ of Corti after exposure to 600 and 800 ppm ethylbenzene. Only the basal part of the cochlea (which transcribes the high frequencies) was partly spared. Also after exposure to 400 ppm considerable outer hair cell loss was found in all animals. After exposure to 200 ppm, significant outer hair cell loss in the third row was seen in 4 out of the 8 exposed animals.

Outer hair cell losses were greatest in the third row. After exposure to 200 ppm the average loss in the animals was 4 %, and the EC<sub>50</sub> was found to be 371 ppm.

Inner hair cell loss was found after exposure to 600 and 800 ppm in the basal part of the organ of Corti. On average, the inner hair cell loss was 32 % at 800 ppm and 14 % at 600 ppm. After exposure to 400 ppm, occasional inner hair cell loss was found in the basal part of the organ of Corti.

In this study the ototoxic effect of ethylbenzene was also tested in combination with two types of mixed xylenes (o-, m-, p-xylene). These co-exposures caused potentiation of the ototoxic effects.

The LOAEC for ototoxic effects was 200 ppm (0.9 mg/L air). However, no NOAEC could be derived. Therefore, the authors calculated theoretical lowest adverse effect levels from the three statistical upper confidence limits of the average losses observed in the controls. All three calculated theoretical lowest adverse effect levels were around 120 ppm (114-130 ppm or 0.5-0.6 mg/L air).

#### *Fechter et al. (2007)*

Inhalation exposure of rats to a mixture of ethylbenzene (660 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 of 93-95 dB resulted in a potentiation of permanent auditory damage.

In contrast to the other investigations, no adverse hearing effects were found after exposure to high solvent exposures alone (660 ppm ethylbenzene plus 400 ppm toluene)

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 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 evidence of glutathione depletion.

The combined exposure to toluene and ethylbenzene produced no effects on DPOAE or compound action potentials at any of the post-exposure 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 showed 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 outer hair cells. Noise alone showed an increase of outer hair cells as compared to controls. This was quite limited but particularly apparent in rows 1 and 2. The outer hair cell loss did not exceed 5 % in any area of the cochlea. The combined treatment with solvent and noise showed after 5 days exposure clear outer hair cell death at 12-24 kHz, greatest in row 1, intermediate row 2 and very limited row 3. Loss of outer hair cells did not exceed 25 % at any locus. A similar pattern of effect was seen after 10 days exposure.

A number of the above studies on ototoxicity following inhalation exposure has also recently been reviewed by Vyskocil et al. (2008), cf. below, section 5.6.5.

### 5.6.3 Repeated dose toxicity: dermal

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

### 5.6.4 Other relevant information

None relevant

### 5.6.5 Summary and discussion of repeated dose toxicity:

No relevant human data is available concerning repeated dose toxicity of ethylbenzene. In experimental animals, repeated doses of ethylbenzene specifically affected the nervous system. Ethylbenzene did not induce overt toxicity in any other organ system.

After **oral exposure** to rats for 90 days, a NOAEL of 75 mg/kg bw/d was found, based on indications of a mild regenerative anaemia and liver changes indicative of microsomal enzyme induction. The LOAEL was 250 mg/kg bw/d. The key study for these data is given by Mellert et al. (2004; 2007). Ototoxicity was found after oral administration; however, only one high dose of 900 mg/kg/day was investigated. No classification results are deduced from these data.

No data is available for repeated **dermal administrations**.

After **repeated inhalation exposure** of rats and mice for 3 months, for general toxicity a NOAEC of 1000 ppm (4.4 mg/L) was established (key study: NTP, 1992; Hard, 2002). Observed increases in liver and kidney weight were interpreted to be associated with metabolic enzyme induction.

Irreversible ototoxicity was found in rats after repeated inhalation exposure to ethylbenzene vapour (key study: Gagnaire et al., 2007).

In neurophysiological measurements effects were found on audiometric thresholds after exposure to ethylbenzene concentrations, ranging from 400 to 800 ppm. The threshold shifts appeared from the fourth week of exposure onwards; there was no significant increase throughout the exposure period and no recovery 8 weeks after exposure.

Morphological examinations after 13 weeks of exposure and 8 weeks of recovery demonstrated losses of outer hair cells at all tested concentrations; the LOAEC was 200 ppm (0.9 mg/L air). Outer hair cell loss was nearly complete after exposure to 600 and 800 ppm and still observable in all animals after 600 ppm; after exposure to 200 ppm significant outer hair cell loss in the third row was seen in 4 out of the 8 exposed animals. Outer hair cell losses were greatest in the third row. After exposure to 200 ppm the average loss in the animals was 4 %, and the EC<sub>50</sub> was found to be 371 ppm (1.6 mg/L air).

These results are in line with those of Cappaert et al. (2000) which showed that the minimum ethylbenzene concentration required to induce significant threshold shifts after exposure for 5 days was 400 ppm, whereas 300 ppm ethylbenzene did not affect the auditory system significantly but caused some minor outer hair cell loss. The fact that hair cell loss is observed at concentrations with only weak or no auditory threshold shifts has already been noted and discussed earlier (e.g., Cappaert et al., 2000). Irreversible hair cell loss may result in a premature onset of signs of normal aging (presbycusis).

At co-exposure to ethylbenzene and high levels of noise, synergistic effects on hearing loss and cell damage of outer hair cells occurred.

Recently, a number of the above studies on ototoxicity following repeated inhalation exposure to ethylbenzene were also reviewed by Vyskocil et al. (2008). The authors considered animal data only when dose levels exceeded current Canadian occupational health thresholds (time-weighted average exposure value, TWAEV) by not more than a factor of 100. They concluded as follows:

*‘[...] Further studies with sufficient data on the ethyl benzene exposure of individual workers are thought necessary to make a definitive conclusion. Given the current evidence from animal studies, we recommend considering ethyl benzene as an ototoxic agent’.*

In the classification criteria (Annex I of CLP-Regulation (EC) No. 1272/2008) category 2 for specific target organ toxicity after repeated exposure is, among others, foreseen when significant toxic effects are observed in a 90 day repeated dose study after inhalation exposure to vapour concentrations ranging from 0.2 to 1.0 mg/L air. The LOAEC for irreversible cell death of outer hair cells of the cochleae was 0.9 mg/L air. Therefore, it is proposed to classify/label ethylbenzene as:

**STOT Rep. 2; H373 - Warning: May cause damage to organs through prolonged or repeated exposure**

## 5.7 Mutagenicity

Mutagenicity has not been considered as part of this dossier.

## 5.8 Carcinogenicity

Carcinogenicity has not been considered as part of this dossier.

## 5.9 Toxicity for reproduction

Toxicity for reproduction has not been considered as part of this dossier.

## 5.10 Other effects

### 5.10.1 Aspiration hazard

Ethylbenzene has a very low kinematic viscosity of 0.63 mm<sup>2</sup>/s as determined at 40 °C following the standard method ASTM D445 (Knothe and Steidley, 2005). This method which - according to <http://www.astm.org/Standards/D445.htm> (as of 2010-10-11) - corresponds to method ISO 3104 directly assesses kinematic viscosity of liquids in the range of 0.2-300000 mm<sup>2</sup>/s..

In the classification criteria for aspiration hazard (Regulation (EC) No. 1272/2008) it is argued that a substance needs to be classified in category 1, if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm<sup>2</sup>/s or less, measured at 40 °C.

The aspiration hazard of ethylbenzene is supported by experimental data (Gerarde and Linden, 1963). Three male Wistar rats, weighing from 250 to 350 g, were anaesthetised to the point of apnoea. The mouth was held open and the tongue pulled forward. With the animals' heads elevated, 0.2 mL (or 0.25 mL, contradictory data) of commercial ethylbenzene were delivered into the mouth. As breathing resumed and became regular, the nostrils were closed with the fingers at the end of the expiration phase of the breathing cycle. This was repeated until the liquid had been aspirated or the animal showed signs of regaining consciousness. All three animals died instantly.

With respect to aspiration toxicity it is therefore proposed to classify/Label ethylbenzene:

- according to Annex I of CLP-Regulation (EC) No. 1272/2008 as:

**Asp.Tox. 1; H304 – Danger: May be fatal if swallowed and enters airways**

- according to the criteria of Directive 67/548/EEC as:

**Xn; R65 - Harmful: May cause lung damage if swallowed**

## 5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not applicable for this dossier.

## 6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

Not relevant for this dossier. It is however noted that according to Annex VI of Regulation (EC) No 1272/2008 ethylbenzene is classified as a Flammable Liquid, Cat. 2.

**7 ENVIRONMENTAL HAZARD ASSESSMENT**

Not relevant for this dossier.

## **JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS**

Ethylbenzene was a priority substance in the existing chemicals program (EEC) 793/93. A classification proposal was not yet discussed at the TC C&L level.

## **OTHER INFORMATION**

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