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3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of ethylbenzene. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

3.2.1.1 Death

No studies were located regarding lethality in humans following inhalation exposure to ethylbenzene. Matsumoto et al. (1992) reported the case of a 44-year-old man who died 9 days after exposure to gasoline vapors for at least 10 hours in an enclosed car. The patient’s clothing was saturated with gasoline, but there was no apparent ingestion of gasoline. The patient suffered chemical burns over 50% of the total body surface area, and the cause of death was reported as multiple organ failure. Ethylbenzene was detected in the patient’s blood (estimated initial blood concentration of 2.6 µg/mL). However, since gasoline contains approximately 1.4% (by weight) ethylbenzene (Agency for Toxic Substances and Disease Registry 1995), it is unlikely that death was due to ethylbenzene exposure.

Studies on the lethality of inhaled ethylbenzene have been conducted using several animal models, with exposure times ranging from a few hours to 2 years. The LC_{50} values obtained from acute inhalation exposure studies were 13,367 ppm following a 2-hour exposure (Ivanov 1962) and 4,000 ppm following a 4-hour exposure (Smyth et al. 1962); 100% mortality was observed following exposure to 16,698 ppm for 2 hours (Ivanov 1962) and exposure to 8,000 ppm for 4 hours (Smyth et al. 1962). Inhalation exposure of Fischer 344 rats and $B6C3F_1$ mice to ethylbenzene for 6 hours/day for 4 days produced lethality at concentrations of 1,200 and 2,400 ppm, respectively, although exposure to concentrations up to 2,400 ppm was not lethal to rabbits (Ethylbenzene Producers Association 1986a). In intermediate-duration exposure studies, inhalation exposure of rats and mice to 782 ppm or rabbits to 1,610 ppm

ethylbenzene for 4 weeks did not produce lethality (Cragg et al. 1989). No mortality was observed in rats or mice exposed to 782 ppm or rabbits exposed to 1,610 ppm ethylbenzene for 4 weeks (Cragg et al. 1989) or in rats or mice exposed to 975 ppm ethylbenzene for 90 days (NTP 1992). Survival of male rats, but not female rats or male or female mice, exposed to 750 ppm ethylbenzene for 2 years was significantly decreased (NTP 1999); survival in male rats exposed to 75 or 250 ppm ethylbenzene was not affected.

The LC_{50} values and all reliable LOAEL values for death in rats and mice following acute- or chronic-duration exposure are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

Little data are available on the systemic effects of inhaled ethylbenzene in humans. Most of the information available is from case reports in which quantitative data on exposure concentrations and durations were not reported. In addition, most of the available studies have confounding factors (e.g., simultaneous exposures to other toxic substances) and insufficient reporting of important study details. In general, the systemic effects observed in humans were respiratory tract and ocular irritation, and possible ototoxicity (hearing loss) and hematological alterations (increased lymphocyte counts and decreased hemoglobin concentration) (Angerer and Wulf 1985; Cometto-Muñiz and Cain 1995; Thienes and Haley 1972; Yant et al. 1930).

Several studies were located on the systemic effects of ethylbenzene in animals following inhalation exposure. Acute- and intermediate-duration exposure to inhaled ethylbenzene is associated with respiratory irritation, changes to the liver (increased organ weights and induction of microsomal enzymes), and effects on the hematological system (decreased platelets and increased leukocyte counts). Chronic exposure is associated with adverse effects to the liver (necrosis and hypertrophy), kidney (nephropathy and hyperplasia), and endocrine system (thyroid and pituitary hyperplasia).

No studies were located describing cardiovascular, gastrointestinal, musculoskeletal, renal, endocrine, dermal, body weight, or metabolic effects in humans, or dermal effects in animals after inhalation exposure to ethylbenzene.

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Fischer- 344)	4 d 6 hr/d				2400 M (100% mortality by day 3)	Ethylbenzene Producers Association 1986a	
2	Rat (NS)	4 hr				4000 M (LC50)	Smyth et al. 1962	
3	Mouse (B6C3F1)	4 d 6 hr/d				1200 M (4/5 animals died by day 3)	Ethylbenzene Producers Association 1986a	
Systemic								
4	Rat (Wistar)	2 wk 5 d/wk 6 hr/d	Hepatic		50 M (induction of UDP-glucuronyl transferase and D-glucuronyllactone dehydrogenase)		Elovaara et al. 1985	
			Renal		600 M (increase in relative kidney weights)			

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
5	Rat (Fischer- 344)	4 d 6 hr/d	Resp	2000 M			Ethylbenzene Producers Association 1986a	No liver or renal histopathological changes were observed.
			Hepatic		400 M	(increased liver weight)		
			Renal	400 M	1200 M	(increased relative kidney weight)		
			Ocular	400 M	1200 M	(lacrimation)		
			Bd Wt	1200 M				
6	Rat (Sprague-Dawley)	3 d 6 hr/d	Resp	2000 M			Toftgard and Nilsen 1982	
			Hepatic		2000 M	(increased relative liver weight, and induction of nadph-cytochrome reductase and 7-ethoxycoumarin o-deethylase)		
			Renal		2000 M	(increased relative kidney weight)		
7	Mouse (Swiss)	5 min	Resp		1432 M	(RD50)	De Ceaurriz et al. 1981	

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
8	Mouse (B6C3F1)	4 d 6 hr/d	Ocular		400 M (lacrimation)		Ethylbenzene Producers Association 1986a	
			Bd Wt	400 M				
9	Mouse (Swiss-Webster)	30 min	Resp		4060 M (RD50)		Nielsen and Alarie 1982	
10	Mouse (CFW)	20 min	Ocular		2000 M (lacrimation and palpebral closure)		Tegeris and Balster 1994	
11	Rabbit (New Zealand)	4 d 6 hr/d	Resp	2400 M			Ethylbenzene Producers Association 1986a	
			Hepatic	2400 M				
			Renal	2400 M				
			Ocular		400 M (lacrimation)			
			Bd Wt	2400 M				
Neurological								
12	Rat (Wag/Rij/Cpb/I	8 hr/d 5 d				800 M (loss of outer hair cells and hearing loss)	Cappaert et al. 1999	
13	Rat (Wag/Rij)	8 hr 5 d		300[b]		400 (loss of outer hair cells and shifts in hearing thresholds)	Cappaert et al. 2000	

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
14	Rat (albino Wag/Rij)	8 hr/d 5 d		300	400 (loss of outer hair cells)		Cappaert et al. 2001	
15	Rat (Wag/Rij)	8 hr/d 5 d			550 F (loss of outer hair cells and shifts in hearing thresholds)		Cappaert et al. 2002	
16	Rat (Fischer- 344)	4 d 6 hr/d		1200 M		2400 M (salivation, prostration)	Ethylbenzene Producers Association 1986a	
17	Rat (CFY)	4 hr		200 M	400 M (moderate activation in motor behavior)	2180 M (narcotic effects)	Molnar et al. 1986	
18	Mouse (B6C3F1)	4 d 6 hr/d		400 M		1200 M (prostration and reduced activity)	Ethylbenzene Producers Association 1986a	
19	Mouse (CFW)	20 min			2000 M (postural changes, decreased arousal and rearing, distrubed gait, decreased mobility, righting reflex, decreased grip strength, increased landing foot splay, impaired psychomotor coordination)		Tegeris and Balster 1994	

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
20	Rabbit (New Zealand)	4 d 6 hr/d		2400 M			Ethylbenzene Producers Association 1986a	
Reproductive								
21	Rat (Fischer- 344)	4 d 6 hr/d		2400 M			Ethylbenzene Producers Association 1986a	
22	Mouse (B6C3F1)	4 d 6 hr/d		1200 M			Ethylbenzene Producers Association 1986a	
23	Rabbit (New Zealand)	4 d 6 hr/d		2400 M			Ethylbenzene Producers Association 1986a	

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE								
Systemic								
24	Rat (Fischer- 344)	4 wk 5 d/wk 6 hr/d	Resp	782			Cragg et al. 1989	
			Cardio	782				
			Gastro	782				
			Hemato	382	782	(increased platelet counts in males; increased mean total leukocyte counts in males and females)		
			Musc/skel	782				
			Hepatic	382	782	(increased absolute and relative liver weight)		
			Renal	782				
			Endocr	782				
			Ocular	99	382	(sporadic incidence of lacrimation)		
			Bd Wt	782				

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
25	Rat (Wistar)	5-16 wk 5 d/wk 6 hr/d	Hepatic		50 M (induction of UDP-glucuronyl transferase and D-glucuronyllactone dehydrogenase)		Elovaara et al. 1985	
			Renal		300 M (increase in microsomal enzyme activity and glutathione)			
			Bd Wt	600 M				
26	Rat (Wistar)	3 wk 5 d/wk 7 hr/d Gd 1-19	Resp	985 F			NIOSH 1981	
			Hepatic	97 F	959 F (increased liver weight)			
			Renal	97 F	959 F (increased kidney weight)			
			Bd Wt	985 F				

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
27	Rat (F344/N)	90 d 5 d/wk 6 hr/d	Resp	99 F	246 F (increased absolute and relative lung weight)		NTP 1992	
			Cardio	975				
			Gastro	975				
			Hemato	975				
			Musc/skel	975				
			Hepatic	99 M	246 M (increased absolute and relative liver weight)			
			Renal	246 M	498 M (<10% increase in absolute and relative kidney weight)			
			Endocr	975				
			Ocular	975				
			Bd Wt	975				
28	Rat SD	6 hr/d Gd 6-20	Hepatic		250 F (increased absolute and relative liver weight, moderate to marked hepatocellular hypertrophy)		Saillenfait et al. 2006, 2007	
			Bd Wt	250 F	1000 F			

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
29	Rat (CD)	6 hr/d 7 d/wk	Hemato	500 F			Stump 2004	
			Bd Wt	500 F				
30	Mouse (B6C3F1)	4 wk 5 d/wk 6 hr/d	Resp	782			Cragg et al. 1989	
			Cardio	782				
			Gastro	782				
			Hemato	782				
			Musc/skel	782				
			Hepatic	382	782 (increased absolute and relative liver weight)			
			Renal	782				
			Endocr	782				
			Ocular	782				
			Bd Wt	782				

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
31	Mouse (B6C3F1)	90 d 5 d/wk 6 hr/d	Resp	975			NTP 1992	
			Cardio	975				
			Gastro	975				
			Hemato	975				
			Musc/skel	975				
			Hepatic	498 M	740 M (increased absolute and relative liver weight)			
			Renal	740 F	975 F (increased relative kidney weight)			
			Endocr	975				
			Ocular	975				
			Bd Wt	975				

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
32	Rabbit (New Zealand)	4 wk 5 d/wk 6 hr/d	Resp	1610			Cragg et al. 1989	
			Cardio	1610				
			Gastro	1610				
			Hemato	1610				
			Musc/skel	1610				
			Hepatic	1610				
			Renal	1610				
			Endocr	1610				
			Ocular	1610				
			Bd Wt	1610				
33	Rabbit (New Zealand)	Gd 1-24 7 d/wk 7 hr/d	Resp	962 F			NIOSH 1981	
			Hepatic	99 F	962 F	(increased absolute and relative liver weights in pregnant rabbits)		
			Renal	962 F				
			Bd Wt	962 F				
			Other	962 F				
Immuno/ Lymphoret								
34	Rat (Fischer- 344)	4 wk 5 d/wk 6 hr/d		782			Cragg et al. 1989	

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
35	Rat (Wistar)	3 wk 5 d/wk 7 hr/d Gd 1-19		97 F	959 F (increased spleen weight)		NIOSH 1981	
36	Rat (F344/N)	90 d 5 d/wk 6 hr/d		975			NTP 1992	NOAEL based on evaluation of thymus weight, gross pathology, histopathology, and hematology.
37	Rat (CD)	6 hr/d 7 d/wk		500 F			Stump 2004	Based on absence of splenic IgM antibody forming response to immunization with T cell-dependent antigen (sheep RBC)
38	Mouse (B6C3F1)	4 wk 5 d/wk 6 hr/d		782			Cragg et al. 1989	NOAEL based on evaluation of thymus and spleen weight, gross pathology, histopathology, and hematology.
39	Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d		975			NTP 1992	NOAEL based on evaluation of thymus weight, gross pathology, histopathology, and hematology.

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
40	Rabbit (New Zealand)	4 wk 5 d/wk 6 hr/d		1610			Cragg et al. 1989	
Neurological								
41	Rat (Fischer- 344)	4 wk 5 d/wk 6 hr/d		99	382 (sporadic incidence of salivation)		Cragg et al. 1989	
42	Rat (SD)	6 hr/d 6 d/wk 13 wk			200[c] M	600 M (complete loss of third row outer hair cells and hearing loss)	Gagnaire et al. 2007	
43	Rat (F344/N)	90 d 5 d/wk 6 hr/d		975			NTP 1992	
44	Mouse (B6C3F1)	4 wk 5 d/wk 6 hr/d		782			Cragg et al. 1989	NOAEL based on clinical observations, organ weights, and histopathology.
45	Mouse (B6C3F1)	90 d 5 d/wk 6 hr/d		975			NTP 1992	NOAEL based on clinical observations, organ weights, and histopathology.

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
46	Rabbit (New Zealand)	4 wk 5 d/wk 6 hr/d		1610			Cragg et al. 1989	NOAEL based on clinical observations, organ weights, and histopathology.
Reproductive								
47	Rat (Fischer- 344)	4 wk 5 d/wk 6 hr/d		782			Cragg et al. 1989	NOAEL based on histopathological examination of reproductive organs.
48	Rat (Crl-CD)	6 hr/d 70 d		500			Faber et al. 2006	NOAEL based on reproductive parameters (mating/fertility indices, gestation length, implantations, births/litter and litter size.
49	Rat (Wistar)	3 wk 5 d/wk 7 hr/d Gd 1-19		985 F			NIOSH 1981	NOAEL assessed by pregnancy rate, implantations, number of litters, resorptions, and live fetuses.
50	Rat (F344/N)	90 d 5 d/wk 6 hr/d		975			NTP 1992	NOAEL based on vaginal cytology and sperm motility, organ weights, and estrous cycle.

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
51	Mouse (B6C3F1)	4 wk 5 d/wk 6 hr/d		782			Cragg et al. 1989	NOAEL based on histopathological examination of reproductive organs.
52	Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d		975			NTP 1992	NOAEL based on vaginal cytology and sperm motility, organ weights, and estrous cycle.
53	Rabbit (New Zealand)	4 wk 5 d/wk 6 hr/d		1610			Cragg et al. 1989	NOAEL based on histopathological assessment of reproductive organs.
54	Rabbit (New Zealand)	Gd 1-24 7 d/wk 7 hr/d		962 F			NIOSH 1981	NOAEL assessed by pregnancy rate, implantations, number of litters, resorptions, and live fetuses.
Developmental								
55	Rat (Crl-CD)	6 hr/d 70 d		500 F			Faber et al. 2006	NOAEL based on fetal survival and gross and histopathological examination of fetuses.

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
56	Rat (Crl-CD)	6 hr/d 70 d		500 F			Faber et al. 2007	NOEAL based on fetal survival, body weights, physical landmarks, and neurobehavioral tests.
57	Rat (Wistar)	3 wk 5 d/wk 7 hr/d Gd 1-19		97 F	959 F (extra rib, supemumerary ribs)		NIOSH 1981	
58	Rat (Sprague-Dawley)	6 hr/d Gd 6-20		500 F	1000 F (decreased fetal body weight)		Saillenfait et al. 2003	
59	Rat (Sprague-Dawley)	6 hr/d Gd 6-20		250 F	1000 F (slight decrease in fetal body weight)		Saillenfait et al. 2006, 2007	
60	Rabbit (New Zealand)	Gd 1-24 7 d/wk 7 hr/d		962 F			NIOSH 1981	NOAEL assessed by pregnancy rate, implantations, number of litters, resorptions, and live fetuses.

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
CHRONIC EXPOSURE								
Death								
61	Rat (F344)	6 hr/d 5 d/wk 2 yr				750 M (decreased survival)	NTP 1999	Survival of male rats was not affected at 75 or 250 ppm. In addition, survival of female rats and male and female mice was not affected at 750 ppm.
Systemic								
62	Rat (F344)	6 hr/d 5 d/wk 2 yr	Resp	750			NTP 1999	
			Cardio	750				
			Hemato	750				
			Musc/skel	750				
			Hepatic	750				
			Renal		75[d] F (increased severity of nephropathy)			
			Endocr	750				
			Dermal	750				
			Bd Wt	750				

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
63	Mouse (F344)	6 hr/d 5 d/wk 2 yr	Resp	750			NTP 1999	
			Cardio	750				
			Gastro	750				
			Musc/skel	750				
			Hepatic	250 M	750 M (hypertrophy and necrosis)			
			Renal	750				
			Endocr	75 F	250 F (hyperplasia in pituitary gland pars distalis)			
					750 (thyroid follicular cell hyperplasia)			
			Dermal	750				
			Bd Wt	750				
Cancer								
64	Rat (F344)	6 hr/d 5 d/wk 2 yr				750 M (CEL: renal tubule adenoma or carcinoma)	NTP 1999	

Table 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)		Reference Chemical Form	Comments
65	Mouse (F344)	6 hr/d 5 d/wk 2 yr				750	(CEL: alveolar/bronchiolar adenoma or carcinoma and hepatocellular adenoma or carcinoma)	NTP 1999	

a The number corresponds to entries in Figure 3-1.

b Used to derive an acute-duration inhalation minimal risk level (MRL) of 5 ppm based on a BMCL using an internal dose metric to simulate time averaged arterial blood concentration of ethylbenzene (81.10 µmol/L); a human equivalent concentration (HEC) of the BMCL was estimated using a human PBPK model. The BMCL(HEC) (154.26 ppm) was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability).

c Used to derive an intermediate-duration inhalation minimal risk level (MRL) of 2 ppm based on a BMCL using an internal dose metric to simulate time averaged arterial blood concentration of ethylbenzene (19.94 µmol/L); a human equivalent concentration (HEC) of the BMCL was estimated using a human PBPK model. The BMCL(HEC) (63.64 ppm) was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability).

d Used to derive a chronic-duration inhalation minimal risk level (MRL) of 0.06 ppm based on an internal dose metric (averaged arterial blood concentration of ethylbenzene, MCA) of the LOAEL; a human equivalent concentration (HEC) of the LOAEL(MCA) was estimated using a human PBPK model. The LOAEL(HEC) (17.45 ppm) was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; F = Female; Gastro = gastrointestinal; Gd = gestation day(s); Hemato = hematological; hr = hour(s); LC50 = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; RD50 = exposure concentration producing a 50% decrease in respiratory rate ; Resp = respiratory; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation

Acute (≤14 days)

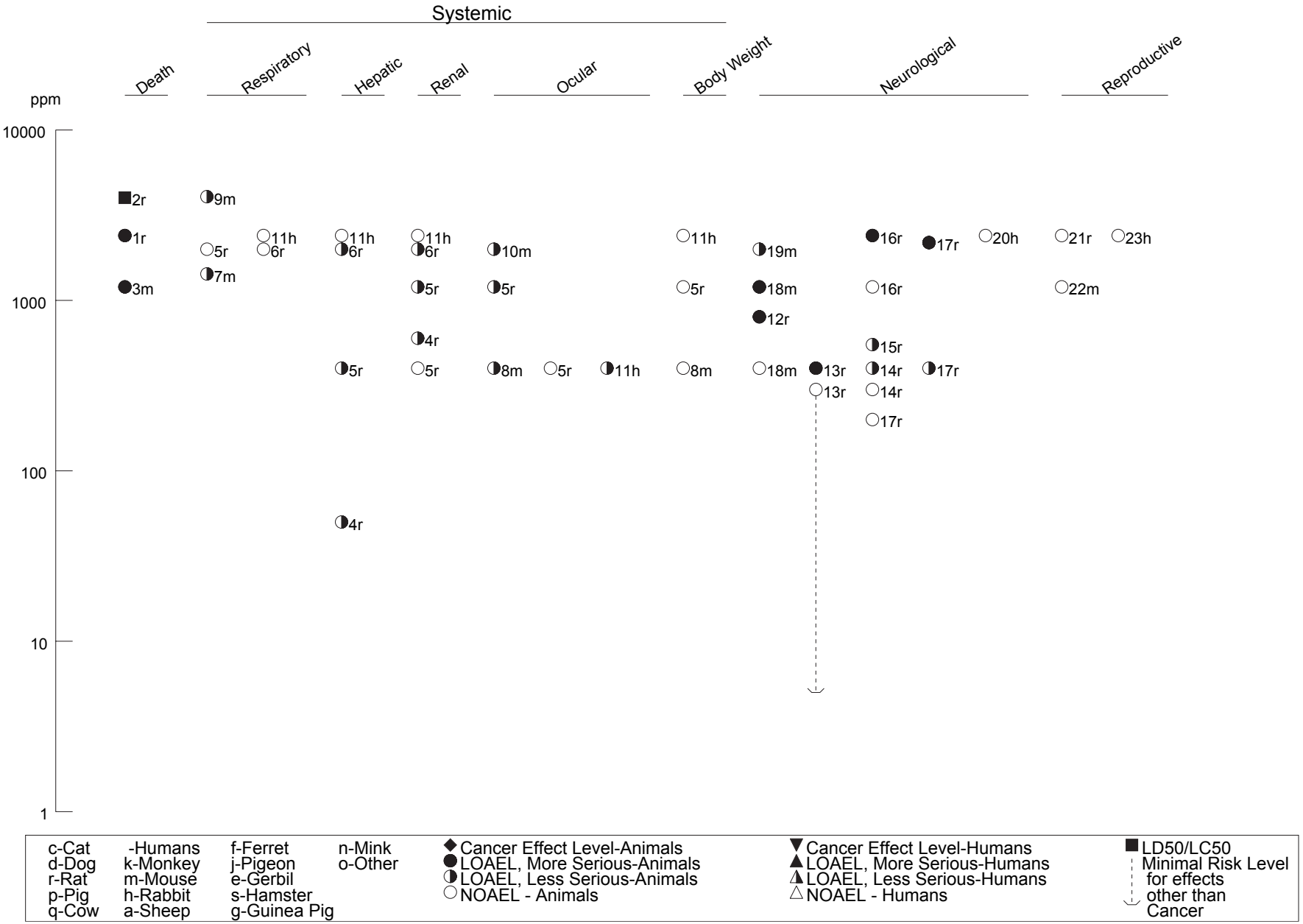


Figure 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation *(Continued)*

Intermediate (15-364 days)

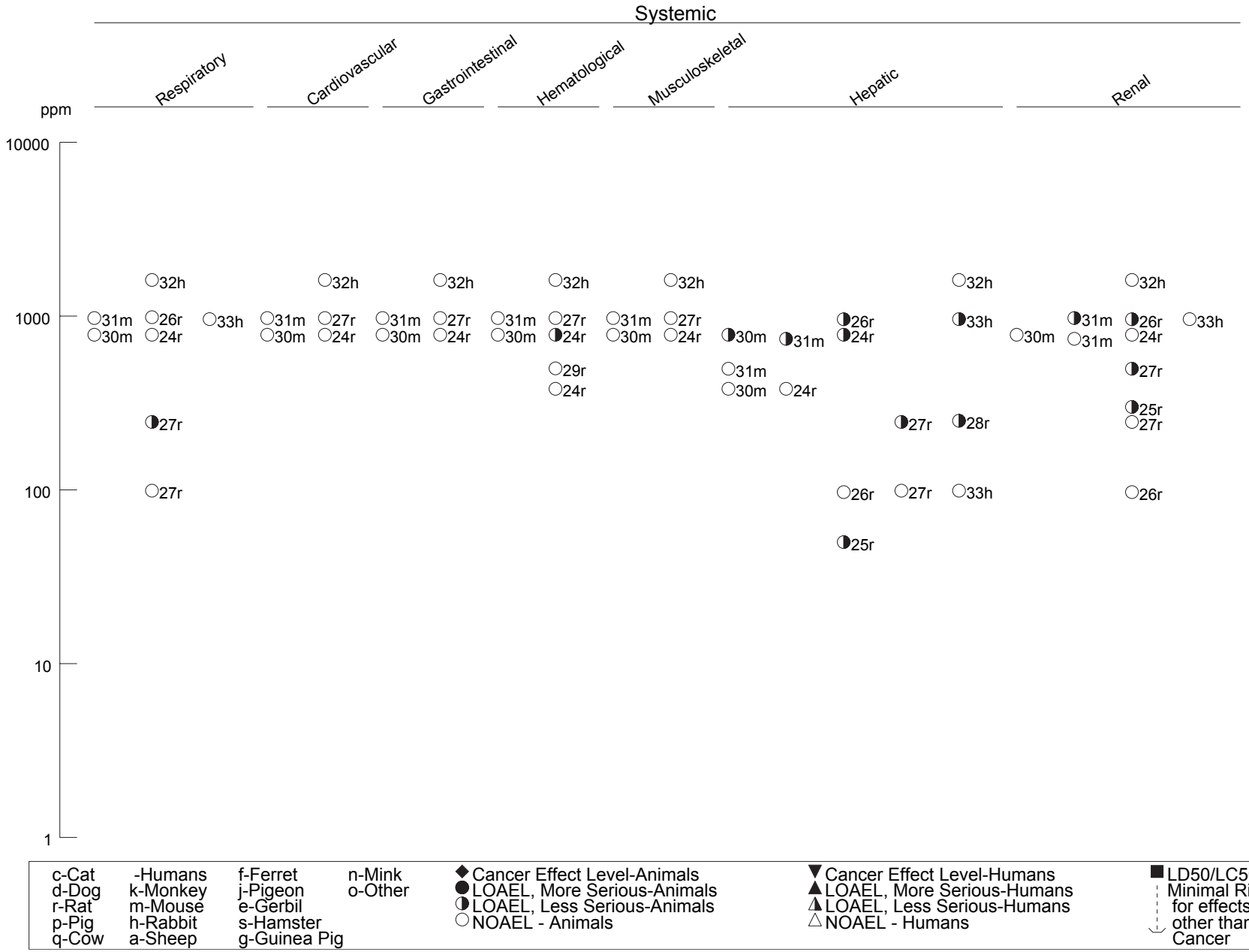


Figure 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation *(Continued)*

Intermediate (15-364 days)

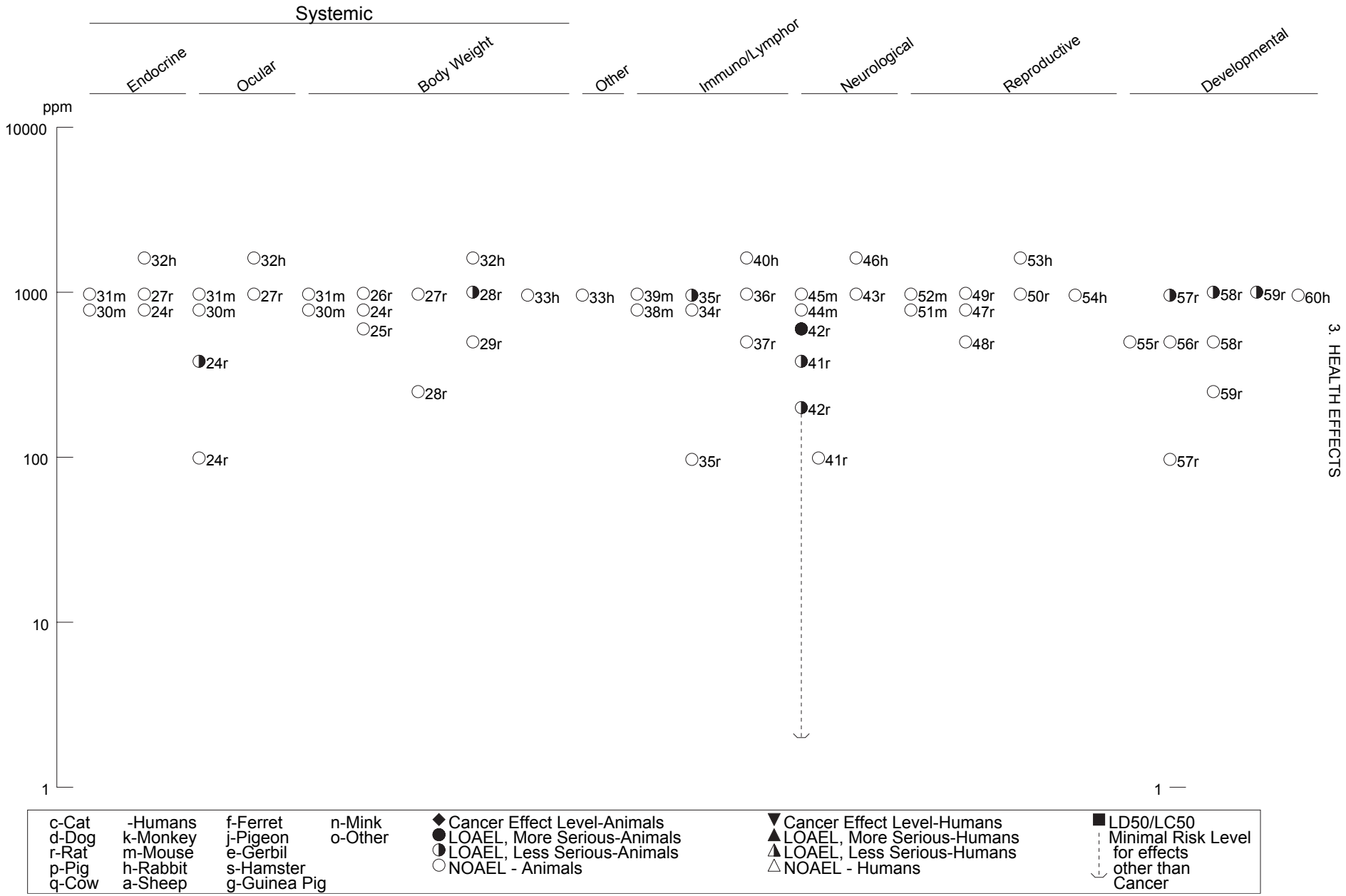
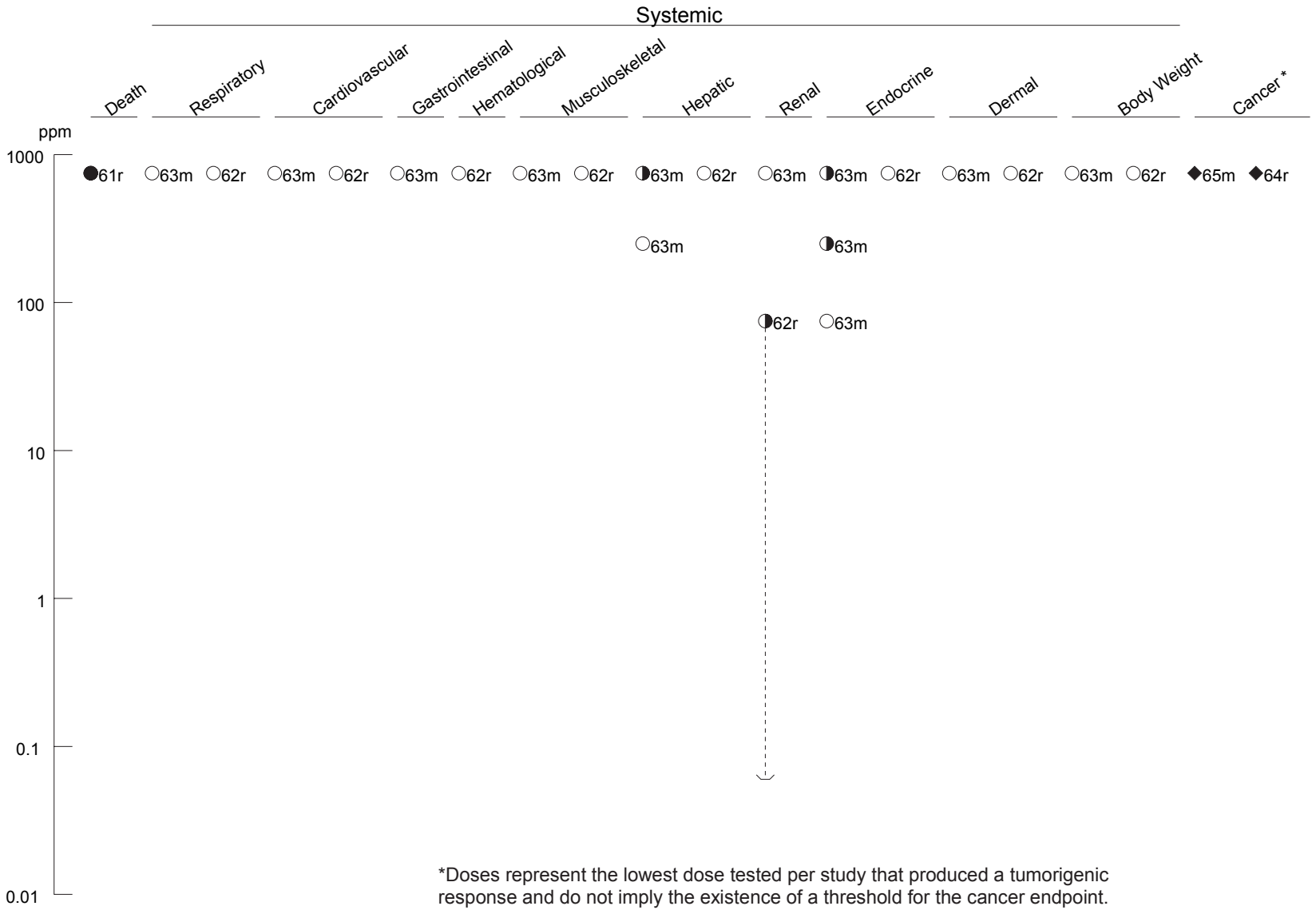


Figure 3-1 Levels of Significant Exposure to Ethylbenzene - Inhalation *(Continued)*

Chronic (≥365 days)



The systemic effects observed after inhalation exposure are discussed below. The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Male volunteers reported throat and nasal irritation and feelings of "chest constriction" during a 6-minute inhalation exposure to 2,000 ppm ethylbenzene (Yant et al. 1930). More severe throat and nasal irritation were reported immediately upon exposure to 5,000 ppm ethylbenzene. No data on pulmonary function were reported (Yant et al. 1930). No bronchospastic response was observed in two subjects challenged with 55 ppm ethylbenzene for 15 minutes in an inhalation chamber, based on comparison of pre- and post-exposure pulmonary function tests (Moscato et al. 1987).

Results of acute studies in animals indicate that inhalation of ethylbenzene produces adverse respiratory effects ranging from irritation to pulmonary congestion. Nasal irritation (based on nose rubbing) was observed in guinea pigs exposed to 1,000 ppm for 3 and 8 minutes and to 2,000, 5,000, and 10,000 ppm for 480, 30, and 10 minutes, respectively (Yant et al. 1930). Gross histopathology revealed congestion and edema in the lungs of animals that died after exposure to 10,000 ppm ethylbenzene, with less severe effects observed in surviving animals. Pulmonary congestion also was observed in rats and mice that died during exposure a 4-day exposure to 2,400 and ≥1,200 ppm ethylbenzene, respectively (Ethylbenzene Producers Association 1986a). A 50% reduction in breathing rate was observed in mice exposed to 1,432 ppm for 5 minutes (De Ceaurriz et al. 1981) and 4,060 ppm for 30 minutes (Nielsen and Alarie 1982). No adverse respiratory effects were observed in rats exposed to 2,000 ppm for 3 days (Toftgard and Nilsen 1982) or in rats, mice, and rabbits exposed to 1,200, 400, or 2,400 ppm, respectively, for 4 days (Ethylbenzene Producers Association 1986a).

Adverse respiratory effects attributed to intermediate- or chronic-duration inhalation exposures to ethylbenzene have been observed in animals. Relative lung weight was increased in male rats exposed to 975 ppm for 90 days, although increased absolute and relative lung weights were observed only in female rats exposed to 246 ppm ethylbenzene, but not to higher concentrations (NTP 1992). Pulmonary lesions (inflammatory cell infiltrate) were observed in male rats exposed to ≥246 ppm for 90 days (NTP 1992). However, the NTP Pathology Working Group considered these effects to be more typical of an infectious agent than a response to the test compound; therefore, pulmonary findings were not attributed to ethylbenzene exposure. No histopathological changes were observed in rats exposed to 2,200 ppm, guinea pigs or rabbits exposed 1,250 ppm, or monkeys exposed to 600 ppm ethylbenzene for 6 months (Wolf et al. 1956); however, the utility of this study is limited by a general lack of study details (e.g., no

exposure or control data were provided) and the small size of exposure groups (1–2 rabbits or monkeys per group). No histopathological findings clearly attributable to ethylbenzene were observed in respiratory tissue of rats or mice exposed to up to 750 ppm ethylbenzene for 2 years (NTP 1999). Although the incidences of edema, congestion, and hemorrhage observed in male rats in the 750 ppm group were increased relative to control rats, observations were considered to be agonal changes in moribund animals and not directly related to chemical toxicity (NTP 1999).

Cardiovascular Effects. Intermediate and chronic exposure of animals to inhaled ethylbenzene does not appear to produce adverse cardiovascular effects, based on results of histopathological examinations. No adverse histopathological effects were reported in cardiac tissue of rats or mice exposed to 782 ppm or rabbits exposed to 1,610 ppm ethylbenzene for 4 weeks (Cragg et al. 1989). Similarly, no cardiovascular effects were observed in rats or mice exposed to 975 ppm ethylbenzene for 90 days (NTP 1992) or in rats exposed to 2,200 ppm, guinea pigs or rabbits exposed to 1,250 ppm, or monkeys exposed to 600 ppm ethylbenzene for 6 months (Wolf et al. 1956). No adverse histopathological findings were observed in cardiovascular tissues of rats or mice exposed to 750 ppm ethylbenzene for 2 years (NTP 1999).

Gastrointestinal Effects. No adverse effects on the gastrointestinal system have been observed following intermediate or chronic inhalation exposure of animals. No changes in gross appearance or adverse histopathological effects were observed in the intestines of rats and mice exposed to 782 ppm or rabbits exposed to 1,610 ppm ethylbenzene for 4 weeks (Cragg et al. 1989). No adverse histopathological changes in gastrointestinal tissue were observed in rats or mice exposed to ≤975 ppm ethylbenzene for 90 days (NTP 1992) or in rats or mice exposed to ≤750 ppm ethylbenzene for 2 years (NTP 1999).

Hematological Effects. Two studies involving long-term monitoring of workers occupationally exposed to ethylbenzene were located (Angerer and Wulf 1985; Bardodej and Cirek 1988). Angerer and Wulf (1985) reported increased lymphocyte count and decreased hemoglobin concentration in male varnish workers exposed to a mixture of solvents, including ethylbenzene, compared to an unexposed control group. Workers were employed for an average of 8.2 years and exposed to an average ethylbenzene concentration of 1.64 ppm. Average lymphocyte levels increased (41.5–68.8%) and average hemoglobin values decreased (5.2–7.1%) in exposed workers, compared to unexposed controls. However, due to concomitant exposure to other chemicals (xylenes, lead, toluene), the relationship of adverse hematological effects to inhaled ethylbenzene cannot be established. No adverse hematological effects were seen in male workers employed at an ethylbenzene manufacturing facility over a 20-year period, compared to unexposed control workers (Bardodej and Cirek 1988). Although no information on

ethylbenzene concentrations was reported, an estimated concentration of 6.4 mg/m^3 was derived from a mean post-shift in urinary mandelic acid concentration in workers, based on the relationship between ethylbenzene concentrations in air and urinary mandelic acid concentration in a chamber-exposed group (Bardodej and Bardodejova 1988). However, given the low exposure concentration, this study had limited power to detect any effect.

Studies using animal models yield conflicting results regarding hematological effects of inhaled ethylbenzene. Platelet count was significantly decreased in male rats and mean total leukocyte count was significantly increased in female rats exposed to 782 ppm ethylbenzene for 4 weeks; hematological effects were not observed in male or female rats exposed to ≤382 ppm, mice exposed to ≤782 ppm, or rabbits exposed to ≤1,610 ppm (Cragg et al. 1989). Similarly, no hematological effects were observed in female rats exposed to ≤500 ppm ethylbenzene vapor daily for 28 days, based results of complete blood count with differential and assessments of erythrocyte morphology and hemoglobin content (Stump 2004). No adverse hematological effects were observed in rats or mice exposed to 975 ppm ethylbenzene for 90 days (NTP 1992), or in rats exposed to ≤2,200 ppm, guinea pigs or rabbits exposed ≤1,250 ppm, or monkeys exposed to ≤600 ppm ethylbenzene for 6 months (Wolf et al. 1956).

Musculoskeletal Effects. No musculoskeletal effects have been observed in laboratory animals exposed to inhaled ethylbenzene for intermediate or chronic durations. No bone tissue abnormalities were observed upon histopathological examination of tissue from rats and mice exposed to 782 ppm and rabbits exposed to 1,610 ppm for 4 weeks (Cragg et al. 1989). Similarly, no adverse effect on bone tissue was observed in rats or mice exposed to 975 ppm ethylbenzene for 90 days (NTP 1992) or 750 ppm for 2 years (NTP 1999).

Hepatic Effects. No adverse effects on liver function, as measured by serum liver enzyme activities, were observed in male workers employed at an ethylbenzene manufacturing facility over a 20-year period (Bardodej and Cirek 1988). Although no information on ethylbenzene concentrations was reported, an estimated concentration of 6.4 mg/m^3 was derived as described above under hematological effects (Section 3.2.1.3). Given the low exposure concentration, this study had limited the power to detect any effect.

Results of studies in laboratory animals have found various effects on the liver, including induction of cytochrome P-450, changes in liver weight (at least in part related to induction of cytochrome P-450), changes in hepatocyte ultrastructure consistent with induction of smooth endoplasmic reticulum, and

histopathological changes, including hepatocyte necrosis. Increased liver weights, induction of hepatic drug metabolizing enzymes, and changes in hepatocyte ultrastructure (consistent with induction of smooth endoplasmic reticulum) have been observed in rats following acute inhalation exposure to ethylbenzene. Concentration-related increases in absolute and relative liver weights were reported in male rats exposed to ≥400 ppm ethylbenzene for 3–4 days, although no histopathological changes were observed (Ethylbenzene Producers Association 1986a). Increased relative liver weight, increased hepatic concentration of cytochrome P-450, and induction of hepatic microsomal enzymes (NADPH-cytochrome reductase, 7-ethoxycoumarin O-deethylase) were reported in rats exposed to 2,000 ppm ethylbenzene concentrations for 3 days (Toftgard and Nilsen 1982). Similarly, hepatic drug metabolizing enzymes (UDP glucuronyl-transferase, D-glucuronolactone dehydrogenase) were increased in male rats exposed to 50 ppm ethylbenzene for 2 weeks, and ethyoxycoumarin o-deethylase was increased at 300 ppm (Elovaara et al. 1985). Electron microscopy showed changes in hepatocyte ultrastructure consistent with induction of cytochrome P-450 (e.g., smooth endoplasmic reticulum proliferation, slight degranulation of rough endoplasmic reticulum) in rats exposed to 600 ppm ethylbenzene for 2 weeks (Elovaara et al. 1985). Hepatic changes following acute exposure to ethylbenzene are consistent with induction of hepatic microsomal enzymes.

Similar hepatic effects (liver weights, induction of hepatic drug metabolizing enzymes, and changes in hepatocyte ultrastructure) also have been reported in laboratory animals exposed to inhaled ethylbenzene for intermediate exposure durations. Significant increases in relative liver weight were observed in female rats exposed to ≥250 ppm ethylbenzene for 15 days (Saillenfait et al. 2006), rats and mice exposed to 782 ppm ethylbenzene for 4 weeks (Cragg et al. 1989), pregnant and nonpregnant rats and pregnant rabbits exposed to 1,000 ppm ethylbenzene for 3 weeks prior to mating and throughout gestation (NIOSH 1981), and rats and mice exposed to 246 and 740 ppm ethylbenzene, respectively, for 90 days (NTP 1992). Increased liver weight was also observed in rats exposed to 400 ppm, and guinea pigs and monkeys exposed to 600 ppm ethylbenzene for 6 months (Wolf et al. 1956). Hepatic microsomal enzymes (total cytochrome P-450 protein, 7-ethoxycoumarin O-deethylase, aminopyrine N-demthylase) were induced in rats exposed to 50 ppm ethylbenzene for 16 weeks and changes in hepatocyte ultrastructure (e.g., smooth endoplasmic reticulum proliferation, slight degranulation of rough endoplasmic reticulum) in rats exposed to ethylbenzene from 2 to 15 weeks (Elovaara et al. 1985).

Other histopathological findings in animals exposed to inhaled ethylbenzene were moderate to marked hypertrophy of periportal hepatocytes with clear cytoplasm in female rats exposed to ≥250 ppm ethylbenzene for 15 days (the investigators noted that minimal hypertrophy was observed in controls)

(Saillenfait et al. 2006) and a cloudy swelling of hepatocytes of rats exposed to 2,200 ppm for 6 months (Wolf et al. 1956). Slight, statistically significant increases in relative liver weights (≤7% compared to controls) were observed in male and female B6C3F1 mice exposed to 750 ppm, but not 75 ppm, ethylbenzene vapor for 1 or 4 weeks (Stott et al. 2003). Histopathological assessment showed hepatocellular hypertrophy, mitotic figures, and S-phase DNA synthesis in mice exposed to 750 ppm ethylbenzene vapor for 1 or 4 weeks, but serum activities of hepatic enzymes (alanine transaminase, asparatate transpeptidase, alkaline phosphatase, and γ-glutamyl trnaspeptidase) were not elevated compared to controls. No histopathological changes in the liver were observed in rats or mice exposed to 246 and 740 ppm, respectively, for 90 days (NTP 1992).

Chronic exposure of mice, but not rats, to inhaled ethylbenzene for 2 years produced hepatic toxicity, as indicated by histopathological changes (syncytial alterations of hepatocytes, hepatocellular hypertrophy, and hepatocyte necrosis) (NTP 1999). Syncytial alterations (enlarged hepatocytes with multiple nuclei), which were concentration-related in incidence and severity, were observed at in male mice exposed to ≥75 ppm ethylbenzene, and hepatocyte hypertrophy and necrosis were observed in male mice exposed to 750 ppm. In female mice exposed to 750 ppm ethylbenzene for 2 years, the incidence and severity of eosinophilic foci, which was considered to be a preneoplastic lesion, was increased.

Renal Effects. Renal effects of acute and intermediate exposure to inhaled ethylbenzene are primarily limited to minimally adverse effects, including increased kidney weight in rats and mice and induction of microsomal enzymes in rats. However, chronic exposure of male and female rats results in more serious renal effects, including nephropathy, renal tubule hyperplasia, and renal tubular adenomas and carcinomas (see Section 3.2.1.7, Cancer). In addition, effects associated with accumulation of $\alpha_{2\mu}$-globulin and hyaline droplets have been observed in male rats exposed to inhaled ethylbenzene. Accumulation of $\alpha_{2\mu}$-globulin in the renal tubule epithelial cells of male rats is associated with tubular epithelial necrosis, regenerative proliferation, and renal tumors. This accumulation is not observed in female rats, mice, or humans (which lack that protein), or in male rats, which are genetically lacking $\alpha_{2\mu}$-globulin. Adverse effects in male rats associated with renal accumulation of $\alpha_{2\mu}$-globulin are therefore not considered relevant to humans (EPA 1991d). However, since renal toxicity of chronic exposure to inhaled ethylbenzene has been observed in female rats, renal effects associated with ethylbenzene are relevant to humans.

Acute exposure to inhaled ethylbenzene had been reported to produce increases in kidney weight, induce renal microsomal enzymes, and enhance renal hyaline droplets in rats of both sexes. In male rats only,

this accumulation includes $\alpha_{2\mu}$-globulin, but evidently other proteins are involved in females, and probably also in the males. Relative kidney weight was significantly increased (11–20%) in male rats exposed to 2,000 ppm for 2 days (Toftgard and Nilsen 1982) and 1,200 ppm ethylbenzene for 4 days (Ethylbenzene Producers Association 1986a), and in male and female rats exposed to 750 ppm ethylbenzene (6–7%), but not at 75 ppm, for 1 week (Stott et al. 2003). Although no change in renal histopathology accompanied increased kidney weight in rats exposed to 1,200 ppm ethylbenzene for 4 days (Ethylbenzene Producers Association 1986a), increased accumulation of $\alpha_{2\mu}$-globulin and hyaline droplets were observed after 1 week in the kidneys of male rats exposed to 750 ppm ethylbenzene compared to controls (Stott et al. 2003). Renal congestion was reported in rats and mice that died during exposure to 2,400 or 1,200 ppm ethylbenzene, respectively (Ethylbenzene Producers Association 1986a). Induction of renal cytochrome P-450 microsomal enzymes (7-ethoxycoumarin, O-deethylase, UDP glucuronyl-transferase, NADPH-cytochrome c reductase) was reported in rats following a 3-day exposure to 2,000 ppm ethylbenzene (Toftgard and Nilsen 1982).

Renal effects of intermediate-duration exposure to inhaled ethylbenzene are similar to those observed following acute exposure (increased kidney weight, induction of renal microsomal enzymes, and changes associated with accumulation of $\alpha_{2\mu}$-globulin). Several studies have shown that exposure of rats or mice to inhaled ethylbenzene for durations of 4 weeks –7 months increases relative kidney weight (Elovaara et al. 1985; NIOSH 1981; NTP 1999; Stott et al. 2003; Wolf et al. 1956). Concentration-related increases in renal microsomal enzymes (7-ethoxycoumarin O-deethylase, UDP glucuronyl-transferase) and renal glutathione concentration were reported in rats following a 5–16-week exposure to ethylbenzene at concentrations ranging from 50 to 600 ppm (Elovaara et al. 1985). Histopathological changes in the kidney include $\alpha_{2\mu}$-globulin-associated changes (nuclear-size and staining variations and vacuolation or decreased amount of cytoplasm) in male rats exposed to 750 ppm for 4 weeks (Stott et al. 2003) and swelling of the tubular epithelium in rats exposed to 600 ppm ethylbenzene for up to 7 months (Wolf et al. 1956).

Chronic exposure of male and female rats, but not mice, to inhaled ethylbenzene for 2 years resulted in nephropathy and renal tubule hyperplasia (NTP 1999). Although age-related nephropathy was observed in control rats, the severity was increased compared to controls in female rats exposed to $\geq$75 ppm and male rats exposed to 750 ppm. The incidence of renal tubular hyperplasia (considered as a preneoplastic effect) was increased in male and female rats exposed to 750 ppm. NTP (1999) concluded that ethylbenzene may have exacerbated the development of age-related nephropathy in rats and that renal

tubular lesions were related to exposure. Additional information pertaining to renal carcinogenesis is provided in Section 3.2.1.7.

Endocrine Effects. Adverse endocrine effects, based on histopathological examinations of endocrine tissues, have not been observed in laboratory animals exposed to inhaled ethylbenzene for 4 weeks to 6 months (Cragg et al. 1989; NTP 1992; Wolf et al. 1956), although chronic exposure is reported to produce hyperplasia of the thyroid and pituitary (NTP 1999). Mice exposed to 750 ppm ethylbenzene for 2 years showed an increased incidence of follicular cell hyperplasia in the thyroid gland. In female mice exposed to ≥250 ppm ethylbenzene, the incidences of hyperplasia of the pituitary gland pars distalis were significantly greater than those in the control group. No effects on other endocrine tissues were observed in mice and no effects on any endocrine tissue were observed in rats exposed to 750 ppm ethylbenzene for 2 years (NTP 1999).

Ocular Effects. Ocular effects observed in humans and animals after inhalation exposure to ethylbenzene are presumed to be due to direct contact of the eyes with ethylbenzene vapor. These effects are discussed in Section 3.2.3.2.

Body Weight Effects. Studies in animals examined body weight effects in acute-, intermediate-, and chronic-duration inhalation exposure to ethylbenzene. Mean body weight was not affected in rats or mice exposed to 400 ppm or in rabbits exposed to 2,400 ppm ethylbenzene for 4 days (Ethylbenzene Producers Association 1986a). Rats exposed to 1,200 ppm showed a mean body weight that was lower than in control animals. No effect on body weight was observed in rabbits after 7 days of exposure to 750 ppm (Romanelli et al. 1986).

No changes in body weight were observed in pregnant rats or rabbits exposed to 985 and 962 ppm ethylbenzene, respectively, for 3 weeks prior to mating and throughout gestation (NIOSH 1981). A decrease in body weight gain of 26–48% at weeks 2, 5, and 9, but not at week 16 was observed in male rats exposed to 600 ppm ethylbenzene for 16 weeks (Elovaara et al. 1985). No adverse effect on body weight was observed in rats or mice exposed to 782 ppm or rabbits exposed to 1,610 ppm ethylbenzene for 4 weeks (Cragg et al. 1989). In female rats exposed to up to 500 ppm ethylbenzene daily for 28 days, no effects on body weight were observed (Stump 2004). No effect on body weight was observed in rats and mice exposed to 975 ppm ethylbenzene for 90 days (NTP 1992). Similarly, no biologically significant effect on body weight was observed in rats and mice exposed to concentrations of ethylbenzene of up to 750 ppm for 2 years (NTP 1999).

3.2.1.3 Immunological and Lymphoreticular Effects

Angerer and Wulf (1985) reported increased lymphocyte counts in male varnish workers exposed to a mixture of solvents, including ethylbenzene, compared to an unexposed control group. Workers were employed for an average of 8.2 years and exposed to an average ethylbenzene concentration of 1.64 ppm. Average lymphocyte levels increased (41.5–68.8%) in exposed workers, compared to unexposed controls. However, due to concomitant exposure to other chemicals (xylenes, lead, toluene), the relationship of adverse hematological effects to inhaled ethylbenzene cannot be established.

Mean total leukocyte count was significantly increased in female rats expose to 782 ppm ethylbenzene, but not ≤382 ppm, for 4 weeks (Cragg et al. 1989). Absolute and relative spleen weights were increased in pregnant rats exposed to 1,000 ppm ethylbenzene during pre-mating and gestation or gestation alone, although histopathological changes were not observed (NIOSH 1981). Spleen weight was not affected in rabbits exposed to 1,000 ppm during gestation (NIOSH 1981). No histopathological changes were observed in bone marrow (sternum), lymph nodes, thymic region, or spleen of rats or mice exposed to 782 ppm or rabbits exposed to 1,610 ppm ethylbenzene for 4 weeks (Cragg et al. 1989). In female rats exposed to ethylbenzene at concentrations up to 500 ppm for 6 hours/day, no effects were observed on humoral immunity (as assessed by the IgM antibody-forming response to the T cell-dependent antigen sheep erythrocytes), thymus or spleen weight, or blood leukocyte populations (Stump 2004). No treatment-related effect on the histopathology of several lymphoreticular tissues, including bronchial lymph nodes, regional lymph nodes, mandibular and mesenteric lymph nodes, mediastinal lymph nodes, spleen, or thymus were observed in rats and mice exposed to 975 ppm ethylbenzene for 90 days (NTP 1992) or 750 ppm for 2 years (NTP 1999).

The highest NOAELs for immunological and lymphoreticular effects in each species for intermediate- or chronic-duration exposure are reported in Table 3-1 and plotted in Figure 3-1.

3.2.1.4 Neurological Effects

Symptoms of dizziness accompanied by vertigo have been observed in humans acutely exposed to air concentrations of ethylbenzene ranging from 2,000 to 5,000 ppm for 6 minutes (Yant et al. 1930).

Workers exposed occupationally for a mean of 13 years to solvent mixtures that include ethylbenzene (mean ethylbenzene exposure level 1.8 ppm) showed a 58% incidence of hearing loss compared to

36% in the reference group (Sliwinska-Kowalska et al. 2001). Hearing losses (expressed as increased hearing thresholds) were observed at all frequencies and appeared to range from 3 to 8 dB. The role of ethylbenzene in the observed losses cannot be ascertained from this study given that ethylbenzene was only one of several solvents, most of which were present at mean concentrations 1.5–3.5 times higher than ethylbenzene.

Neurological effects have been observed in several animals following acute-duration exposure to inhaled ethylbenzene, although there is considerable variability in species sensitivity. In general, central nervous system depression is associated with acute exposure to higher concentrations, whereas stimulation of the motor nervous system is associated with lower concentrations. The most serious adverse neurological effect associated with acute- and intermediate-duration inhalation exposure to ethylbenzene is ototoxicity, characterized by deterioration in auditory thresholds and alterations of cochlear morphology.

Clinical signs of general central nervous system depression or increased motor activity have been observed in animals acutely exposed to inhaled ethylbenzene. Moderate activation in motor behavior was observed in rats following a 4-hour inhalation exposure to levels of ethylbenzene ranging from 400 to 1,500 ppm, whereas narcotic effects were observed at higher ethylbenzene concentrations (2,180–5,000 ppm) (Molnar et al. 1986). This study is limited by a lack of methodological detail and appropriate statistical analysis. Central nervous system depression (unconsciousness) and ataxia were observed in guinea pigs exposed to 2,000 ppm ethylbenzene for acute-duration periods (Yant et al. 1930). Salivation, prostration, and/or reduced activity were observed in rats and mice exposed to 2,400 or 1,200 ppm ethylbenzene, respectively, for 4 days (Ethylbenzene Producers Association 1986a). However, rabbits exposed to 2,400 ppm ethylbenzene for the same period showed no adverse behavioral effects. Exposure of mice to ethylbenzene for 20 minutes to ≥2,000 ppm produced changes in posture; decreased arousal and rearing; increased ease of handling; disturbances of gait, mobility, and righting reflex; decreased forelimb grip strength; increased landing foot splay; and impaired psychomotor coordination (Tegeris and Balster 1994). These acute effects were short-lived and more pronounced during exposure than after exposure, with recovery beginning within minutes of removal from the exposure chamber. Sensorimotor reactivity also decreased. Acute exposure of rats and mice to 245 and 342 ppm, respectively (Frantik et al. 1994), resulted in a 30% depression of evoked electrical activity in the brain immediately after exposure.

General signs of neurotoxicity have not been observed in animals exposed to inhaled ethylbenzene for intermediate or chronic durations, although ethylbenzene concentrations evaluated in intermediate- and

chronic-duration studies were lower than those evaluated in acute studies. No behavioral changes, clinical signs of neurotoxicity or histopathological alterations of neurological tissues were observed in rats or mice exposed to concentrations of up to 782 ppm or rabbits exposed to concentrations up to 1,610 ppm ethylbenzene for 4 weeks. Sporadic salivation was noted in rats at doses of ≥382 ppm (Cragg et al. 1989). In a 90-day study (NTP 1992), rats and mice showed no adverse histopathological effects on brain tissue at doses up to 975 ppm. No adverse effects were noted in the brain tissues of rats and mice exposed to concentrations of ethylbenzene of up to 750 ppm for 2 years (NTP 1999).

Ototoxic effects of inhaled ethylbenzene have been observed following acute- or intermediate-duration exposure of rats. Effects are characterized by deterioration in auditory thresholds and alterations of cochlear morphology. Male rats exposed to ethylbenzene at 800 ppm, 8 hours/day for 5 days showed significant deterioration in auditory thresholds (threshold shifts were 10–20 and 17–28 dB using two methods) 1 and 4 weeks after the exposure had ceased (Cappaert et al. 1999). The magnitude of the increase in thresholds did not change between assessments conducted on post-exposure weeks 1 and 4; threshold shifts were evident at all tested frequencies (1–24 kHz). Eight to 11 weeks after exposure, a significant loss (52–66%) of outer hair cells (OHCs) in the organ of Corti in the auditory region corresponding to 11–21 kHz was observed. No loss of inner hair cells (IHCs) was found in the exposed animals (Cappaert et al. 1999). Similarly, auditory threshold shifts (approximately 15–30 dB) and OHC losses (25–75%) were observed 3–6 weeks after exposure in male rats exposed to 400 or 550 ppm ethylbenzene, 8 hours/day for 5 days. Auditory thresholds and OHC counts were not affected in rats exposed to 300 ppm ethylbenzene (Cappaert et al. 2000, 2001). Cappaert et al. (2002) demonstrated a significant species difference in the susceptibility of rats and guinea pigs to the ototoxic effects of ethylbenzene. Guinea pigs exposed to ethylbenzene at 2,500 ppm, 6 hours/day for 5 days did not show auditory deficits or losses in OHCs. There was no loss of IHCs in either species (Cappaert et al. 2002).

Auditory deficits persisted unchanged throughout the 13-week exposure period and the 8-week post-exposure recovery period in rats exposed to ≥400 ppm ethylbenzene (Gagnaire et al. 2007). Histological assessments conducted after the 8-week recovery period showed significant losses in OHCs in rats exposed to ≥200 ppm ethylbenzene, 6 hours/day, 6 days/week for 13 weeks (Gagnaire et al. 2007). IHC losses (14–32%) were observed in rats exposed to 600 and 800 ppm ethylbenzene, but only occasionally in rats exposed to 400 ppm (Gagnaire et al. 2007).

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following inhalation exposure to ethylbenzene.

No adverse effects on reproduction were observed in female rats and rabbits exposed to approximately 100 or 1,000 ppm ethylbenzene for 3 weeks prior to mating and throughout gestation (NIOSH 1981). Reproductive function and outcome was assessed in a 2-generation reproductive toxicity study in rats exposed to 25, 100, or 500 ppm ethylbenzene starting with a pre-mating exposure of ≥70 days and continuing through gestation day 20, lactation day 5–21, and postnatal day 21 (Faber et al. 2006). On lactational days 1–4 for F0 and F1 dams, inhalation exposure was discontinued and oral ethylbenzene (in corn oil at doses of 0, 26, 90, or 342 mg/kg/day) was administered; inhalation exposure was re-initiated on lactational day 5 and maintained for the remainder of the exposure period. Oral exposure on lactational days 1–4 was intended to provide the same maternal blood concentrations as inhalation exposure. Estrous cycle length was significantly reduced in F0 rats in the 500-ppm group (4.0 days vs. 4.4 days in controls), but was not altered in the F1 females. Reproductive parameters (mating or fertility indices, gestation length, number of implantation sites, number of births/litter, and litter size) were not affected in F0 or F1 females (or males, as appropriate) exposed to 25–500 ppm ethylbenzene.

No treatment-related histopathological changes were noted in the testes of rats, mice, or rabbits exposed to concentrations as high as 2,400 ppm ethylbenzene for 4 days (Ethylbenzene Producers Association 1986a). No testicular histopathological abnormalities were reported in rats and mice exposed to 782 ppm and rabbits exposed to ethylbenzene concentrations as high as 1,610 ppm for 4 weeks (Cragg et al. 1989). NTP (1992) reported no effect on sperm or testicular morphology or on the length of the estrous cycle in rats or mice exposed to 975 ppm ethylbenzene for 90 days. Mice showed a decrease in epididymal weight in the 1,000-ppm group; however, this observation was not considered biologically significant since there was no significant difference in spermatid counts, sperm motility, or weight of the caudal epididymis among treated and control animals. Inhalation exposure of male monkeys and rabbits to 600 ppm ethylbenzene for 6 months produced degeneration of germinal epithelium in the testes of one monkey and one rabbit (Wolf et al. 1956). No adverse histopathological effects were seen in the testes of rats or guinea pigs exposed to concentrations up to 1,250 or 600 ppm, respectively, for 6–7 months (Wolf et al. 1956). In a 2-generation reproduction study, no effects were observed on sperm number, motility, and morphology in F0 and F1 rats exposed to 25–500 ppm ethylbenzene (Faber et al. 2006).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species for acute-, intermediate-, and chronic-duration are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans following inhalation exposure to ethylbenzene.

The developmental effects of inhaled ethylbenzene have been studied in rats (NIOSH 1981; Ungváry and Tátrai 1985), mice (Ungváry and Tátrai 1985), and rabbits (NIOSH 1981; Ungváry and Tátrai 1985) exposed during gestation or pre-mating and gestation; rats exposed from pre-gestation through lactation (Faber et al. 2006); and in a 2-generation reproduction study in rats (Faber et al. 2007). Results of studies in rats indicate that ethylbenzene produces reduced fetal weight, skeletal anomalies, and anomalies and delayed development of urogenital tract; skeletal and urogenital anomalies were observed in the presence of maternal toxicity (Faber et al. 2006; NIOSH 1981; Ungváry and Tátrai 1985). Studies conducted by Faber et al. (2006, 2007) and NIOSH (1981) are of high quality and guideline-compliant. However, usefulness of the Ungváry and Tátrai (1985) study is hampered by incomplete descriptions of the results and because an analysis of the results on a per litter basis was not provided.

In rats, continuous inhalation exposure to ethylbenzene on gestational days 7–15 to concentrations ranging from 138 to 552 ppm resulted in fetal resorption and skeletal retardation (Ungváry and Tátrai 1985). Retarded skeletal development was observed in 13, 26, 30, and 35% of fetuses exposed to 138, 276, and 552 ppm ethylbenzene, respectively. Increased incidence of extra ribs and anomalies of the urinary tract (specific effects not reported) were observed in 7% of fetuses at 552 ppm, compared to 1% in controls. Maternal toxicity was reported to be moderate and dose-dependent, but data were not presented. In this same study, the incidence of anomalies of the uropoietic apparatus (not specified) was observed in the offspring of mice exposed to 115 ppm ethylbenzene on gestation days 6–15. Anomalies of the uropoietic apparatus were not observed in other developmental studies (Faber et al. 2006, 2007; Saillenfait et al. 2003, 2006, 2007). In rabbits continuously exposed to ethylbenzene on gestation days 7–20, decreases in female fetal body weights were observed at 115 ppm and an increase in abortions (3/3 does aborted compared to 0/60 in controls) were observed at 230 ppm. The Ungváry and Tátrai (1985) study did not include sufficient details regarding the adverse fetal effects, dictating caution in the interpretation of study findings.

In a NIOSH sponsored study conducted by Andrews et al. (NIOSH 1981), increases in the incidences of fetuses with extra ribs were observed in the offspring of rats exposed to 100 or 1,000 ppm ethylbenzene through gestation day 19 or 1,000 ppm during pre-mating and gestation. The incidence of supernumerary ribs was increased only in the offspring of rats exposed to 1,000 ppm during gestation. Reduced pup crown-rump length and an increased incidence of supernumerary ribs were observed in the offspring of rats exposed to 1,000 ppm during gestation, but not in the offspring of rats exposed to 1,000 ppm during pre-mating and gestation. No other significant increases in major malformations or minor anomalies were observed. There was some evidence of maternal toxicity (increased relative liver, kidney, and spleen weights) in rats exposed to 1,000 ppm ethylbenzene during pre-mating and gestation or gestation only (NIOSH 1981).

Statistically significant reductions in fetal body weight were observed in the offspring of pregnant rats exposed to ≥1,000 ppm ethylbenzene during gestation (Saillenfait et al. 2003, 2006, 2007), but not in rats exposed to 500 ppm (Saillenfait et al. 2003) or 250 ppm (Saillenfait et al. 2006, 2007). A significant increase in the number of fetuses with fetal malformations (mostly skeletal variations) was observed in the offspring of rats exposed to ≥1,000 ppm ethylbenzene (Saillenfait et al. 2003, 2006, 2007). On a per litter basis, a significant increase in the incidence of fetal malformations was observed only at 2,000 ppm (Saillenfait et al. 2003). Maternal toxicity was observed in rats exposed to ≥1,000 ppm, as indicated by significant reductions in maternal weight gain compared to control animals (Saillenfait et al. 2003, 2006, 2007).

Survival from birth to postnatal days (PND) 4 and 21 was not affected in F1 or F2 offspring of pregnant rats exposed to ethylbenzene at 25, 100, or 500 ppm during premating, mating, gestation, and lactation (Faber et al. 2006). A statistically significant delay in balanopreputial separation was observed in F1 males in the 500 ppm group, although the mean age at separation in that group (44.7 days) was similar to that observed in historical controls (44.8 days). No exposure-related macroscopic findings or changes in organ weight in F1 pups necropsied on PND 21 were observed.

Results of a 2-generation reproduction study in rats show that the mean age at acquisition of vaginal patency was significantly reduced in F1 females at 25, 100, and 500 ppm; however, the values in the treated groups (33.3–33.9 days) were similar to the historical control value (33.4 days) in the conducting laboratory (Faber et al. 2006). F2 generation pups (which were not exposed to ethylbenzene by inhalation) did not show differences from controls in the age at preputial separation or vaginal patency

(Faber et al. 2007). Developmental landmarks (pinna detachment, hair growth, incisor eruption, and eye opening) were not affected in F1 male or female pups exposed to 25–500 pm ethylbenzene (Faber et al. 2006); however, in the F2 generation, statistically significant delays in hair growth were observed in males and females in all exposure groups and eye opening was significantly delayed in males the 25 and 100 ppm groups, but not in the 500 ppm group (Faber et al. 2007). Neurodevelopmental tests conducted on subsets of the F2 offspring (Faber et al. 2007) did not show statistically significant differences from controls in a functional observational battery assessment or in fore- or hind-limb grip strength. Although the data suggest an increase in motor activity in F2 males and females in the 25–500 ppm groups on PNDs 13 and 17, there were no statistical differences in motor activity on PNDs 13, 17, and 21 between exposed and control animals (Faber et al. 2007). A statistically significant increase in motor activity was observed on PND 61 in F2 females in the 25 ppm group only. Startle response was not affected in F2 rats of either sex on PND 20 or females on PND 60; F2 males showed statistically lower startle responses, but this was attributed to highly variable and abnormal responses in some control animals. Swimming ability, learning, and memory assessments conducted in a Biel water maze did not reveal any significant effects in F2 animals in any of the exposure groups (Faber et al. 2007). No morphometric or histologic effects in brains of F2 animals were observed from any exposure group on PND 21 or 72 (Faber et al. 2007).

Reduced fetal weight was observed in female rats exposed to 115 ppm during gestation (Ungváry and Tátrai 1985). In the offspring of rabbits exposed to ethylbenzene, no treatment-related effects were observed in fetal size, placental weight, or intrauterine growth retardation and there were no significant incidences of major malformations, minor anomalies, or common variants observed in the absence of maternal toxicity (NIOSH 1981).

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.7 Cancer

No association has been found between the occurrence of cancer in humans and occupational exposure to ethylbenzene. No cases of malignancy were observed in workers exposed to ethylbenzene monitored for 20 years (Bardodej and Cirek 1988). No information on ethylbenzene concentrations was reported, although an estimated concentration of 6.4 mg/m^3 was derived as described above under hematological effects (Section 3.2.1.3). However, no clear conclusions can be drawn from this study due to the lack of measured ethylbenzene concentrations. Furthermore, the low exposure concentration limited the power

of this study to detect any effect. No other studies were found regarding cancer effects in humans exposed to ethylbenzene by inhalation.

Information concerning the carcinogenicity of ethylbenzene in animals comes from an NTP-sponsored bioassay in male and female rats and mice exposed to 0, 75, 250, or 750 ppm ethylbenzene for up to 2 years (NTP 1999). NTP (1999) concluded that ethylbenzene showed clear evidence of carcinogenic activity in male rats based on increased incidences of renal tubule neoplasms and testicular adenomas, some evidence of carcinogenic activity in female rats based on increased incidences of renal tubule adenomas, some evidence of carcinogenic activity in male mice based on increased incidences of alveolar/bronchiolar neoplasms, and some evidence of carcinogenic activity in female mice based on increased incidences of hepatocellular neoplasms (NTP 1999).

Pathological findings in male and female rats exposed to 750 ppm ethylbenzene showed significant increases in the incidence of renal tubule adenoma and adenoma or carcinoma (combined) compared to control animals. An extended histopathological evaluation of the kidneys showed significant increases in the incidence of nephropathy and of renal tubular hyperplasia (a preneoplastic lesion) in male rats exposed to 750 ppm; in female rats, nephropathy was observed at concentrations ≥75 ppm and renal tubular hyperplasia was only observed at a concentration of 750 ppm. In a reevaluation of the histopathology of rat kidneys from the NTP study, Hard (2002) confirmed the NTP (1999) findings and suggested that the increase incidence of kidney tumors in rats in the high-dose group was related to a chemical-induced exacerbation of chronic progressive nephropathy (CPN), with a minor contributing factor in male rats being $\alpha_{2\mu}$-globulin nephropathy. However, in an analysis of the association between CPN and renal tubule cell neoplasms in male F344 rats, Seely et al. (2002) concluded that the association between CPN and renal tubule cell neoplasms is marginal. Results of this analysis suggest that the number of renal tubule cell neoplasms secondary to CPN would be few (Seely et al. 2002). The incidence of interstitial cell adenoma in the testes of males exposed to 750 ppm was significantly greater than in the control group and slightly exceeded the historical control range for inhalation studies. The incidence of bilateral testicular adenoma was also significantly increased in males exposed to 750 ppm.

In male mice, the incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) were significantly greater in males exposed to 750 ppm than in the controls. No significant increased in the incidence of neoplastic lung lesions was observed in female rats. The incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were

significantly greater in female mice exposed to 750 ppm than in the control group. Hepatocellular adenomas or carcinomas were not observed in male mice.

3.2.2 Oral Exposure

3.2.2.1 Death

No studies were located regarding death in humans following oral exposure to ethylbenzene.

Lethality has been observed in laboratory animals following ingestion of ethylbenzene. The oral LD_{50} for gavage administration of ethylbenzene was reported to be 4,769 mg/kg ethylbenzene in rats (Smyth et al. 1962). In another oral study with rats exposed to ethylbenzene, the LD_{50} was reported to be approximately 3,500 mg/kg ethylbenzene (Wolf et al. 1956). The usefulness of these data is limited since the study methodology was not provided.

An oral LD_{50} value for rats is recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located describing respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, body weight, or metabolic effects in humans or gastrointestinal, musculoskeletal, endocrine, dermal, ocular, body weight, or metabolic effects in animals after oral exposure to ethylbenzene.

The highest NOAEL values and all reliable LOAEL values are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. No clinical signs of respiratory effects or histopathological findings in respiratory tissues were observed in male and female rats exposed to 750 mg/kg/day by gavage for 4 or 13 weeks (Mellert et al. 2007). No respiratory effects were observed in female rats orally exposed to 680 mg/kg ethylbenzene by gavage for 6 months (Wolf et al. 1956). The utility of this study is limited because of poor protocol description.

Table 3-2 Levels of Significant Exposure to Ethylbenzene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Carworth-Wistar)	once (G)				4769 M (LD50)	Smyth et al. 1962	
Neurological								
2	Rat (SD)	1 x/d 5 d/wk 2 wk (G)				900 M (complete loss of outer hair cells in cochlea)	Gagnaire and Langlais 2005	
INTERMEDIATE EXPOSURE								
Systemic								
3	Rat (Wistar)	4 wk 7 d/wk 2 x/d (GO)	Resp	750			Mellert et al. 2007	
			Cardio	750				
			Hemato	750				
			Hepatic	75 M	250 M (increased absolute and relative liver weights, increased incidence of centrilobular hepatocyte hypertrophy)			
			Renal	75 M	250 M (hyaline droplet nephropathy)			

Table 3-2 Levels of Significant Exposure to Ethylbenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
4	Rat (Wistar)	13 wk 7 d/wk 2 x/d (GO)	Resp	750			Mellert et al. 2007	
			Cardio	750				
			Hemato	250	750 (increased mean corpuscular volume in males and females, decreased platelet counts in females)			
			Hepatic	75[b]	250 (increased serum liver enzymes in males, increased absolute and relative liver weights in males and females, increased incidence of centrilobular hepatocyte hypertrophy in males and females)			
			Renal	75 M	250 M (hyaline droplet nephropathy)			

Table 3-2 Levels of Significant Exposure to Ethylbenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
5	Rat	6 mo 5 d/wk 1 x/d (GO)	Hemato	680 F			Wolf et al. 1956	Quality of study is poor, with inadequate descriptions of study methods and results.
			Hepatic	136 F	408 F (increased liver weight; cloudy swelling of parenchymal liver cells)			
			Renal	136 F	408 F (increased kidney weight; cloudy swelling of kidney tubular epithelium)			
Neurological								
6	Rat (CD)	2 x/d 13 wk (G)		500			Barnett 2006	Based on negative findings for functional observational battery and motor activity assessments and for histopathology to neurological tissues
7	Rat (Wistar)	4 wk 7 d/wk 2 x/d (GO)		750			Mellert et al. 2007	

Table 3-2 Levels of Significant Exposure to Ethylbenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
8	Rat (Wistar)	13 wk 7 d/wk 2 x/d (GO)		750			Mellert et al. 2007	NOAEL based on absence of histopathological findings of neurological tissues and negative findings for assessment of motor activity and FOB.
Reproductive								
9	Rat (Wistar)	4 wk 7 d/wk 2 x/d (GO)		750			Mellert et al. 2007	
10	Rat (Wistar)	13 wk 7 d/wk 2 x/d (GO)		750			Mellert et al. 2007	NOAEL based on absence of histopathological findings of reproductive tissues.

a The number corresponds to entries in Figure 3-2.

b Used to derive an intermediate-duration oral MRL of 0.4 mg/kg/day based on a BMDL using an internal dose metric to simulate time averaged liver concentration of ethylbenzene (6.61 µmol/L); a human equivalent dose (HED) of the BMDL was estimated using a human PBPK model. The BMDL(HED) (10.68 mg/kg/day) was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability).

Cardio = cardiovascular; d = day(s); F = Female; (G) = gavage; (GO) = gavage in oil; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); x = time(s)

Figure 3-2 Levels of Significant Exposure to Ethylbenzene - Oral

Acute (≤14 days)

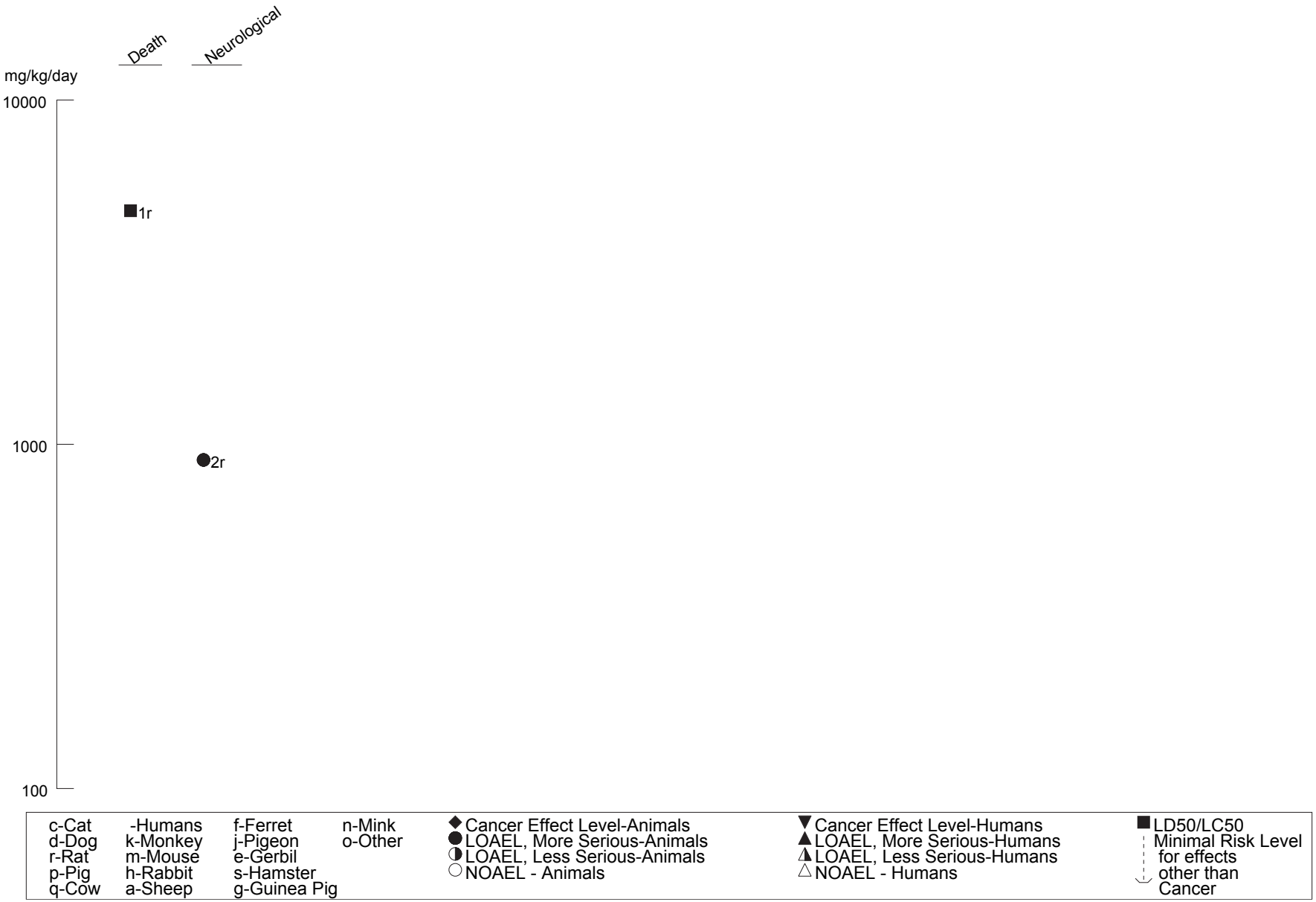
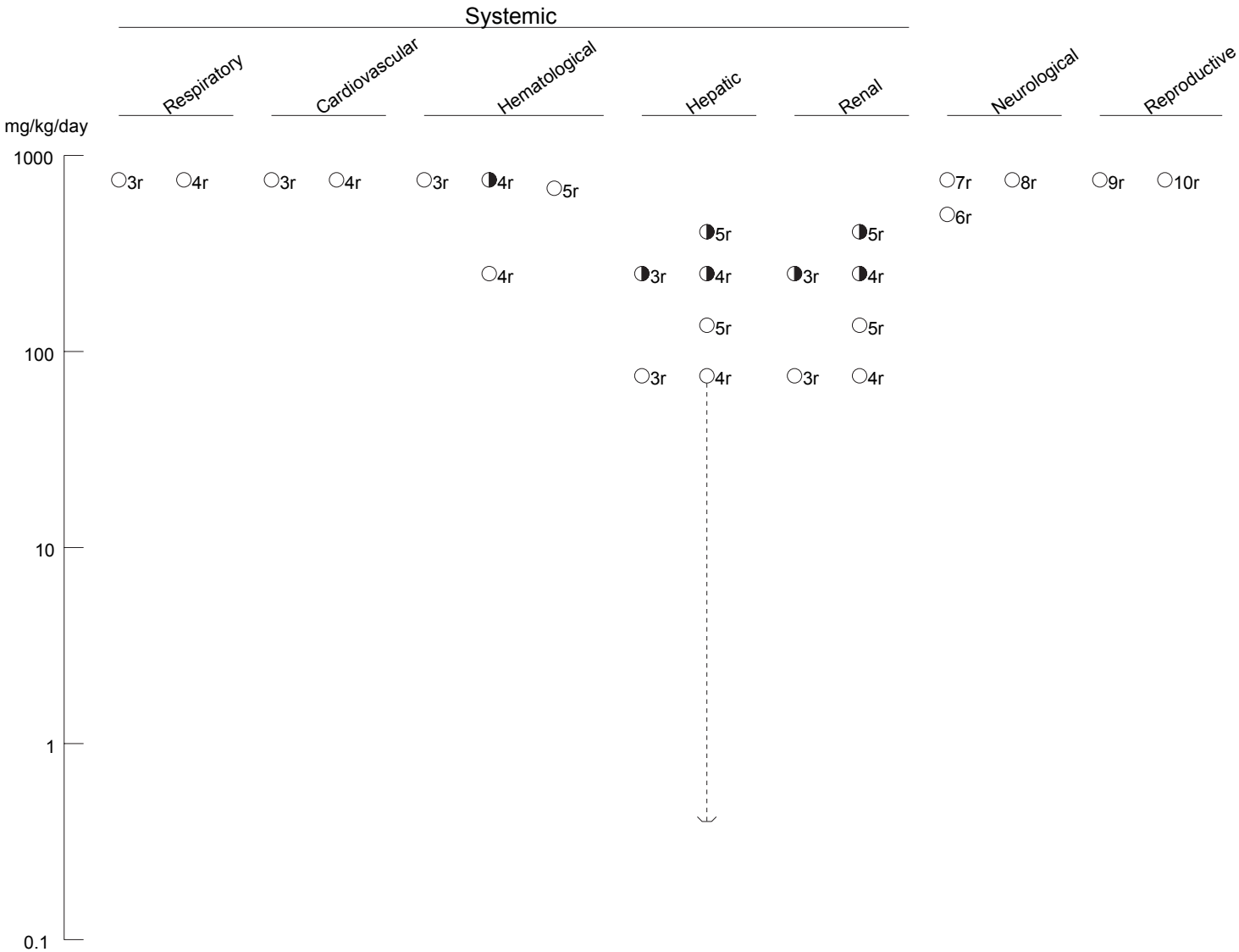


Figure 3-2 Levels of Significant Exposure to Ethylbenzene - Oral *(Continued)*

Intermediate (15-364 days)



Cardiovascular Effects. No histopathological findings were made in cardiac tissue from male and female rats exposed to 750 mg/kg/day by gavage for 4 or 13 weeks (Mellert et al 2007) or from female rats exposed to 13.6–680 mg/kg via gavage for 6 months (Wolf et al. 1956)

Hematological Effects. Decreased platelet count was observed in females administered 750 mg/kg/day and increased mean corpuscular volume was observed in males and females exposed to ≥250 mg/kg/day ethylbenzene by gavage for 13 weeks (Mellert et al 2007). No effects on hematological parameters were observed in rats treated for 4 weeks (Mellert et al. 2007). No adverse effects on bone marrow counts or histopathology were reported in female rats orally exposed to 13.6–680 mg/kg ethylbenzene by gavage for 6 months (Wolf et al. 1956). The usefulness of this study is limited by the poorly described and limited results that were provided.

Hepatic Effects. Effects indicative of liver toxicity were observed in male and female rats exposed to oral ethylbenzene for 4 and 13 weeks (Mellert et al. 2007). After 4 weeks of exposure, observed effects consistent with hepatotoxicity included increased absolute and relative liver weights (≥250 mg/kg/day in males and 750 mg/kg/day in females), increased incidence of hepatocyte centrilobular hypertrophy (≥250 mg/kg/day in males and 750 mg/kg/day in females), and increase serum liver enzyme activity (alanine aminotransferase) (750 mg/kg/day in males and females) (Mellert et al. 2007). After 13 weeks of exposure, increased activity of serum liver enzymes (alanine aminotransferase and γ-glutamyl transferase) in males (≥250 mg/kg/day) and females (750 mg/mg/day), increased absolute and relative liver weights (≥250 mg/kg/day in males and females), and a dose-related increase in the incidence of centrilobular hepatocyte hypertrophy (≥250 mg/kg/day in males and females) were observed. Increased bilirubin (≤250 mg/kg/day in males and 750 mg/kg/day in females), total protein (750 mg/kg/day in females), albumin (750 mg/kg/day in males and females), globulins (750 mg/kg/day in females), and cholesterol (≤250 mg/kg/day in males and females), and decreased prothrombin time (750 mg/kg/day in males and ≥250 mg/kg/day in females) were considered by study investigators as adaptive effects in the liver. In males in the 75 mg/kg/day group, relative liver weight was significantly increased by (4% compared to controls); however, no histopathological changes, or increases in absolute liver or serum liver enzyme activities were observed at this dosage. Given that ethylbenzene is a microsomal enzyme inducer, and the absence of histopathology and other evidence of liver injury at the 75 mg/kg/day dosage, the small increase in relative liver weight in male rats was at this dosage not considered evidence for an adverse effect on the liver (Mellert et al. 2007). Histopathological changes characterized by cloudy swelling of parenchymal cells of the liver and an increase in liver weight were observed in female rats administered 408 mg/kg/day for 6 months (Wolf et al. 1956). No other hepatic changes were reported. No liver effects

were observed in female rats administered 136 mg/kg/day. No conclusions could be drawn from these results because of serious weaknesses in the methodology and reporting of the data, including incidence data and statistical analyses.

Renal Effects. Renal effects in males administered ethylbenzene by gavage for 13 weeks included increased serum creatinine (750 mg/kg/day), increased incidences of transitional epithelial cells and granular and epithelial cell casts in the urine (≥250 mg/kg/day), increased absolute and relative kidney weights (≥250 mg/kg/day), and a dose-related increase in severity of hyaline droplet nephropathy (≥250 mg/kg/day) (Mellert et al. 2007). Adverse renal effects in males were most likely related to accumulation of α2μ-globulin accumulation, and, therefore, considered not relevant to humans. Similar renal findings were observed in male rats exposed for 4 weeks (administered ethylbenzene by gavage for 13 weeks). Absolute kidney weight was significantly increased by 7 and 13% in females administered 250 and 750 mg/kg-day, respectively, compared to controls. However, since no histopathological findings or alterations in urinalysis parameters were observed, increased kidney weight in females was not considered evidence for renal toxicity in female rats. The only animal study that investigated renal effects following ethylbenzene exposure involved female rats administered 13.6–680 mg/kg/body weight ethylbenzene by gavage for 6 months (Wolf et al. 1956). Histopathological changes characterized as cloudy swelling of the tubular epithelium in the kidney and an increase in kidney weight were observed at the 408 mg/kg/day dose level. No other renal changes were reported. As in hepatic effects, no conclusions could be drawn from these results because of serious weaknesses in the methodology and reporting of the data (e.g., no data on the number of animals with renal effects). Furthermore, no statistical analysis was performed.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans following oral exposure to ethylbenzene.

Absolute and relative thymus weights were significantly decreased in females treated with ≥250 mg/kg/day for 13 weeks, but no histopathological findings were observed (Mellert et al. 2007). Therefore, effects were not considered adverse.

3.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans following oral exposure to ethylbenzene.

Significant ototoxic effects were observed in male rats administered 900 mg/kg/day (the only dose tested) by gavage for 2 weeks (Gagnaire and Langlais 2005). The authors reported an almost complete loss of the three rows of OHCs in the organ of Corti 10 days after the last exposure to ethylbenzene. This study did not have a control group to clearly establish the magnitude of the effects relative to unexposed animals; nevertheless, this study showed that the losses observed in ethylbenzene-exposed animals were among the highest observed among 21 organic solvents tested (Gagnaire and Langlais 2005). No ethylbenzene-related behavioral changes were observed in female rats administered 13.6–680 mg/kg/day ethylbenzene by gavage for 6 months (Wolf et al. 1956). No other parameters were investigated. The utility of this study is limited because the monitored behavioral changes were not reported, and the study protocol was poorly described.

In male and female rats exposed to 75–750 mg/kg/day ethylbenzene by gavage for 13 weeks, no neurological effects were observed, based on negative results of motor activity tests and a functional observational battery (FOB) (Mellert et al. 2007). Similarly, no neurological effects were observed in male or female rats admininistered 50–500 mg/kg/day ethylbenzene by gavage for 13 weeks, based on negative findings for FOB and motor activity assessments and for histopathology to neurological tissues (Barnett 2006). However, assessments of ototoxicity were not conducted in these studies.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following oral exposure to ethylbenzene.

The only available reproduction study reported that acute oral exposure to 500 or 1,000 mg/kg ethylbenzene decreases peripheral hormone levels and may block or delay the estrus cycle in female rats during the diestrus stage (Ungváry 1986). Decreased levels of hormones, including luteinizing hormone, progesterone, and 17 β-estradiol, were accompanied by uterine changes, which consisted of increased stromal tissue with dense collagen bundles and reduced lumen. No dose response was noted. The study is limited by the absence of statistical analysis of the data.

No histopathological findings of reproductive tissues were observed in male and female rats exposed to 750 mg/kg/day by gavage for 4 or 13 weeks (Mellert et al. 2007).

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals following oral exposure to ethylbenzene.

3.2.2.7 Cancer

No studies were located regarding carcinogenic effects in humans following oral exposure to ethylbenzene.

The carcinogenicity of ethylbenzene by the oral route has been evaluated in a chronic-duration study in Sprague-Dawley rats (Maltoni et al. 1985). A statistically significant increase in total malignant tumors was reported in females and in combined male and female groups exposed to 500 mg kg/day via gavage for 104 weeks and observed until after week 141. No data on specific tumor type were presented, only one dose was tested, and no information on survival was provided.

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding lethal effects in humans following only dermal exposure to ethylbenzene. Matsumoto et al. (1992) reported the case of a 44-year-old man who died 9 days after being massively exposed to gasoline (which contained ethylbenzene) dermally and by inhalation for ≥10 hours.

The dermal LD_{50} in rabbits exposed to liquid ethylbenzene (applied to clipped skin subsequently covered with an impervious plastic film) was calculated to be 15,433 mg/kg/body weight (Smyth et al. 1962). No additional studies were located regarding death in animals following dermal exposure to ethylbenzene.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, body weight, or metabolic effects in humans or animals after dermal exposure to ethylbenzene.

The systemic effects observed after dermal exposure to ethylbenzene are discussed below. The highest NOAEL values and all reliable LOAEL values for each species and duration category are recorded in Table 3-3.

Dermal Effects. No studies were located regarding dermal effects in humans following dermal exposure to ethylbenzene.

Liquid ethylbenzene applied directly to the skin of an unspecified number of rabbits caused irritation characterized by reddening, exfoliation, and blistering (Wolf et al. 1956). Mild dermal irritation (grade 2 on a scale of 10) was also noted in New Zealand White rabbits 24 hours after application of ethylbenzene to clipped skin (Smyth et al. 1962).

Ocular Effects. Ocular effects observed in humans and animals after inhalation exposure are assumed to be due to exposure of the mucous membranes of the eye to ethylbenzene vapor. Volunteers reported eye irritation and burning, and profuse lacrimation, which gradually decreased with continued exposure to 1,000 ppm for 1–6 minutes (Yant et al. 1930). Upon entering the chamber with an ethylbenzene concentration of 2,000 or 5,000 ppm, the volunteers also experienced severe eye irritation. Cometto-Muñiz and Cain (1995) reported eye irritation in humans after exposure to ethylbenzene vapor. Eye irritation was observed at 10,000 ppm.

Liquid ethylbenzene applied directly to the eyes of rabbits for an unspecified duration caused slight irritation of conjunctival membranes (Wolf et al. 1956) and slight corneal injury (Smyth et al. 1962; Wolf et al. 1956).

Irritant effects from exposure to ethylbenzene vapor have been reported in animals. Tegeris and Balster (1994) reported lacrimation and palpebral closure in mice after 20 minutes of exposure to 2,000 ppm ethylbenzene. Eye irritation was observed in guinea pigs exposed to 1,000 ppm for 8 minutes, and in animals exposed to 2,000, 5,000, and 10,000 ppm for 480, 30, and 10 minutes, respectively (Yant et al. 1930). Lacrimation was observed in rats exposed to 1,200 ppm ethylbenzene and in mice and rabbits exposed to 400 ppm ethylbenzene for 4 days (Ethylbenzene Producers Association 1986a). After 4 weeks of exposure to 382 ppm, rats showed sporadic lacrimation, whereas mice and rabbits showed no ocular effects at 782 and 1,610 ppm, respectively (Cragg et al. 1989). No ocular effects were seen in rats or mice after a 13-week exposure to 975 ppm ethylbenzene (NTP 1992).

Table 3-3 Levels of Significant Exposure to Ethylbenzene - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL				Reference Chemical Form	Comments
				Less Serious		Serious			
ACUTE EXPOSURE									
Death									
Rabbit (New Zealand)	24 hr					15433 M mg/kg/day	(LD50)	Smyth et al. 1962	
Systemic									
Rabbit (New Zealand)	24 hr	Dermal		8.67 mg	(grade 4 skin irritation)			Smyth et al. 1962	
INTERMEDIATE EXPOSURE									
Systemic									
Rat (F344/N)	13 wk 5 d/wk 6 hr/d	Ocular	975 B ppm					NTP 1992	
Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d	Ocular	975 B ppm					NTP 1992	

B = males and females; d = day(s); hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; ppm = parts per million; wk = week(s)

No studies were located regarding the following health effects in humans or animals after dermal exposure to ethylbenzene:

3.2.3.3 Immunological and Lymphoreticular Effects

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.3 GENOTOXICITY

Holz et al. (1995) reported no increase in sister chromatid exchanges, DNA adduct formation, micronuclei, or DNA single-strand breaks in the peripheral lymphocytes of workers exposed to low levels of ethylbenzene and other aromatic hydrocarbons (benzene, toluene, and xylene) in a styrene plant. Chromosomal aberrations were observed in peripheral blood lymphocytes of workers concomitantly exposed to ethylbenzene (0.2–13.1 mg/m^3) and benzene (0.4–15.1 mg/m^3) (Sram et al. 2004). Although a reduction in exposure (reduced ethylbenzene and benzene concentrations not reported) reduced chromosomal aberrations, due to concomitant exposure to benzene, an association between chromosomal damage and ethylbenzene exposure cannot be established. No significant alterations in micronuclei formation were observed in peripheral blood erythrocytes of B6C3F1 mice exposed to 500–1,000 ppm ethylbenzene 6 hours/day, 5 days/week for 13 weeks (NTP 1992, 1999) or in polychromatic erythrocytes in the bone marrow of NMRI mice administered two daily doses of 0.37–0.75 mL/kg via intraperitoneal injection (Mohtashamipur et al. 1985). Similarly, inhalation exposure of B6C3F1 mice to 375–1,000 ppm ethylbenzene for 6 hours did not induce DNA repair (as assessed by unscheduled DNA synthesis) in hepatocytes (Clay 2001). The *in vivo* genotoxicity data in laboratory animals are shown in Table 3-4.

The genotoxic potential of ethylbenzene has been investigated primarily using *in vitro* assays in *Salmonella typhimurium*, *Escherichia coli*, *Saccharomyces cerevisiae*, Chinese hamster ovary cells, mouse lymphoma cells, Syrian hamster embryo cells, and human lymphocytes. Results of these *in vitro* genotoxicity studies are shown in Table 3-5. The available data indicate that ethylbenzene is not mutagenic in bacteria (Dean et al. 1985; Degirmenci et al. 2000; Florin et al. 1980; Kubo et al. 2002; Nestmann et al. 1980; NTP 1986, 1999; Zeiger et al. 1992) or yeast cells (Dean et al. 1985; Nestmann and Lee 1983) in the presence or absence of metabolic activation. Ethylbenzene was found to induce forward

Table 3-4. Genotoxicity of Ethylbenzene *In Vivo*

Species (test system)	End point	Results	Reference
B6C3F1 mice (inhalation exposure 6 hours/day, 5 days/week for 13 weeks)	Micronuclei formation in peripheral blood erythrocytes	–	NTP 1992, 1999
NMRI mice (intraperitoneal injection)	Micronuclei formation in polychromatic bone marrow erythrocytes	–	Mohtashamipur et al. 1985
B6C3F1 mice (inhalation 6 hours)	Unscheduled DNA synthesis in hepatocytes	–	Clay 2001

– = negative result; DNA = deoxyribonucleic acid

Table 3-5. Genotoxicity of Ethylbenzene *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium (plate incorporation assay)	Gene mutation	–	–	Dean et al. 1985[a]; Florin et al. 1980[b]; Nestmann et al. 1980[c]
S. tymphimurium (plate incorporation assay; strains TA87, TA98, TA100, TA1537; TA1538)	Gene mutation	–	–	NTP 1986[d]
S. tymphimurium (plate incorporation assay; strains TA97, TA98, TA100, TA1535)	Gene mutation	–	–	Zeiger et al. 1992[d] (results also published in NTP 1992, 1999)
Escherichia coli WP_2, WP_2uvrA	Gene mutation	–	–	Dean et al. 1985[a]
Eukaryotic organisms:				
Saccharomyces cerevisiae JD1 gene conversion assay	Gene mutation	–	–	Dean et al. 1985
S. cerevisiae Dy, XV185-14C	Gene mutation	–	No data	Nestmann and Lee 1983
Mammalian cells:				
Mouse lymphoma cells	Gene mutation	No data	+	McGregor et al. 1988 (results also published in NTP 1992, 1999)
Mouse lymphoma cells	Gene mutation	–	–	Seidel et al. 2006
Mouse lymphoma cells	Gene mutation	–	–	Wollny 2000
Rat liver (RL4) epithelial type cells	Chromosome damage	–	–	Dean et al. 1985
Chinese hamster ovary cells	Chromosome damage	–	–	NTP 1992, 1999
Syrian hamster embryo cells	Micronuclei formation	No data	+	Gibson et al. 1997
Human lymphocytes	Sister chromatid exchange	Not applicable	±[e]	Norppa and Vainio 1983a
Chinese hamster ovary cells	Sister chromatid exchange	–	–	NTP 1986, 1992, 1999
Syrian hamster embryo cells	Cell transformation	No data	+	Kerckaert et al. 1996

Table 3-5. Genotoxicity of Ethylbenzene *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Human lymphocytes	DNA single strand breaks	No data	+	Chen et al. 2008
Human lymphocytes	DNA double strand breaks	No data	–	Chen et al. 2008

[a]Concentrations of ethylbenzene tested: 0, 0.2, 2, 20, 500, or 2,000 µg/plate (>99% pure)
[b]Concentrations of ethylbenzene tested: 0, 3, 31, 318, or 3,184 µg/plate (0, 0.03, 0.3, 3, or 30 µmole/plate)
[c]Concentrations of ethylbenzene tested: 0.4 mg/plate
[d]Concentrations of ethylbenzene tested: 0, 10, 33, 110, 333, 666, or 1,000 µg/plate
[e]Also cytotoxic.

— = negative result; + = positive result; ± = weakly positive

mutations in mouse lymphoma cells at 80 mg/L without metabolic activation (McGregor et al. 1988; results also reported in NTP 1992, 1999); this concentration was near the lethal concentration of 100 mg/L. Similarly, no alterations in the occurrence of forward mutations in mouse lymphoma cells (with or without activation) were observed at concentrations of ≤50 mg/L (Seidel et al. 2006). A third study examining the occurrence of forward mutations in mouse lymphoma cells (Wollny 2000) found a positive response at cytotoxic concentrations (34.4 and 68.8 µg/mL without metabolic activation and 825 µg/mL with activation); however, two replications of the experiment failed to find a positive mutagenic response. A weak positive response was observed when ethylbenzene was tested for sister chromatid exchanges in human lymphocytes (Norppa and Vainio 1983a); however, the positive response was only observed at a cytotoxic concentration. Ethylbenzene also failed to induce sister-chromatid exchanges in Chinese hamster ovary cells (NTP 1986, 1999) or chromosomal aberrations in Chinese hamster cells (NTP 1986, 1999) or rat liver cells (Dean et al. 1985). A positive dose-related increase in the occurrence of micronuclei formation were observed in Syrian hamster embryo cells tested at ethylbenzene concentrations of 25–200 µg/mL (Gibson et al. 1997). A significant increase in morphological cells transformations was also observed in Syrian hamster embryo cells exposed to 150 or 200 µg/mL for 7 days (Kerckaert et al. 1996); the 200 µg/mL concentration resulted in significant cytotoxicity. In this same study, no significant alterations the percent cell transformations were observed when the cells were incubated with ethylbenzene for 24 hours. A series of studies conducted by Chen et al. (2008) evaluated the potential of ethylbenzene to induce DNA damage in human lymphocytes. A significant increase in single strand breaks was observed at 100 and 200 µM; no alterations in double strand breaks were found at 200 µM. The study also found a significant decrease in DNA damage in cells pre-treated with spin traps, suggesting that the DNA damage may be due to the generation of free radicals.

In general, the results of available *in vivo* and *in vitro* genotoxicity studies suggest that ethylbenzene is not genotoxic. Excluding studies that found results at cytotoxic or near cytotoxic concentrations, a few studies have found positive results. The significance of these positive findings is not known.

3.4 TOXICOKINETICS

Ethylbenzene is absorbed from the lungs, gastrointestinal tract, and through the skin. Absorbed ethylbenzene is rapidly eliminated (blood $t_{1/2} \leq 1$ hour) by metabolism and excretion of metabolites. The major metabolic pathways are side chain and ring hydroxylation with subsequent formation of O-glucuronide and sulfate conjugates. Conjugates, mandelic acid and phenylglyoxylic acid are the

primary excreted metabolites. The distribution of ethylbenzene to tissues has been modeled as a flow-limited process (i.e., clearance from blood to tissues can be predicted by tissue blood flow) with rapid equilibrium achieved between blood and tissues. Measured blood:tissue partition coefficients in the rat are approximately as follows: fat, 36; liver, 2; and muscle, 0.6. These values predict the same order for tissue concentrations for any given blood concentration; however, this order has not been verified experimentally with simultaneously measured blood and tissue ethylbenzene concentrations *in vivo*.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Inhalation studies in humans demonstrate that ethylbenzene is rapidly absorbed via this route (Bardodej and Bardodejova 1970; Gromiec and Piotrowski 1984; Knecht et al. 2000; Tardif et al. 1997). A steady-state blood:alveolar air concentration ratio of approximately 30 was achieved within 1 hour of initiating exposure (Tardif et al. 1997). Volunteers exposed for 8 hours to ethylbenzene at concentrations of 23–85 ppm were shown to retain 64% of the inspired vapor, with only trace amounts detected in expired air at the end of the exposure period (Bardodej and Bardodejova 1970). Another inhalation study that involved humans exposed to similar levels of ethylbenzene demonstrated mean retention rates of 49% (Gromiec and Piotrowski 1984). The differences may be attributable to human variability in absorption rates although they could also be due to differences in methodology.

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.

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, 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.

Ethylbenzene concentrations in whole blood collected from workers at the end of their work shifts correlated significantly with the average concentrations of occupational exposure to ethylbenzene (Kawai

et al. 1992). The maximum ethylbenzene concentration in air in the workplace was 5 ppm (Kawai et al. 1992).

Inhalation studies in animals exposed to ethylbenzene showed results similar to those found in humans (Chin et al. 1980b; Freundt et al. 1989; Fuciarelli 2000; Romer et al.1986; Tardif et al. 1997). Harlan-Wistar rats rapidly absorbed radiolabeled ethylbenzene during respiration, with a retention rate of 44% (Chin et al. 1980b). This absorption value may have been slightly overestimated, however, because possible contributions from dermal exposure were not addressed. Concentrations of ethylbenzene in the blood of rats and guinea pigs exposed to ethylbenzene at 550 ppm for 8 hours reached 23 and 3 µg/mL, respectively. Ethylbenzene concentrations in blood after the last of three daily exposures (8 hours each) had diminished to 6 µg/mL in rats and to <2 µg/mL in guinea pigs (Cappaert et al. 2002). In mice, steady-state blood ethylbenzene concentrations achieved within 2 hours of initiating inhalation exposures were approximately 0.71 mg/L at 75 ppm, 2.3 mg/L at 200 ppm, and 20 mg/L at 500 ppm (Charest-Tardif et al. 2006). These data indicate that the disposition of inhaled ethylbenzene between guinea pigs may be different from that in rats and mice. Ethylbenzene concentrations in blood in adult rats and their offspring increased at a rate that was greater than proportional to dose (Faber et al. 2006). Ethylbenzene levels in maternal blood, collected on PND 22, after a 6-hour exposure to 25, 100, or 500 ppm, 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 (Faber et al. 2006).

No studies describing factors specifically affecting absorption of ethylbenzene following inhalation exposure were available.

3.4.1.2 Oral Exposure

No studies were located regarding the absorption of ethylbenzene in humans following oral exposure. Studies in animals, however, indicate that ethylbenzene is quickly and effectively absorbed by this route. Recovery of ethylbenzene metabolites in the urine of rabbits administered a single dose of 593 mg/kg was between 72 and 92% of the administered dose 24 hours following exposure (El Masry et al. 1956). Similarly, 84% of the radioactivity from a single oral dose of 30 mg/kg ethylbenzene administered to female rats was recovered within 48 hours (Climie et al. 1983). Ethylbenzene was detected at 0.49, 3.51, and 18.28 mg/L in maternal blood of rats 1 hour after the last of four daily administrations of 26, 90, or 342 mg/kg/day (distributed over three equal doses), respectively; however, ethylbenzene was not detected

(limit of detection: 0.006 mg/L) in blood of weanlings culled from the same dams (Faber et al. 2006). It is unclear if the latter finding is due to low transfer of ethylbenzene to milk or if the 1-hour exposure-to-sampling time-lapse was too long to allow detection of ethylbenzene.

3.4.1.3 Dermal Exposure

Studies in humans dermally exposed to liquid ethylbenzene demonstrate rapid absorption through the skin, but absorption of ethylbenzene vapors through the skin appears to be minimal (Dutkiewicz and Tyras 1967; Gromiec and Piotrowski 1984). Absorption rates of 24–33 mg/cm^2/hour and 0.11–0.23 mg/cm^2/hour have been measured for male subjects exposed to liquid ethylbenzene and ethylbenzene from aqueous solutions, respectively (Dutkiewicz and Tyras 1967). 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. These results indicate that skin absorption could be a major route of uptake of liquid ethylbenzene or ethylbenzene in water. In contrast, ethylbenzene metabolite levels in urine following dermal exposure to 150–300 ppm (650–1,300 mg/m^3) ethylbenzene vapors for two hours did not differ from values taken prior to exposure, indicating minimal, if any, dermal absorption of ethylbenzene vapors (Gromiec and Piotrowski 1984).

Susten et al. (1990) conducted *in vivo* percutaneous absorption studies of ethylbenzene in mice. Results showed total absorption (sums of radioactivity found in the excreta, carcass, skin application site, and expired breath) was 3.4% of the nominal dose. The total percentage recovered (includes wipe of skin area, ethylbenzene 0.03%) was 95.2%. The amount of ethylbenzene absorbed at an estimated contact time of 5 minutes was 148.55 μg with an absorption rate of 37 μg/cm^2/minute.

The volumes of ethylbenzene absorbed in rats treated dermally for 24 hours with aqueous solutions of ethylbenzene (neat [99% pure], saturated, 2/3 or 1/3 saturated), were 0.24, 0.20, 0.18, and 0.17 mL, respectively (Morgan et al. 1991). Peak blood level during exposure to neat ethylbenzene was reported at 5.6 μg/mL, attained after 1 hour of exposure, which decreased during the remainder of the exposure period. The concentration of ethylbenzene in the blood was highest after exposure to saturated aqueous solutions, followed by the 2/3 and 1/3 saturated solutions.

Results of dermal penetration studies in excised rat skin indicate that the penetration rate of pure ethylbenzene (Tsuruta 1982) is faster than that of ethylbenzene from JP-8 jet fuel (McDougal et al. 2000). The penetration rates of pure liquid ethylbenzene following 3-, 4-, and 5-hour exposure durations in rat

skin were approximately 2.38, 3.12, and 3.22 µg/cm^2/hour (calculated by ATSDR from reported data on exposure area, exposure time, and mass transfer); these rates are substantially lower than the rate of dermal absorption determined for humans (Tsuruta 1982). This might be attributed to differences between *in vitro* and *in vivo* testing and/or differences in rat versus human skin. McDougal et al. (2000) reported an ethylbenzene flux of 0.377 µg/cm^2/hour (0.0004 mg/cm^2/hour) for ethylbenzene and a permeability coefficient of 3.1x10^{-4} cm/hour during a 4-hour period in excised rat skin which had received 2 mL of JP-8 fuel containing approximately 1,200 µg ethylbenzene/mL fuel (0.15% w/w). It was recognized that the choice of vehicle (JP-8) could affect dermal penetration of ethylbenzene. 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/hour, 0.06x10^{-3} cm/hour, and 715x10^{-6} cm^2/hour (Muhammad et al. 2005).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

In humans exposed for 2 hours to a mixture of industrial xylene containing 40.4% ethylbenzene, the estimated solvent retention in adipose tissue was 5% of the total uptake (Engstrom and Bjurstrom 1978). 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. No studies were located concerning the distribution of ethylbenzene in humans following exposure to ethylbenzene alone. However, studies by Pierce et al. (1996) suggest that *in vitro,* the partitioning of ethylbenzene from air into human adipose tissue is similar to that observed in rats.

Studies conducted in rats and mice have shown that inhaled ethylbenzene accumulates in adipose tissue (Elovaara et al. 1982; Engstrom et al. 1985; Fuciarelli 2000). In rats and mice exposed to 750 ppm ethylbenzene for 6 hours/day, concentrations of ethylbenzene in mesenteric adipose were 20–60 times higher than steady-state blood concentrations, whereas concentration in liver were similar to blood (Fuciarelli 2000). Concentrations of ethylbenzene in perirenal adipose tissue were reported to increase, although not linearly, with increasing concentrations of ethylbenzene (Engstrom et al. 1985) and in a mixture of solvent vapors containing ethylbenzene (Elovaara et al. 1982). The less-than-linear increase of ethylbenzene in adipose tissue with increasing dose was partially attributed to the induction of drug-metabolizing enzymes occurring with increasing exposure concentrations, altered blood flow to adipose tissue, changes in lung excretion, and changes in the distribution of ethylbenzene in different tissues. Ethylbenzene was shown to be efficiently distributed throughout the body in rats following inhalation exposure to radiolabeled ethylbenzene (Chin et al. 1980b). The highest amounts of radioactivity in

tissues 42 hours after exposure to 230 ppm ethylbenzene for 6 hours were found in the carcass, liver, and gastrointestinal tract, with lower amounts detected in the adipose tissue.

3.4.2.2 Oral Exposure

No studies were located regarding distribution of ethylbenzene in humans or animals following oral exposure.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans following dermal exposure to ethylbenzene.

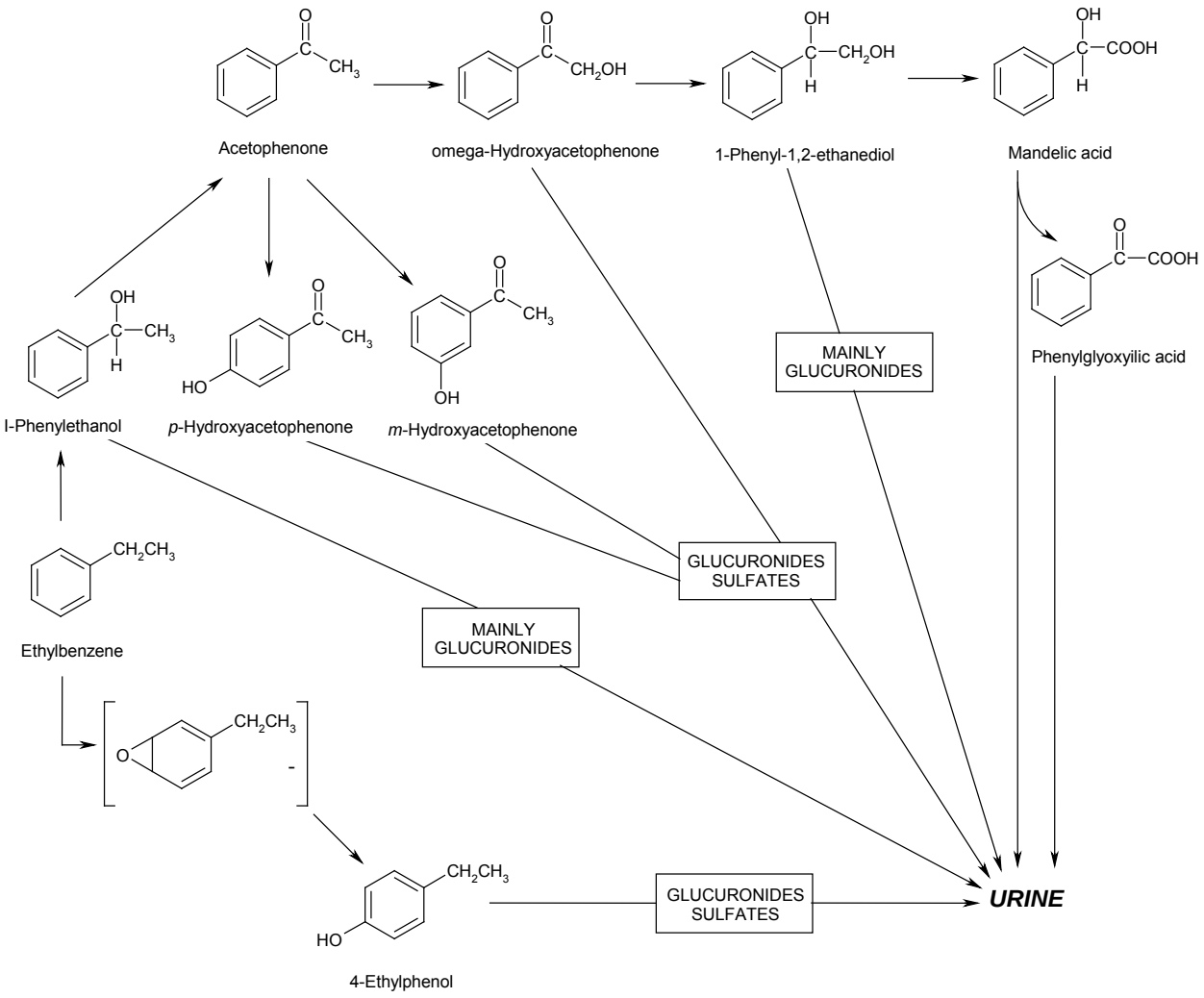
The percentages of absorbed doses following dermal application of [^{14}C]-ethylbenzene in hairless mice were: 15.5%, carcass; 4.5%, application site; 14.3%, expired breath; and 65.5%, excreta (Susten et al. 1990).

3.4.3 Metabolism

The metabolism of ethylbenzene has been studied in humans and other mammalian species. The data demonstrate that ethylbenzene is metabolized mainly through hydroxylation and then through conjugation reactions from which numerous metabolites have been isolated. Figure 3-3 summarizes the proposed metabolic pathway for ethylbenzene in humans (Engstrom et al. 1984). The major urinary metabolites have been identified (Kiese and Lenk 1974; Sullivan et al. 1976). Comparisons of *in vitro* data with data from intact animals indicate that liver microsomal enzymes participate in ethylbenzene hydroxylation (McMahon and Sullivan 1966; McMahon et al. 1969; Sams et al. 2004). In microsomes prepared from human liver, hydroxylation of ethylbenzene to 1-phenylethanol is catalyzed by cytochrome P-450 isoforms CYP2E1 and CYP2B6 (Sams et al. 2004). Adrenal cortex may be a major site of extra-hepatic ethylbenzene metabolism (Greiner et al. 1976). No significant qualitative differences in metabolism between oral and inhalation routes were reported in humans or animals. The metabolism of ethylbenzene has been found to vary with species, sex, and nutritional status. These differences are described below.

In humans exposed via inhalation, the major metabolites of ethylbenzene are mandelic acid (approximately 64–71%) and phenylglyoxylic acid (approximately 19–25%) (Bardodej and Bardodejova 1970; Engstrom et al. 1984; Knecht et al. 2000; Jang et al. 2001; Tardif et al. 1997). Based on data from human, animal, and *in vitro* studies, the metabolic pathway for ethylbenzene in humans was proposed

Figure 3-3. Metabolic Scheme for Ethylbenzene in Humans



Source: Engstrom et al. 1984

(Engstrom et al. 1984). This pathway is shown in Figure 3-3. Evidence indicates that the initial step in this metabolic pathway is oxidation (hydroxylation) of the side chain of ethylbenzene to produce 1-phenylethanol. Microsomal preparations from rat liver have shown that the oxidation of ethylbenzene proceeds with the incorporation of atmospheric oxygen, as opposed to oxygen from water molecules (McMahon et al. 1969). Filipovic et al. (1992) have shown that cytochrome P-$450_{(cam)}$ from *Pseudomonas putida* provides a useful metabolic model for ethylbenzene hydroxylation, converting ethylbenzene to 1-phenylethanol at 98%. 1-Phenylethanol is conjugated to glucuronide, which then is either excreted or converted to subsequent metabolites. Oxidation of 1-phenylethanol yields acetophenone, which is both excreted in the urine as a minor metabolite and further transformed. Continued oxidation of the side chain leads to the sequential formation of 2-hydroxyacetophenone, 1-phenyl-1,2-ethanediol, mandelic acid, and phenylglyoxylic acid. Minor pathways (e.g., ring hydroxylation) include glucuronide and sulfate conjugation with hydroxylated derivatives to form glucuronides and sulfates that are excreted in the urine. Analysis of urine from humans exposed to ethylbenzene via the inhalation route showed that approximately 70 and 25% of the retained dose of ethylbenzene is excreted as mandelic acid and phenylglyoxylic acid, respectively (Bardodej and Bardodejova 1970; Engstrom et al. 1984). Additional metabolites detected in human urine include 1-phenylethanol (4%), *p*-hydroxyacetophenone (2.6%), *m*-hydroxyacetophenone (1.6%), and trace amounts of 1-phenyl-1,2-ethanediol, acetophenone, 2-hydroxyacetophenone, and 4-ethylphenol. Following dermal exposure of humans, however, excretion of mandelic acid was shown to be only 4.6% of the absorbed dose (Dutkiewicz and Tyras 1967), which may indicate differences in the metabolic fate between inhalation and dermal exposure routes. However, the small percentage of absorbed dose accounted for limits the interpretation. No animal data were located which could confirm these metabolic differences following dermal exposure. Generally, ethyl-benzene metabolites and intermediates are thought to be only slightly toxic, since no adverse effects from human experimental exposure have been reported (Bardodej and Bardodejova 1970).

Qualitative and quantitative differences in the biotransformation of ethylbenzene in animals as compared to humans have been reported (Bakke and Scheline 1970; Climie et al. 1983; El Masry et al. 1956; Engstrom et al. 1984, 1985; Smith et al. 1954a, 1954b; Sollenberg et al. 1985). The major metabolites of ethylbenzene differ from species to species, and different percentages of the metabolites are seen in different species. The principal metabolic pathway in rats is believed to begin with oxidation (hydroxylation) of the side chain as in humans (Climie et al. 1983; Engstrom et al. 1984, 1985; Smith et al. 1954a). In rats exposed by inhalation or orally to ethylbenzene, the major metabolites were identified as benzoic acids and glycine conjugates (e.g., hippuric acid; approximately 38%), 1-phenylethanol (approximately 25%), and mandelic acid (approximately 15–23%), with phenylglyoxylic acid making up

only 10% of the metabolites (Climie et al. 1983; Engstrom et al. 1984, 1985; Fuciarelli 2000). The urinary excretion rate of mandelic acid in rats exposed to 250 ppm ethylbenzene, 6 hours/day for 15 days was the same after the first exposure as it was after the last exposure. In contrast, excretion in the 1,000 ppm group was 2–5 times higher after the last exposure than it was after the first exposure (Saillenfait et al. 2006). Both *in vivo* studies using rats and *in vitro* studies using rat liver microsomes showed that 4-ethylphenol was also produced from ethylbenzene, perhaps by rearrangement of corresponding arene oxides (Bakke and Scheline 1970; Kaubisch et al. 1972). Kaubisch et al. (1972) also showed that 2-hydroxyethylbenzene was produced from ethylbenzene *in vitro* in the presence of rat liver microsomes. The level of ethylbenzene exposure was shown to affect the metabolic pattern. This was thought to be due either to selective enzymatic induction in the biotransformation of ethylbenzene or to delayed excretion of certain metabolites with increasing doses.

Acetophenone was detected (quantitative data were not provided) in blood of rats and guinea pigs exposed to ethylbenzene in air at 500 ppm, 8 hours/day for 3 days (Cappaert et al. 2002). Further clarification of ethylbenzene metabolic pathways was provided by Sullivan et al. (1976). Using intraperitoneally dosed rats, the authors demonstrated that the conversion of 1-phenylethanol to mandelic acid initially involves oxidation to acetophenone. Acetophenone was considered to be the precursor of mandelic acid, benzoylformic acid, and benzoic acid. A similar study in which rabbits were intraperitoneally injected with a single dose of 250 mg ethylbenzene/kg body weight was conducted by Kiese and Lenk (1974). This study showed that between 1 and 10% of the dose was excreted as 1-phenylethanol in the urine and <1% was excreted in the urine as 2-hydroxyacetophenone, *p*-hydroxy-acetophenone, and *m*-hydroxyacetophenone.

Rabbits given an oral dose of ethylbenzene showed the major metabolic pathway to be hydroxylation of the α-carbon to 1-phenylethanol, which is oxidized further to a number of intermediates and metabolites (El Masry et al. 1956; Smith et al. 1954a). Many of these intermediates are subsequently conjugated to glucuronides and sulfates and excreted. In rabbits, the most important metabolite is hippuric acid, which is probably formed by oxidative decarboxylation of phenylglyoxylic acid (El Masri et al. 1958). Oxidation of the methyl group of ethylbenzene was also shown to occur, as evidenced by the presence of phenaceturic acid in the urine. A slight increase in the excretion of thioether suggests that glutathione conjugation may also play a minor role.

The nutritional status of animals was demonstrated to have a marked effect on ethylbenzene metabolism in rats (Nakajima and Sato 1979). The *in vitro* metabolic activity of liver microsomal enzymes on ethyl-

benzene was shown to be significantly enhanced in fasted rats despite a marked loss of liver weight. No significant increases in the microsomal protein and cytochrome P-450 contents were detected in fasted rats compared with fed rats. In addition, the metabolic rate in fasted males was significantly higher than in fasted females, but the difference in rates decreased following food deprivation for 3 days. These results suggest possible sex differences in the rate of ethylbenzene metabolism. However, it is not known if such differences exist in the normally fed rats.

Metabolism of ethylbenzene has not been studied in children or immature animals. However, some members of two of the enzyme superfamilies involved in conjugation of phase I ethylbenzene metabolites are known to be developmentally regulated. In humans, UDP glucuronosyltransferase activity does not reach adult levels until about 6–18 months of age, although the development of this activity is isoform specific. Activity of sulfotransferases seems to develop earlier, although again, it is isoform specific. The activity of some sulfotransferase isoforms may even be greater during infancy and early childhood than in adults (Leeder and Kearns 1997).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Excretion of ethylbenzene has been studied in humans and in a number of animal species. Ethylbenzene has been shown to be rapidly metabolized and then eliminated from the body, primarily as urinary metabolites. The major metabolic products have been previously described in Section 3.4.3.

Elimination of ethylbenzene has been studied in volunteers exposed by inhalation (Bardodej and Bardodejova 1970; Dutkiewicz and Tyras 1967; Engstrom and Bjurstrom 1978; Gromiec and Piotrowski 1984; Knecht et al. 2000; Tardif et al. 1997; Yamasaki 1984) and in humans exposed by inhalation in the occupational setting (Holz et al. 1995; Jang et al. 2001; Kawai et al. 1991, 1992; Ogata and Taguchi 1988). Elimination of ethylbenzene in exhaled air in volunteers exposed to 33 ppm ethylbenzene exhibited multi-phasic kinetics with an early-phase half time of <1 hour (Tardif et al. 1997). This elimination rate is similar to the rate of elimination of ethylbenzene from blood following cessation of exposure (Knecht et al. 2000; Tardif et al. 1997) and is considerably faster than the rate of elimination (i.e., urinary excretion) of the metabolites, mandelic acid ($t_{1/2}$≈3–5 hours) and phenylgloxylic acid ($t_{1/2}$≈10–12 hours) (Gromiec and Piotrowski 1984; Knecht et al. 2000; Tardif et al. 1997). Elimination of mandelic acid was reported to be biphasic, with half-lives of 3.1 hours for the rapid phase and 25 hours for the slow phase (Gromiec and Piotrowski 1984). During the 8-hour exposure, 23% of the retained

ethylbenzene was eliminated in the urine, and 14 hours following termination of exposure an additional 44% of the retained ethylbenzene was eliminated. The highest excretion rate of urinary metabolites in humans exposed to ethylbenzene by inhalation occurred 6–10 hours after the beginning of exposure (Gromiec and Piotrowski 1984; Yamasaki 1984).

Concentrations of ethylbenzene metabolites in before-shift and after-shift urine were significantly higher in workers exposed to 85 to >921 ppm ethylbenzene in a styrene plant than in control workers exposed to 33.4–66.8 ppm (Holz et al. 1995).

A statistically significant correlation was observed between urinary excretion of mandelic acid and ethylbenzene exposure in workers exposed to mixed solvents (including an ethylbenzene time-weighted average [TWA] of 0.9 ppm) in a metal-coating factory (Kawai et al. 1991). No correlation was observed between ethylbenzene exposure and phenylglyoxylic acid urinary excretion. In a study of chronic-duration exposure at lower levels (2.1 and 2.3 ppm for geometric and arithmetic mean, respectively), no significant correlation was observed between ethylbenzene exposure and urinary excretion of phenylglyoxylic acid and mandelic acid (Kawai et al. 1992).

In animals, elimination of ^{14}C-ethylbenzene following inhalation exposure is rapid and occurs primarily via urinary excretion (Chin et al. 1980a, 1980b; Engstrom et al. 1984, 1985) and to a much lesser degree via the feces, and expired “gasses” and carbon dioxide (Chin et al. 1980b). Rats exposed to 230 ppm radiolabeled ethylbenzene for 6 hours via inhalation excreted virtually all of the radioactivity within 24 hours after the onset of exposure (Chin et al. 1980a, 1980b). Ninety-one percent of the radioactivity was recovered, primarily in the form of urinary metabolites. In a similar inhalation experiment using rats exposed to 300 or 600 ppm, urinary excretion was reported to be 83 and 59% of the absorbed dose within 48 hours after the onset of exposure, with 13% eliminated during the first 6 hours of exposure (Engstrom et al. 1984).

Quantitative differences between species in the percentages of metabolites excreted in the urine were also reported by Chin et al. (1980a). In this report, urinary metabolites in dogs and rats exposed to ethylbenzene by inhalation were studied. 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. These results were attributed to differences in metabolism between dogs and rats.

Elimination of inhaled ethylbenzene from blood, fat, liver, and lung tissue following inhalation exposures is biphasic in rats and mice (Fuciarelli 2000). The terminal phase for blood is approximately 4–7 times slower than the initial phase. For example, in male rats exposed to 75 ppm for 6 hours, the initial and terminal elimination half-times were approximately 77 and 493 minutes, respectively. Blood elimination kinetics of inhaled ethylbenzene are dependent on exposure concentration, with decreasing clearance of ethylbenzene from blood in association with increasing exposure concentration. In female mice, rate constants for ethylbenzene measured at the conclusion of 4-hour exposures to concentrations ranging from 75 to 1,000 ppm were 0.21 $minute^{-1}$ ($t_{1/2}$=3.3 minute) at 75 ppm and 0.011 $minute^{-1}$ ($t_{1/2}$=63 minutes) at 1,000 ppm; similar decreases in elimination rate were observed in male mice (Charest-Tardif et al. 2006). Elimination half-life values increased approximately 2-fold, with the 10-fold increase in exposure concentration, and the ratio of the area under the curve (AUC) for ethylbenzene in blood/exposure concentration increased 10–20-fold (Fucareli 2000). The concentration dependence on elimination is consistent with a capacity limitation in metabolism of ethylbenzene. Elimination rates measured in mice after 1 or 7 days of 4-hour exposures to 75 ppm ethylbenzene were similar; however, elimination rates increased following 7 days of exposure to 750 ppm (e.g., 0.016 $minute^{-1}$, $t_{1/2}$=43.3 minutes, female mice), compared to 1 day of exposure (0.061 $minute^{-1}$, $t_{1/2}$=11.4 minutes, female mice; Charest-Tardif et al. 2006). These observations are consistent with induction of metabolic clearance of ethylbenzene in association with repeated exposures to 750 ppm. Elimination rates of ethylbenzene from blood were faster in mice compared to rats. In rats exposed to 75 ppm ethylbenzene for 6 hours, the rate for the initial phase of elimination was 0.00902 $minute^{-1}$ ($t_{1/2}$=76.8 minutes) in male rats and 0.0102 $minute^{-1}$ ($t_{1/2}$=68.1 minutes) in female rats. In mice the elimination rates were 0.0596 $minute^{-1}$ ($t_{1/2}$=11.6 minutes) in male mice, and 0.109 $minute^{-1}$ ($t_{1/2}$=6.36 minutes) in female mice (Fuciarelli 2000).

3.4.4.2 Oral Exposure

No studies were located regarding the excretion of ethylbenzene metabolites in humans following oral exposure to ethylbenzene.

Elimination of ethylbenzene and its metabolites in animals after oral exposure has been shown to be similar to that following inhalation exposure. Female rats administered a single oral dose of 30 mg radiolabeled ethylbenzene/kg/body weight showed very rapid elimination, mostly in the urine (Climie et al. 1983). Eighty-two percent of the radioactivity was detected in the urine, while 1.5% was detected in the feces. The major metabolites were mandelic acid (23%) and hippuric acid (34%), with 1-phenylethyl glucuronide detected as a minor metabolite. Relatively minor metabolites (e.g., 4-ethylphenol, 2-phenyl-

ethanol, 1-phenylethanol) were shown to be excreted in the urine of male rats exposed to a single oral dose of 100 mg/kg ethylbenzene administered by gavage in oil (Bakke and Scheline 1970). No data on the major metabolites were provided in this study.

In a similar study in which male rats were given single oral doses of 350 mg/kg/body weight ethylbenzene, the excretion of mandelic acid and phenylglyoxylic acid was detected in the first urine sample after exposures. Peak concentration was reached within 17 hours, and ethylbenzene was virtually eliminated 48 hours following the onset of exposure (Sollenberg et al. 1985).

As in inhalation experiments, quantitative and qualitative differences between species were shown to exist in the percentages of metabolites excreted in the urine. Rabbits orally exposed to ethylbenzene excreted large amounts of glucuronide conjugates in the urine (El Masry et al. 1956; Smith et al. 1954a, 1954b) instead of mandelic acid, hippuric acid, and phenylglyoxylic acid, which are the major metabolites in rats (see above). Glucuronide conjugates accounted for 32% of the administered dose, with mandelic acid making up only 2% of the administered dose (El Masry et al. 1956). These results were confirmed in a study by Smith et al. (1954a, 1954b), who detected 32% of a single oral dose of ethylbenzene (433 mg/kg) administered to rabbits as glucuronide conjugates excreted in the urine.

3.4.4.3 Dermal Exposure

In humans, the pattern of excretion of ethylbenzene metabolite following dermal exposure has been shown to differ significantly from the pattern in which humans have been exposed by inhalation. Dermal absorption of ethylbenzene in aqueous solutions was estimated as the difference of the ethylbenzene concentrations in solution before and after exposure. The urinary excretion of mandelic acid in humans dermally exposed to ethylbenzene for 2 hours was only 4.6% of the absorbed ethylbenzene (Dutkiewicz and Tyras 1967). Urine was collected periodically during the 2-hour exposure period and a 10-hour follow-up. Interpretation is difficult due to the small percentage of absorbed dose accounted for. No ethylbenzene was reported to be excreted in exhaled air. No further details on the excretion patterns were provided.

Susten et al. (1990) conducted *in vivo* percutaneous absorption studies of ethylbenzene in Hairless mice. Results showed total absorption (sums of radioactivity found in the excreta, carcass, skin application site, and expired breath) was 3.4% of the nominal dose. The absorbed dose collected in expired breath during the first 15 minutes of ethylbenzene application was 9.3%. The percentage of absorbed doses following

dermal application of [^{14}C]-ethylbenzene are as follows: 15.5% in the carcass; 4.5% at the application site; 14.3% in expired breath; and 65.5% in excreta.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

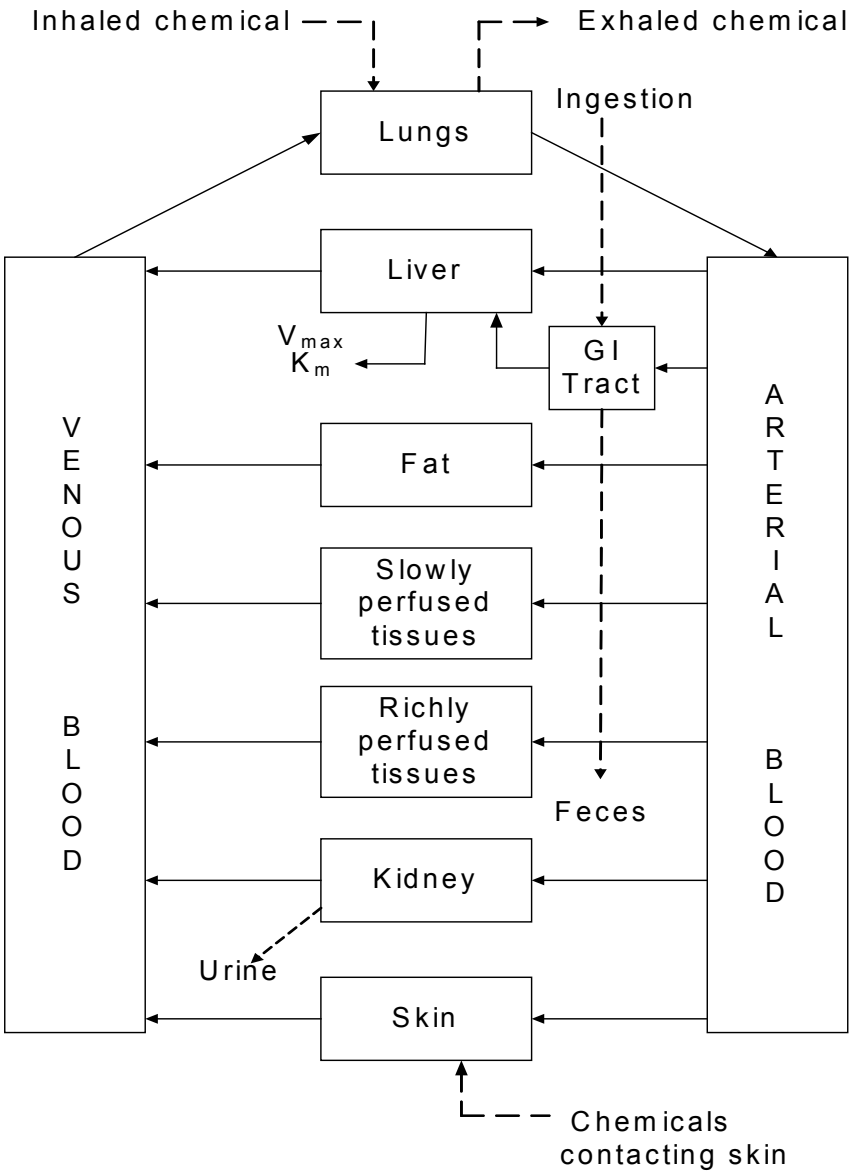
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

If PBPK models for ethylbenzene exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Several models have been developed that simulate the kinetics of inhaled ethylbenzene in animals and humans (Dennison et al. 2003; Haddad et al. 1999, 2000, 2001; Jang et al 2001; Nong et al. 2007; Tardif et al. 1997). The Dennison et al. (2003) and Tardif et al. (1997) inhalation models have been incorporated into models of gasoline component mixture models (Dennison et al. 2004, 2005; Haddad et al. 2001). An extension of the Tardif et al. (1997) model has been reported that includes simulation of gastrointestinal absorption (i.e., transfer of ingested ethylbenzene to liver) of ethylbenzene in rats (Faber et al. 2006). A model of dermal absorption ethylbenzene in human has also been reported (Shatkin and Brown 1991). Models that simulate kinetics of ingested ethylbenzene have not been reported. In general, all of the inhalation models of have similar structures, with the major conceptual differences being the simulation of metabolism and excretion of metabolites. The Nong et al. (2007) mouse model simulates metabolism of ethylbenzene in liver, lung, and richly perfused tissues, whereas all other models attribute all ethylbenzene metabolism to the liver. The Nong et al. (2007) mouse model also simulated changes in ethylbenzene blood kinetics in mice that occurred with repeated exposure to ethylbenzene at exposure concentrations >75 ppm. These changes were attributed to induction of cytochrome P450 and were simulated as an increase in V_{max} for metabolism of ethylbenzene in liver. The Jang et al. (2001) human model simulates excretion of the ethylbenzene metabolite, mandelic acid, whereas other models do not

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan and Andersen 1994

simulate the urinary elimination pathway. The Tardif et al. (1997), Dennison et al. (2003), and Nong et al. (2007) models are described in greater detail in the sections that follow. Complete documentation of the Jang et al. (2001) model was not available for providing a detailed description of the model, although it appears to be conceptually similar to the Tardif et al (1997) and Dennison et al. (2003) models.

The Tardif et al. (1997; Haddad et al. 1999, 2000, 2001) Model

Description of the Model. Tardif et al. (1997) developed a PBPK model for simulating the kinetics of ethylbenzene, toluene, and xylene in blood following inhalation exposures to the individual chemicals or the ternary mixture (in rats and humans). The structure of the model is essentially identical the generic model depicted in Figure 3-4, with tissue compartments limited to lungs, liver, fat, richly perfused tissues (RPT), and poorly perfused tissues (SPT). All metabolism was attributed to the liver and represented as functions of the concentration of parent compound in liver venous blood, affinity constant (K_m), and maximum reaction velocity (V_{max}). Transfers of parent compound between blood and tissues were assumed to be flow-limited, with clearance represented by tissue blood flow (L/hour). Parameters for the ethylbenzene model are presented in Table 3-6. Tissue: air partition coefficients for ethylbenzene were derived from vial equilibrium studies of isolated rat tissues. Tissue:blood partition coefficients were derived from measured tissue:air and blood:air partition coefficients (i.e., tissue:blood=tissue:air/ blood:air; Tardif et al. 1997). Metabolism parameter values for ethylbenzene were derived by fitting the model to observations of blood ethylbenzene kinetics measured in rats during and following 4-hour exposures to ethylbenzene in a dynamic exposure chamber (Tardif et al. 1996). Physiological parameters, cardiac output (QC), alveolar ventilation rate (QP), and metabolism V_{max} were allometrically scaled across species (i.e., rat to human) as a function of body weight (BW, scaling factor=$BW^{0.75}$). A blood:air partition coefficient for humans was estimated from vial equilibrium measurements made on isolated human blood; human tissue:blood partition coefficients were derived based on tissue:air coefficients for the rat (Tardif et al. 1997).

Subsequent enhancements of the Tardif et al. (1997) model included simulation of kinetics of an inhaled quaternary mixture that included benzene, ethylbenzene, toluene, and xylene (Haddad et al. 1999) or mixtures of the latter quaternary mixture with dichloromethane (Haddad et al. 2000, 2001). In the development of the quaternary mixture model, values for V_{max} and K_m for ethylbenzene were re-estimated by fitting data on kinetics of ethylbenzene in venous blood following 4-hour exposures to ethylbenzene (50, 100, or 200 ppm; Haddad et al. 1999; Tardif et al. 1997). The resulting estimates were similar to the original estimates reported in Tardif et al. (1997): V_{max}=6.39 mg/hour-kg) and K_m=1.04 mg/L.

Table 3-6. Parameter Values for Tardif et al. (1997) Ethylbenzene PBPK Models

Parameter	Definition	Rat model	Human model
Physiological parameters			
VLC	Liver volume (fraction of body)	0.049	0.026
VFC	Fat volume (fraction of body)	0.09	0.19
VSC	Slowly-perfused tissue volume (fraction of body)	0.72	0.62
VRC	Richly-perfused tissue volume (fraction of body)	0.05	0.05
QCC	Cardiac output (L/hour-kg BW)	15[a]	18[a]
QPC	Alveolar ventilation rate (L/hour-kg body weight)	15[a]	18[a]
QLC	Liver blood flow (fraction of cardiac output)	0.25	0.26
QFC	Fat blood flow (fraction of cardiac output)	0.09	0.05
QSC	Slowly-perfused blood flow (fraction of cardiac output)	0.15	0.25
QRC	Richly-perfused blood flow (fraction of cardiac output)	0.51	0.44
Chemical parameters			
PB	Blood:air partition coefficient	42.7	28.0
PL	Liver:blood partition coefficient	1.96[b] (83.8)	2.99[b]
PF	Fat:blood partition coefficient	36.44[b] (1,556)	55.57[b]
PS	Slowly-perfused partition coefficient	0.61[b] (26.0)	0.93[b]
PR	Richly-perfused partition coefficient	1.41[b] (60.3)	2.15[b]
$V_{max}C$	Maximum rate of metabolism (mg/hour-kg body weight)	7.3[a,c]	7.3[a,c]
K_mC	Michaelis-Menten coefficient for metabolism (mg/L)	1.39[c]	1.39[c]

[a]Scaled to body weight ($BW^{0.75}$)
[b]Tissue:blood partition coefficients calculated based on reported, experimentally determined (vial equilibrium), tissue:air partition coefficients for rats (values in parentheses) and blood:air coefficient (PB) measured in rat or human blood; tissue:blood=tissue:air/blood:air.
[c]Values derived by optimization to data on blood ethylbenzene concentrations in rats exposed to ethylbenzene (dynamic chamber).

Although the Tardiff et al. (1997) model was developed to simulate kinetics of inhaled ethylbenzene, Faber et al. (2006) reported an extension of the model that includes simulation of gastrointestinal absorption (i.e., transfer of ingested ethylbenzene to liver) of ethylbenzene in rats. The gastrointestinal absorption rate constant of 0.18 $hour^{-1}$ was estimated by iteratively adjusting the ate constant to achieve agreement with observed blood ethylbenzene kinetics in following a gavage dose of ethylbenzene (180 mg/kg), in corn oil, administered to adult female rats.

Validation of the Model. Optimization of the metabolism parameters against observed blood kinetics of ethylbenzene in rats exposed for 4 hours to 100 or 200 ppm ethylbenzene achieved predicted blood concentration of ethylbenzene that were within 1–2 standard deviations (SD) of the observations. Most of the validation efforts reported in Tardif et al. (1997) were directed at exploring how well the mixture model predicted observed blood kinetics of ethylbenzene, toluene, or xylene in rats during and following exposures to binary or ternary mixtures of the chemicals. Interactions were simulated as competitive, noncompetitive, or uncompetitive inhibition of metabolism, with the values of the inhibition constants derived by fitting the model to observed blood kinetics during and following exposures to binary mixtures. Although the mixture model achieved predictions similar to observations (i.e., within 1–2 SD of observations), this outcome would have been highly influenced by the parameter values selected for each of the individual chemicals, including fitted interaction constants. A more direct evaluation of the ethylbenzene model was explored by comparing predictions of the human model (allometrically scaled from the rat) to ethylbenzene concentrations in blood and in exhaled air observed in human subjects who were exposed to ethylbenzene (33 ppm) for 7 hours/day on 4 different days (Tardif et al. 1991, 1997). Predicted blood and exhaled air concentrations of ethylbenzene (at steady-state and following cessation of exposure) in subjects exposed to ethylbenzene were within 1–2 SD of observations. The Tardif et al. (1997) model has been incorporated into a mixtures model of JP-8 vapor and its components (Campbell and Fisher 2007). The model included simulations of the kinetics of m-xylene and ethylbenzene, with other aromatic components of JP-8 vapor represented as a lumped component. Interactions between *m*-xylene and ethylbenzene were simulated as competitive inhibition of metabolism. The mixtures model predicted kinetics liver concentrations of ethylbenzene in rats during and following 4-hour exposures to JP-8 vapor (380–2,700 mg/m^3) that were similar to observed kinetics (i.e., predicted liver concentrations were within 1–2 SD of observations).

Risk Assessment. The ethylbenzene model predicts blood kinetics of ethylbenzene and kinetics of metabolism of ethylbenzene that occur in association with inhalation exposures. These predictions are

potentially useful for predicting inhalation-derived internal doses of ethylbenzene in rats and/or humans (e.g., blood concentrations, liver concentrations of parent compound, or amounts of total metabolites formed in the liver), and for making extrapolations of these internal dose metrics across species. The applicability any of the above dose metrics to risk assessment will depend on the mechanism for the specific toxicity end point being assessed, and contribution of the parent compound and/or metabolism metabolites to toxicity (see Section 3.5.2, Mechanisms of Toxicity).

The Tardif et al. (1997) ethylbenzene model has been incorporated into a mixtures model for alkylbenzenes (ethylbenzene, toluene, *m*-xylene), benzene, and dichloromethane (DCM), (Haddad et al. 2001). This mixture model has been applied to the derivation of interaction coefficients for various endpoints of dichloromethane toxicity (i.e., hypoxia, central nervous system effects, and cancer) based on predictions of corresponding relevant DCM internal dose metrics (i.e., carboxyhemoglobin concentration, area under concentration-time curve for DCM in richly perfused tissues; time-integrated amount of DCM conjugated with GSH). Interactions were simulated as dose addition, with internal dose metrics representing dose and the sole interaction mechanism assumed to be competitive inhibition of cytochrome P-450-mediated metabolism of DCM. The Tardif et al. (1997) model also has been incorporated into a mixtures model of JP-8 vapor and its components (Campbell and Fisher 2007). The model included simulations of the kinetics of *m*-xylene and ethylbenzene, with other aromatic components of JP-8 vapor represented as a lumped component. Interactions between *m*-xylene and ethylbenzene were simulated as competitive inhibition of metabolism. The Tardif et al. (1997) mixtures model was also utilized to evaluate occupational exposure limits for ethylbenzene, toluene, *m*-xylene for hypothetical exposures to ternary mixtures of the chemicals (Dennison et al. 2005). These analyses demonstrate the potential utility of the ethylbenzene model for predicting metabolic interactions with other chemicals that share a common mechanism of metabolic elimination, and the potential impacts of such interactions on risk (e.g., hazard index, cancer risk, occupation exposure limits).

Target Tissues. The Tardif et al. (1997) ethylbenzene model was calibrated and evaluated to predict blood kinetics of ethylbenzene that occur in association with inhalation exposures. Essential to this prediction is the accurate prediction of rates of metabolism of ethylbenzene, which, in the model, is attributed solely to the liver. The model has potential utility for predicting, in addition to blood kinetics of ethylbenzene, kinetics of concentrations of ethylbenzene in important toxicity target tissues, including liver and richly perfused tissues (which includes kidney and central nervous system), and rates of metabolism of ethylbenzene and total amount of metabolites formed in liver. The model does not simulate, specifically, the kidney or central nervous system.

Species Extrapolation. The Tardif et al. (1997) model was initially developed and calibrated to simulate kinetics of inhaled ethylbenzene in the rat, and was allometrically scaled to the human. The scaled human model was evaluated for predicting ethylbenzene blood concentrations observed in human subjects who were exposed to ethylbenzene (33 ppm) for 7 hours/day on 4 different days (Tardif et al. 1991). Predicted blood concentrations of ethylbenzene in subjects exposed to ethylbenzene were within 1–2 SD of observations. Studies of the robustness of the model for predicting ethylbenzene kinetics in other species (after allometric scaling to those species) were not located.

Interroute Extrapolation. The Tardiff et al. (1997) model was developed to simulate kinetics of inhaled ethylbenzene in rats and humans. An extension of the model that includes a gastrointestinal absorption rate constant enables simulation of gavage dosing in rats administered ethylbenzene in corn oil (Faber et al. 2006). The gastrointestinal model has not been evaluated for applications to human oral exposures. Studies that evaluated the model for predicting ethylbenzene kinetics following dermal exposure were not located.

The Nong et al. (2007) Model

Description of the Model. Nong et al. (2007) developed a PBPK model for simulating the kinetics of inhalation exposures of ethylbenzene in mice. The structure of the model is essentially identical to the Tardif et al. (1997) model (Figure 3-4, with tissue compartments limited to lungs, liver, fat, richly perfused tissues, and poorly perfused tissues), with the addition of metabolism of ethylbenzene in richly and poorly perfused tissues (K_m, V_{max}). Parameter values are presented in Table 3-7. Values for V_{max} in naive (not induced) liver were derived my allometric scaling ($BW^{0.75}$) of values estimated for the rat and values for K_m in liver were assumed to be the same as in the rat (Haddad et al. 2000). The value for V_{max} was subsequently optimized against data on venous blood kinetics in mice exposed to ethylbenzene concentrations ranging from 75 to 1,000 ppm (Nong et al. 2007). The model overpredicted observations in animals exposed to 750 ppm; adequate fit was achieved by upward adjustment of the V_{max} by a factor of 3. This adjustment achieved adequate fit to blood kinetics observed in animal repeatedly exposed (12 days) to 75 or 750 ppm and was adopted to account for induction of cytochrome P450 in animals exposed repeatedly to ethylbenzene at concentrations >75 ppm (Nong et al. 2007). The value for V_{max} in lung was derived from estimates made in *in vitro* preparation of mouse lung microsomes (Nong et al. 2007) and was scaled to lung weight. Values for K_m in lung and V_{max} and K_m in richly perfused tissues were derived by optimization against data on venous blood kinetics in mice exposed to ethylbenzene

Table 3-7. Parameter Values for Nong et al. (2007) Ethylbenzene Mouse Physiologically-based Pharmacokinetic Model

Parameter	Definition	Male	Female
Physiological parameters			
VLC	Liver volume (fraction of body)	0.06	0.06
VLUC	Lung volume (fraction of body)	0.0073	0.0073
VFC	Fat volume (fraction of body)	0.1	0.1
VSC	Slowly-perfused tissue volume (fraction of body)	0.70	0.70
VRC	Richly-perfused tissue volume (fraction of body)	0.05	0.05
QCC	Cardiac output (L/hour-kg BW)	24[a]	24[a]
QPC	Alveolar ventilation rate (L/hour-kg body weight)	16[a]	16[a]
QLC	Liver blood flow (fraction of cardiac output)	0.25	0.25
QLUC	Lung blood flow (fraction of cardiac output)	1.00	1.00
QFC	Fat blood flow (fraction of cardiac output)	0.09	0.09
QSC	Slowly-perfused blood flow (fraction of cardiac output)	0.15	0.15
QRC	Richly-perfused blood flow (fraction of cardiac output)	0.51	0.51
Chemical parameters			
PB	Blood:air partition coefficient	42.8	65.4
PL	Liver:blood partition coefficient	1.38[b] (72.9)	1.12[b] (72.9)
PLU	Lung:blood partition coefficient	1.21[b] (63.8)	0.98[b] (63.8)
PF	Fat:blood partition coefficient	26.84[b] (1417)	21.67[b] (1417)
PS	Slowly-perfused partition coefficient	0.86[b] (45.6)	0.70[b] (45.6)
PR	Richly-perfused partition coefficient	1.38[b,c] (72.9)	1.12[b,c] (72.9)
V_{max}CL	Maximum rate of metabolism in liver for exposures ≤75 ppm (mg/hour-kg body weight)	7.3[d]	7.3[d]
K_mCL	Michaelis-Menten coefficient for metabolism in liver (mg/L)	1.04	1.04
V_{max}CL	Maximum rate of metabolism in liver for repeated exposures >75 ppm (mg/hour-kg body weight)	19.2[d]	19.2[d]
K_mCL	Michaelis-Menten coefficient for metabolism in liver (mg/L)	1.04	1.04
V_{max}CLU	Maximum rate of metabolism in lung (mg/hour-kg body weight)	13.4[d]	13.4[d]
K_mCLU	Michaelis-Menten coefficient for metabolism in lung (mg/L)	5.57	4.35

Table 3-7. Parameter Values for Nong et al. (2007) Ethylbenzene Mouse Physiologically-based Pharmacokinetic Model

Parameter	Definition	Male	Female
V_{max}CRP	Maximum rate of metabolism in rapidly perfused tissue (mg/hour-kg body weight)	17.4[d]	12.9[d]
K_mCRP	Michaelis-Menten coefficient for metabolism in rapidly perfused tissue (mg/L)	2.33	1.15

[a]Scaled to body weight ($BW^{0.75}$).
[b]Tissue:blood partition coefficients calculated based on reported, experimentally determined (vial equilibrium), tissue:air partition coefficients for rats (values in parentheses) and blood:air coefficient (PB) measured in rat or human blood; tissue:blood=tissue:air/blood:air.
[c]Values shown are for liver; values reported for tissue:air were as follows: brain, 51.44; heart, 61.16; and kidney, 68.53 corresponding values for tissue:blood were: brain, 0.97 (male), 0.79, female); heart, 1.16 (male), 0.94 (female); and kidney, 1.30 (male), 1.05 (female).
[d]Scaled to body weight ($BW^{0.74}$).

concentrations ranging from 75 to 1,000 ppm (Nong et al. 2007). Tissue:air partition coefficients for ethylbenzene were derived from vial equilibrium studies of isolated tissues, and included blood:air coefficients determined in male (52.8±3 standard error [SE]) and female (65.4±5 SE) mice. Tissue:blood partition coefficients for male and female mice were derived from measured gender-specific blood:air partition coefficients and tissue:air coefficitent (i.e., tissue:blood=tissue:air/blood:air). Physiological parameters, cardiac output (QC) and alveolar ventilation rate (QP), and metabolism V_{max} were allometrically scaled as a function of body weight.

Validation of the Model. Metabolism parameters were optimized against observed blood kinetics of ethylbenzene in mice exposed for 4 hours to 75 or 1,000 ppm ethylbenzene. The optimized model was evaluated against observations of blood, liver, lung, and fat concentrations of ethylbenzene in male or female mice repeatedly exposed to 750 ppm ethylbenzene (12 days, 6 hours/day) and predictions were within 1–2 SDs of the observations.

Risk Assessment. The Nong et al. (2007) model predicts blood kinetics of ethylbenzene and kinetics of metabolism of ethylbenzene that occur in association with inhalation exposures. These predictions are potentially useful for predicting inhalation-derived internal doses of ethylbenzene in mice and/or humans (e.g., blood concentrations, liver concentrations of parent compound, or amounts of total metabolites formed in the liver), and for making extrapolations of these internal dose metrics across species. However, a human model that includes simulation of metabolism of ethylbenzene in lung and rapidly perfused tissue has not been reported. The applicability any of the above dose metrics to risk assessment will depend on the mechanism for the specific toxicity end point being assessed, and contribution of the parent compound and/or metabolism metabolites to toxicity (see Section 3.5.2, Mechanisms of Toxicity).

Target Tissues. The Nong et al. (2007) ethylbenzene model was calibrated and evaluated to predict blood, liver, lung, and fat kinetics of ethylbenzene that occur in association with inhalation exposures to mice. The model has potential utility for predicting, in addition to blood kinetics of ethylbenzene, kinetics of concentrations of ethylbenzene in important toxicity target tissues, including liver and richly perfused tissues (which includes kidney and central nervous system), and rates of metabolism of ethylbenzene and total amount of metabolites formed in liver, lung, and other rapidly perfused tissues (e.g., brain, heart, kidney). The model does not simulate, specifically, the kidney or central nervous system.

Species Extrapolation. The Nong et al. (2007) model was initially developed and calibrated to simulate ethylbenzene kinetics in the mouse. The model could be allometrically scaled to other species (e.g., humans). Studies of the robustness of the model for predicting ethylbenzene kinetics in other species (after allometric scaling to those species) were not located.

Interroute Extrapolation. The Nong et al. (2007) model was developed to simulate kinetics of inhaled ethylbenzene. Studies that evaluated the model for predicting ethylbenzene kinetics following oral or dermal exposure were not located.

The Dennison et al. (2003, 2004) Model

Description of the Model. Dennison et al. (2003, 2004) developed a PBPK model for simulating the blood and elimination kinetics of components of gasoline, including benzene, ethylbenzene, *n*-hexane, toluene, *o*-xylene, and other volatile components (represented as a single lumped composition) in rats. The structure of the model is essentially identical the generic model depicted in Figure 3-4, with tissue compartments limited to lungs, liver, fat, richly perfused tissues (RPT), and slowly perfused tissues (SPT). All metabolism was attributed to the liver and represented as functions of the concentration of parent compound in liver venous blood, affinity constant (K_m), and maximum reaction velocity (V_{max}). Transfers of parent compound between blood and tissues were assumed to be flow-limited, with clearance represented by tissue blood flow (L/hour). Parameters for the ethylbenzene portion of the model are presented in Table 3-8. Partition coefficients for ethylbenzene were derived from vial equilibrium studies of isolated rat tissues (Tardif et al. 1997). Metabolism parameter values for ethylbenzene were derived by fitting the model to observations of blood ethylbenzene kinetics measured in rats during closed-chamber exposure to ethylbenzene (approximately 2,000 ppm starting chamber concentration; Dennison et al. 2003). Physiological parameters, cardiac output (QC), and alveolar ventilation rate (QP), and metabolism V_{max} were allometrically scaled to body weight (BW, scaling factor=$BW^{0.74}$)

Validation of the Model. Optimization of the metabolism parameters for rats against observed closed chamber air concentration kinetics achieved predicted elimination kinetics of ethylbenzene that were similar to observations (Dennison et al. 2003). Validation efforts reported in Dennison et al. (2004) were directed at exploring how well the mixture model predicted observed closed chamber air kinetics of ethylbenzene, toluene, or xylene during exposures of rats to gasoline (1, 10, 100, 1,000 ppm). Interactions were simulated as competitive inhibition of metabolism, with the values of the inhibition constants derived by fitting to chamber air concentration kinetics during exposures to binary mixtures.

Table 3-8. Parameter Values for Dennison et al. (2003) Ethylbenzene PBPK Model

Parameter	Definition	Rat model
Physiological parameters		
VLC	Liver volume (fraction of body)	0.037
VFC	Fat volume (fraction of body, scaled to body weight)	0.036(body weight)+0.205
VSC	Slowly-perfused tissue volume (fraction of body)	0.91-remaining[a]
VRC	Richly-perfused tissue volume (fraction of body)	0.054
VLBV	Lung blood volume (fraction of body)	0.002
QCC	Cardiac output (L/hour-kg body weight)	15[b]
QPC	Alveolar ventilation rate (L/hour-kg body weight)	15[b,c]
QLC	Liver blood flow (fraction of cardiac output)	0.183
QSC	Fat blood flow (fraction of cardiac output)	0.07
QFC	Slowly-perfused blood flow (fraction of cardiac output)	0.237[d]
QRC	Richly-perfused blood flow (fraction of cardiac output)	0.51
Chemical parameters		
PB	Blood:air partition coefficient	42.7[e]
PL	Liver:blood partition coefficient	1.96[e]
PF	Fat:blood partition coefficient	36.4[e]
PS	Slowly-perfused partition coefficient	0.609[e]
PR	Readily-perfused partition coefficient	1.96
$V_{max}C$	Maximum rate of metabolism (mg/hour-kg body weight)	7.6[b,f]
K_mC	Michaelis-Menten coefficient for metabolism (mg/L)	0.10[f]

[a]VSC=0.91-(VLC+VFC+VRC+VLB)
[b]Scaled to body weight ($BW^{0.74}$)
[c]QPC varied with exposure: 14.9 at 500 ppm, 13.1 at 1,000 ppm, and 12.5 at 1,500 ppm.
[d]QSC=1-(QLC+QFC+QRC)
[e]Tissue:blood partition coefficients calculated based on reported, experimentally determined (vial equilibrium) tissue:air partition coefficients for rats (Tardif et al. 1997).
[f]Values derived by optimization to data on blood ethylbenzene concentrations in rats exposed to ethylbenzene (closed chamber).

Although the mixture model achieved predictions similar to observations, this outcome would have been highly influenced by the parameter values selected for each of the individual chemicals (or lumped chemicals), including fitted interaction constants and, therefore, does not directly address the validity of the ethylbenzene model.

Risk Assessment. The Dennison et al. (2003) ethylbenzene model predicts blood kinetics of ethylbenzene and kinetics of metabolism of inhaled ethylbenzene. These predictions are potentially useful for predicting internal doses of inhaled ethylbenzene in rats (e.g., blood concentrations, liver concentrations of parent compound, or amounts of total metabolites formed in the liver). The applicability any of the above dose metrics to risk assessment will depend on the mechanism for the specific toxicity endpoint being assessed, and contribution of the parent compound and/or metabolism to toxicity. Current knowledge of the mechanism of toxicity of ethylbenzene does not include an understanding of the relative contributions of parent compound or metabolites as proximate toxic agents in the major end points of ethylbenzene toxicity (i.e., kidney, liver, otic, cancer).

The Dennison et al. (2003) ethylbenzene model has been incorporated into a mixtures model for gasoline (benzene, ethylbenzene, *n*-hexane, toluene, *o*-xylene, and other volatile components). In the mixtures model, chemical interactions were attributed to competitive inhibition of metabolism and inhibition constants were derived by fitting model predictions of closed chamber air concentration kinetics during exposures to binary mixtures. The model was used to predict the effect of increasing exposure concentration to gasoline on blood concentrations and amounts of individual mixture components metabolized (Dennison et al. 2004).

Target Tissues. The Dennison et al. (2003) ethylbenzene model was calibrated and evaluated to predict elimination (i.e., metabolism) kinetics of ethylbenzene that occur in association with inhalation exposures. Essential to this prediction is the accurate prediction of rates of metabolism of ethylbenzene, which, in the model, is attributed solely to the liver. The model has potential utility for predicting, in addition to elimination kinetics of ethylbenzene, kinetics of concentrations of ethylbenzene in important toxicity target tissues, including liver, richly perfused tissues (which includes kidney and central nervous system), and rates of metabolism of ethylbenzene and total amount of metabolites formed in liver. The model does not simulate, specifically, the kidney or central nervous system.

Species Extrapolation. The Dennison et al. (2003) ethylbenzene model was developed and calibrated to simulate ethylbenzene kinetics in the rat; however, it could be allometrically scaled to the

human. Studies of the robustness of the model for predicting ethylbenzene kinetics in species other than the rat (after allometric scaling to those species) were not located.

Interroute Extrapolation. Studies that evaluated the model for predicting ethylbenzene kinetics following oral or dermal exposure were not located.

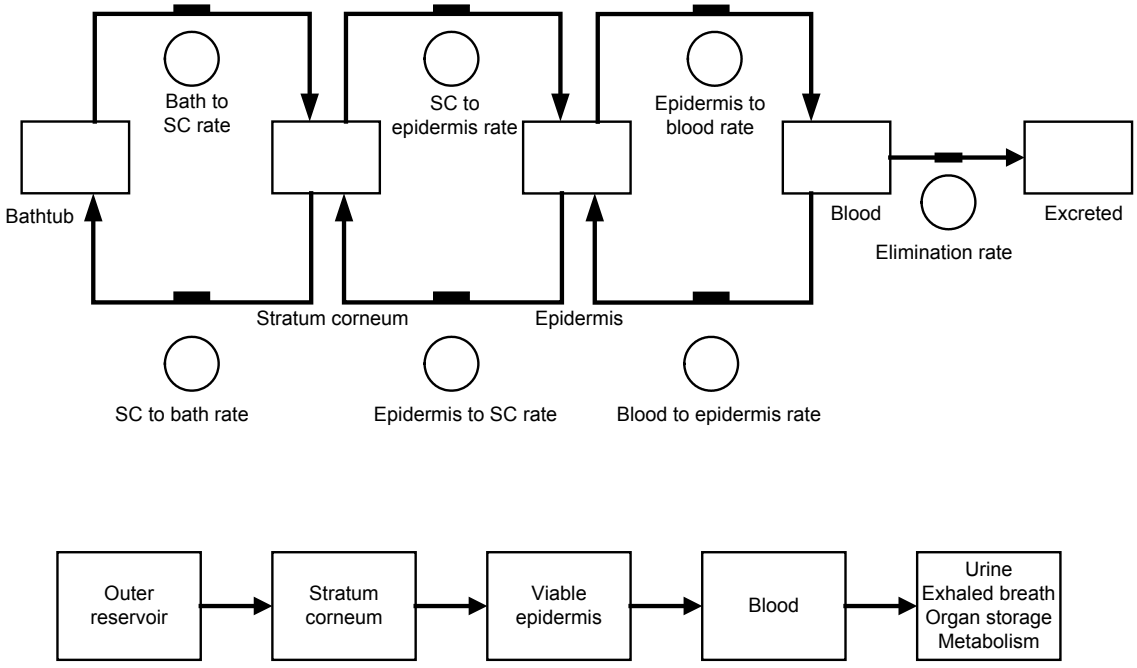
The Shatkin and Brown (1991) Model

Description of the Model. 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 structure of the model is depicted in Figure 3-5; model parameters are presented in Table 3-9. The model includes three compartments: stratum corneum, viable epidermis, and blood. Transfers of ethylbenzene in solution through the fully hydrated stratum corneum and viable epidermis are assumed to be diffusive, with passage through the stratum corneum being the rate-limiting step. A uniform thickness of 40 μm was assumed for the stratum corneum, with adjustments for different body parts. Immersion of the hand or of the full body was assumed for the predicted models. The viable epidermis was assumed to be 200 μm, although the thickness was varied to test the outcome of the model. Transfer from the viable epidermis to blood was assumed to be flow-limited. Elimination from blood (i.e., distribution to tissues, excretion to urine and air, and metabolism) was represented with a single first-order rate constant. A sensitivity analysis revealed a relatively high influence of epidermal blood flow (as expected for flow-limited transfer to blood), epidermal thickness (increasing thickness decreased absorption), and stratum corneum fat (increasing fat content decreased absorption).

Validation of the Model. Model predictions were compared to the estimates of dermal absorption of ethylbenzene in humans (Dutkiewicz and Tyras 1967, 1968). In this study, adult males (n=7) immersed their hands into a bath solution of 151 mg/L ethylbenzene for 1 hour and the absorbed dose was estimated from the change in concentration of the exposure bath. The model predicted absorption of 34–37% of the bath ethylbenzene in 1 hour, 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.

Risk Assessment. The Shatkin and Brown (1991) model is potentially useful for predicting dermal absorption of ethylbenzene in risk assessment applications. An example of one application is prediction of the dermal-absorbed dose of ethylbenzene for a bathing scenario (20-minute immersion in 0.1 mg ethylbenzene/L); the predicted absorbed dose, 0.47–0.50 mg, was higher than the absorbed dose predicted

Figure 3-5. Schematic Representation of the Model of Dermal Absorption



Source: Shatkin and Brown 1991

Table 3-9. Parameters Used in the Shatkin and Brown PBPK Model of Dermal Absorption of Ethylbenzene

Parameter[a]	Value	Reference
Stratum corneum/water partition coefficient (K_m)	NG	Calculated from Roberts et al. 1975
Stratum corneum diffusion coefficient (D_{sc})	NG	Calculated from Guy and Maibach 1984
Skin surface	320 cm^2 (adult, hands and forearms)	Dutkiewicz and Tyras 1967, 1968
	NG (adult, body)	Guy and Maibach 1984
Skin surface (infant)	1,900 cm^2	Guy and Maibach 1984
Epidermis diffusion coefficient (D_e)	$3.6x10^{-4}$ cm^2/minute	Scheuplein 1976; Scheuplein et al. 1969
Stratum corneum thickness (H_{sc})	0.004 cm	Blank and Scheuplein 1969
Epidermis thickness (H_e)	0.02–0.1 cm	Blank and McAuliffe 1985; Blank and Scheuplein 1969; Guy et al. 1982
Epidermal blood flow (F_{eb}) (adult, at rest)	280 mL/minute–m^2	Wade et al. 1962
Epidermal blood flow (F_{eb}) (adult, heavy exercise)	4,000 mL/minute–m^2	Rowell 1986
Epidermis/blood partition coefficient (K_{eb})	2.75	Shatkin and Brown 1991
Stratum corneum/epidermis partition coefficient ($K_{sc/e}$)	NG	Shatkin and Brown 1991
Blood volume (V_b) (adults)	5,000 mL	Shatkin and Brown 1991
Blood volume (V_b) (infants)	693 mL	Shatkin and Brown 1991
Fat in blood	0.7–0.9%	Brown and Hattis 1989
Fat in stratum corneum	3–6%	Raykar et al. 1988
Fat in epidermis	2–2.5%	Scheuplein 1976
Elimination rate constant (K_e)	0.1 $minute^{-1}$	Hagemann 1979
Octanol/water partition coefficient (K_{ow})	2,230	Shatkin and Brown 1991

[a]Taken from Shatkin and Brown 1991. All parameters used were either taken from published experimental work of others or calculated from previously reported mathematical relationships.

NG = value not given

for ingestion of 2 L of water at the same concentration, or from inhalation during a 20-minute shower (Shatkin and Brown 1991). The model may be useful in predicting absorbed doses of ethylbenzene resulting from bathing, swimming, and other activities (Shatkin and Brown 1991).

Target Tissues. The Shatkin and Brown (1991) model was developed to simulate dermal absorption of ethylbenzene (and other volatile organics); it does not predict concentrations of ethylbenzene in specific tissues, other than skin. However, if the model were integrated into PBPK models of the distribution and elimination of absorbed ethylbenzene (e.g., Dennison et al. 2003; Tardif et al. 1997), the combined models might be useful for predicting internal doses of ethylbenzene associated with dermal exposures.

Species Extrapolation. The Shatkin and Brown (1991) model was developed to simulate dermal absorption of ethylbenzene in humans and has been evaluated with observations in humans. The major physiological parameters in the model are scalable or could be determined in different species (e.g., dermal thickness, dermal fat content, dermal blood flow), and the chemical parameters could be evaluated in other species (e.g., partition coefficients) or predicted from physical-chemical properties (e.g., diffusion coefficients). These features improve the feasibility of scaling the model to other species.

Interroute Extrapolation. The Shatkin and Brown (1991) model, in combination with existing PBPK ethylbenzene inhalation models (e.g., Dennison et al. 2003; Tardif et al. 1997), may have utility for estimating dermal exposures (i.e., concentration, time) that would be expected to yield equivalent absorbed doses from inhalation exposures.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Studies of the *in vitro* metabolism of ethylbenzene in microsomes prepared from human liver have identified high and low affinity catalytic pathways for the initial hydroxylation to 1-phenylethanol (Sams et al. 2004). In human liver microsomes, the high affinity pathway exhibited a lower K_m and lower V_{max} (K_m=8 μM, V_{max}=689 pmol/minute/mg protein) than the low affinity pathway (K_m=391 μM, V_{max}=3,039 pmol/min/mg protein) and was inhibited by diethyldithiocarbamate. Studies conducted in microsomes prepared from insect cells expressing recombinant human isoforms of cytochrome P-450 also revealed a relatively high affinity, low V_{max} catalysis by CYP2E1and lower affinity, higher V_{max} catalysis

by CYP1A2 and CYP2B6, suggesting that the latter two isoforms may contribute to the low affinity pathway observed in human liver microsome (Sams et al. 2004).

Blood kinetics of inhaled ethylbenzene have been successfully modeled with assumptions of flow-limited transfer of ethylbenzene to tissues and capacity-limited elimination by metabolism (Dennison et al. 2003, 2004; Tardif et al. 1997, see Section 3.4.5). These models have been successfully scaled from the rat to humans by applying species physiological parameter values (e.g., tissue volumes) and partition coefficients (i.e., blood:air partition coefficient) and allometrically scaling flows (i.e., cardiac output, alveolar ventilation rate) and metabolism parameters (i.e., K_m, V_{max}) to body weight (Tardif et al. 1997). These studies suggest a general similarity of the distribution and elimination kinetics in rats and humans. The robustness of these models for predicting ethylbenzene kinetics in other species has not been reported; however, the blood elimination kinetics of inhaled ethylbenzene in the mouse show similarities to that in the rat (e.g., nonlinearity of clearance with exposure concentration, similar elimination half-times (Charest-Tardif et al. 2006; Tardif et al. 1997).

3.5.2 Mechanisms of Toxicity

Mechanisms of ototoxicity, toxicity to the liver and kidney, and carcinogenicity have not been identified. However, studies on ethylbenzene and ethylbenzene metabolites provide some insights regarding the potential roles of parent compound and metabolites in ethylbenzene-induced effects. As reviewed in Section 3.2.1.4 (Inhalation Exposure, Neurological Effects), inhalation exposure of animals to ethylbenzene produces hearing loss through irreversible loss of OHC in the organ of corti (Cappaert et al. 1999, 2000, 2001, 2002; Gagnaire and Langali 2005; Gagnaire et al. 2007). Cappaert et al. (2002) attributed the lack of ototoxicity (based on auditory thresholds and histological assessment of cochlea) in guinea pigs exposed to inhaled ethylbenzene to lower circulating levels of ethylbenzene, relative to levels producing ototoxicity in rats. Results of a 3-month oral study on phenylglyoxylic acid, a major ethylbenzene metabolite, show that this metabolite did not produce ototoxicity, based on electrophysiological tests, in rats exposed to drinking water at approximately 293 mg/kg/day (Ladefoged et al. 1998). Although this study provides supporting evidence that phenylglyoxylic acid is not ototoxic, animals were not evaluated for OHC loss in this study. Furthermore, Pryor et al. (1991) proposed that hearing loss caused by toluene, which is structurally similar to ethylbenzene, was caused by parent compound, rather than metabolites; pretreatment of rats with phenobarbital, which induces cytochrome P450 metabolism of toluene, prevented ototoxicity. Taken together, results of these studies suggest that ethylbenzene, rather than metabolites, may be responsible for ototoxicity.

Regarding possible mechanisms of carcinogenicity, results of the NTP (1999) bioassay on ethylbenzene provided clear evidence of carcinogenicity in male rats based on renal tubule neoplasms, some evidence in female rats based on renal tubule adenomas, some evidence in male mice based on alveolar/bronchiolar neoplasms, and some evidence in female mice based on hepatocellular neoplasms; in addition, testicular neoplasms were increased in male rats. In contrast, results of an NTP (1990) 2-year bioassay on 1-phenylethanol, a primary oxidative metabolite of ethylbenzene, provided some evidence of carcinogenicity in male rats based on increased incidences of renal tubular cell adenomas and adenomas or adenocarcinoma (combined), but no evidence of carcinogenicity in female rats or male or female mice. These results suggest that carcinogenic activity of ethylbenzene may be, at least in part, attributed to the parent compound and/or reactive oxidative metabolites in the 4-ethylphenol pathway, rather than the 1-phenylethanol pathway.

Ethylbenzene has been shown to exert adverse central nervous system effects on both humans (Yant et al. 1930) and animals (Cragg et al. 1989; Ethylbenzene Producers Association 1986a; Molnar et al. 1986; Tegeris and Balster 1994; Yant et al. 1930). *In vivo* animal studies of ethylbenzene toxicity at the cellular level indicate that changes in brain levels of dopamine and other biochemical alterations, and in evoked electrical activity in the brain may be involved in ethylbenzene central nervous system toxicity (Andersson et al. 1981; Frantik et al. 1994; Mutti et al. 1988; Romanelli et al. 1986).

The molecular mechanism(s) of ethylbenzene-induced ototoxicity has not been established. Results of a recent *in vitro* study suggest that ototoxicity induced by low concentrations of ethylbenzene may be mediated through nicotinic acetylcholine receptors (van Kleef et al. 2008). Using human heteromeric α9α10 nicotinic acetylcholine receptors expressed in *Xenopus* oocytes, ethylbenzene inhibited acetylchloline-mediated ion currents under conditions of low receptor occupancy. Based on *in vitro* studies conducted with toluene, Cappaert et al. (2001) briefly speculated that increased intracellular calcium levels might be responsible for the obliteration of outer hair cells exposed to solvents such as ethylbenzene.

In vitro studies of the mechanism of toxicity have focused on the effect of ethylbenzene on cell membranes, particularly that of the astrocyte (Engelke et al. 1993; Naskali et al. 1993, 1994; Sikkema et al. 1995; Vaalavirta and Tähti 1995a, 1995b). In a review by Sikkema et al. (1995), changes in the structure and integrity of the cell membrane after partitioning of ethylbenzene into the lipid bilayer may be a mechanism of toxicity. Changes in the integrity of the cell membrane may subsequently affect the

function of membrane, particularly as a barrier and in energy transduction, and in the formation of a matrix for proteins and enzymes.

The work of Vaalavirta and Tähti (1995a, 1995b) and Naskali et al. (1993, 1994) has investigated the effect of ethylbenzene on the membrane of the rat astrocyte, as an *in vitro* model for the membrane-mediated effects of solvents on the central nervous system. Cultured astrocytes from the cerebella of neonatal Sprague-Dawley rats were sensitive to the effects of ethylbenzene, as measured by the inhibition of activity of Na+, K+-ATPase, and Mg++-ATPase (Vaalavirta and Tähti 1995a, 1995b). This effect was found to be dose-dependent (Naskali et al. 1994). Inhibition of these membrane-bound enzymes that regulate the ion channels of the membrane may disturb the ability of the cells to maintain homeostasis. Experiments with rat synaptosome preparations, similar to those using microsomal preparations by Engelke et al. (1993), showed that membrane fluidity was increased after exposure to ethylbenzene. ATPase and acetylcholinesterase activity were also decreased, as seen in the astrocyte preparations.

3.5.3 Animal-to-Human Extrapolations

Species differences have been shown for ethylbenzene metabolism. In humans exposed via inhalation, the major metabolites of ethylbenzene are mandelic acid (approximately 70% of the absorbed dose) and phenylglyoxylic acid (approximately 25% of the absorbed dose), which are excreted in the urine (Bardodej and Bardodejova 1970; Engstrom et al. 1984). Evidence indicates that the initial step in this metabolic pathway is oxidation of the side chain of ethylbenzene to produce 1-phenylethanol. In rats exposed by inhalation or orally to ethylbenzene, the major metabolites were identified as hippuric and benzoic acids (approximately 38%), 1-phenylethanol (approximately 25%), and mandelic acid (approximately 15–23%), with phenylglyoxylic acid making up only 10% of the metabolites (Climie et al. 1983; Engstrom et al. 1984, 1985). In rabbits, the most important metabolite is hippuric acid, which is probably formed by oxidative decarboxylation of phenylglyoxylic acid (El Masri et al. 1958). Rabbits have been shown to excrete higher levels of glucuonidated metabolites than do humans or rats (El Masry et al. 1956; Smith et al. 1954a, 1954b). Thus, there are no animal models of ethylbenzene metabolism that are completely consistent with human metabolism. However, of the experimental models investigated, rats appear to be a more appropriate model than rabbits.

Models of the pharmacokinetic mechanisms and mechanisms of toxicity of ethylbenzene have focused on cellular processes (see Sections 3.5.1 and 3.5.2, above). In these, humans and animals appear to be similar. Although some species differences exist with respect to toxicity, adverse effects observed after

ethylbenzene exposure in both humans and animals seem to be similar in scope (i.e., respiratory, hepatic, renal, and neurological). Rats may be more sensitive than mice or rabbits (Cragg et al. 1989; NTP 1992). Thus, the rat may be the most appropriate animal model for studying the mechanism of toxicity of ethylbenzene as it relates to human health effects assessment.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997b). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No studies were located regarding endocrine disruption in humans or animals after exposure to ethylbenzene. Nishihara et al. (2000) concluded that ethylbenzene was not estrogenic as established in an *in vitro* yeast two-hybrid assay. No additional *in vitro* studies were located regarding endocrine disruption of ethylbenzene.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and

sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

There are no data describing the effect of exposure to ethylbenzene in children. Respiratory and eye irritation, and dizziness are the most prevalent signs of exposure to high levels of ethylbenzene (Yant et al. 1930), and it is expected that children would also exhibit these effects, as well as other effects observed in adults. Minor birth defects have occurred in newborn rats, but not rabbits, whose mothers were exposed by breathing air contaminated with ethylbenzene (NIOSH 1981; Ungváry and Tátrai 1985). These defects consisted of urinary tract anomalies (not specified) and supernumerary ribs. Supernumerary ribs were observed in the presence of minimal maternal changes. Although developmental effects were reported in the offspring of animals exposed to <500 ppm ethylbenzene (Ungváry and Tátrai 1985) several longer duration studies have shown developmental effects at biologically or statistically significant levels only in the offspring of rats exposed to >500 ppm ethylbenzene (Faber et al. 2006, 2007; Saillenfait et al. 2003, 2006, 2007). Furthermore, the report by Ungváry and Tátrai (1985) lacks pertinent experimental details, including specific data on the urinary tract anomalies, dictating caution in the interpretation of study findings. Section 3.2.1.6, Developmental Effects, contains a more detailed discussion of these results. It is not known whether these developmental effects observed in animals would be observed in people. Ethylbenzene has been detected in human breast milk at unspecified concentrations (Pellizzari et al. 1982), but no pharmacokinetic experiments have been done to confirm that it is actually transferred to breast milk in mammals. No specific information was found concerning ethylbenzene concentrations in placenta, cord blood, or amniotic fluid.

Since there is no information about health effects in children, it is unknown whether they differ from adults in their susceptibility to health effects from ethylbenzene. However, in general, the principle that early-in-life exposures may increases susceptibility to carcinogens may apply to ethylbenzene (EPA 2005).

There is no specific information about the metabolism of ethylbenzene in children or immature adults. However, since two of the enzyme families responsible for the conjugation and elimination of ethylbenzene metabolites are developmentally regulated, it is possible that the activity of these enzymes would differ in children or immature animals compared to adults. In humans, UDP glucuronosyltransferase activity does not reach adult levels until about 6–18 months of age, although the development of this activity is isoform specific. Activity of sulfotransferases (which is also isoform specific) seems to develop earlier. The activity of some sulfotransferase isoforms may even be greater during infancy and early childhood than in adulthood (Leeder and Kearns 1997). In addition, age-dependence of elimination kinetics (e.g., glomerular filtration and tubular secretion of organic anion metabolites of ethylbenzene), potentially, could contribute to age-related differences in sensitivity to ethylbenzene.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids

(e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to ethylbenzene are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by ethylbenzene are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Ethylbenzene

Information on ethylbenzene concentrations in human tissue or fluids is available. Exposure to ethylbenzene may be determined by the detection of mandelic acid and phenylglyoxylic acid in urine (Bardodej and Bardodejova 1970) or by direct detection of ethylbenzene in whole human blood (Antoine et al. 1986; Ashley et al. 1992; Cramer et al. 1988) or urine (Wang et al. 2007). However, mandelic acid and phenylglyoxilic acid are also metabolites of styrene (Agency for Toxic Substances and Disease Registry 1992).

The 1982 National Human Adipose Tissue Survey conducted by EPA measured ethylbenzene in 96% of the 46 composite samples analyzed for volatile organic compounds (EPA 1986). A wet tissue concentration range of not detected (detection limit=2 ng/g) to 280 ng/g was reported, but an average concentration was not provided.

Numerous studies indicate that environmental exposures to ethylbenzene can result in detectable levels in human tissues (Antoine et al. 1986; Cramer et al. 1988; Pellizzari et al. 1982; Wolff 1976; Wolff et

al. 1977) and in expired air (Conkle et al. 1975; Engstrom and Bjurstrom 1978; EPA 1984e). Analysis of blood specimens from a test population of 250 patients (Antoine et al. 1986) and composite samples obtained from blood donations of laboratory personnel with potentially low-level exposure (Cramer et al. 1988) indicated ethylbenzene concentrations in the blood to range from below detection limits to 59 ppb. Similarly, ethylbenzene was detected in 8 of 12 milk samples from lactating women living in various urban areas of the United States with high probability of emissions of pollutants (Pellizzari et al. 1982). Subcutaneous fat samples taken from individuals exposed to an average of 1–3 ppm ethylbenzene in the workplace contained ethylbenzene levels as high as 0.7 ppm (Wolff 1976; Wolff et al. 1977).

Studies examining the correlation of ethylbenzene concentrations in ambient air with concentrations measured in expired or alveolar air have also been conducted (Conkle et al. 1975; Engstrom and Bjurstrom 1978; EPA 1984e). Ethylbenzene concentrations in breath samples were reported to correlate well with ethylbenzene concentrations in indoor samples taken with personal air monitors (EPA 1984e). A correlation was also found between ethylbenzene uptake and ethylbenzene concentrations in alveolar air during, but not after, inhalation exposure in volunteers (Engstrom and Bjurstrom 1978). Rates of ethylbenzene expiration measured in volunteers with no known previous exposure to ethylbenzene ranged from 0.78 to 14 μg/hour, with higher rates detected in smokers than in nonsmokers (Conkle et al. 1975).

3.8.2 Biomarkers Used to Characterize Effects Caused by Ethylbenzene

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (Agency for Toxic Substances and Disease Registry 1990) and for information on biomarkers for neurological effects see OTA (1990).

No specific biomarkers of effect for ethylbenzene were identified. Most of the information on humans is from case reports in which the effects are general and non-specific, such as eye and throat irritation and chest constriction (Yant et al. 1930).

3.9 INTERACTIONS WITH OTHER CHEMICALS

The metabolism of ethylbenzene includes pathways involving mono-oxygenases (e.g., cytochrome P-450) and formation of glucuronide and sulfate conjugates. Therefore, the metabolism of ethylbenzene could be markedly altered by inhibitors (e.g., carbon monoxide, SKF 525A) and inducers (e.g., phenobarbital, described above) of drug-metabolizing enzymes (Gillette et al. 1974) and by the availability of conjugation reactants (e.g., glucuronic acid, sulfate) that facilitate the excretion of ethylbenzene

metabolites. Mono-oxygenases (MOs) are a class of enzymes involved in the detoxication of xenobiotics, including ethylbenzene. Substances that induce or inhibit MO enzymes may alter the toxicity of ethylbenzene by increasing the rate of production of its metabolites. Compounds that affect glucuronic acid availability could also affect the excretion rate of ethylbenzene metabolites.

Numerous studies have demonstrated interactions between ethylbenzene and chemicals that inhibit cytochrome P-450 (e.g., carbon monoxide; Maylin et al. 1973), compete with ethylbenzene for metabolism by cytochrome P-450 (e.g., alkylbenzenes, ethanol; Angerer and Lehnert 1979; Elovaara et al. 1984; Engstrom et al. 1984; Romer et al. 1986), or induce cytochrome P-450 (e.g. phenobarbital; Maylin et al. 1973; McMahon and Sullivan 1966). Competitive inhibition of metabolism by alkylbenzenes, including ethylbenzene, has been introduced into PBPK models of alkylbenzene mixtures and gasoline component mixtures (Dennison et al. 2003, 2004; Haddad et al. 2001; Tardif et al. 1997).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to ethylbenzene than will most persons exposed to the same level of ethylbenzene in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of ethylbenzene, or compromised function of organs affected by ethylbenzene. Populations who are at greater risk due to their unusually high exposure to ethylbenzene are discussed in Section 6.7, Populations with Potentially High Exposures.

Even though ethylbenzene is not known to bioaccumulate (Aster 1995; Meylan et al. 1999; Nuns and Benville 1979; Ogata et al. 1984), human and animal studies suggest that several factors can contribute to an increased probability of adverse health effects following ethylbenzene exposure (NIOSH/OSHA 1978). Exposure to ethylbenzene has been shown to produce hearing loss and animals; therefore, individuals with pre-existing hearing loss (e.g., congenital or infection-related) or individuals participating in activities that may result in hearing loss (e.g., sharpshooting) may be more susceptible to the ototoxic effects of ethylbenzene. Exposure of individuals with impaired pulmonary function to ethylbenzene in air has been shown to exacerbate symptoms because of ethylbenzene's irritant properties. Because ethylbenzene is detoxified primarily in the liver and excreted by the kidney, individuals with liver or kidney disease might be more susceptible to ethylbenzene toxicity, as would persons taking medications or other drugs (e.g., alcohol) that are known hepatotoxins. Persons with dermatitis or other

skin diseases may be at greater risk, since ethylbenzene is a defatting agent and may aggravate these symptoms. Children's susceptibility is discussed in Section 3.7.

In summary, groups that might be more susceptible to the toxic effects of ethylbenzene are individuals with hearing loss and diseases of the respiratory system, liver, kidney, or skin; young children; fetuses; pregnant women; and individuals taking certain medications such as hepatotoxic medications or drugs.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to ethylbenzene. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to ethylbenzene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to ethylbenzene:

Leikin JB, Poloucek FP. 2002. Poisoning and toxicology handbook. Hudson, OH: Lexi-Comp, Inc., 554.

Proctor NH, Hughes JP. 1978. Chemical hazards of the workplace. Philadelphia, PA: J.B. Lippincott Company, 251-252.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to ethylbenzene can occur by inhalation, oral, or dermal contact. General recommendations for reducing absorption of ethylbenzene following exposure include removing the exposed individual from the contaminated area and removing the contaminated clothing. Removal of the patient from the source of contamination is an initial priority along with proper ventilation and cardiac monitoring. If the eyes and skin were exposed, they should be flushed with water. Emesis or lavage is not recommended following oral exposure due to the risk of aspiration pneumonia (Leikin and Paloucek 2002). Activated charcoal may be administered to adsorb ethylbenzene (Leikin and Paloucek 2002). Benzodiazepines may be used to control seizures (Leikin and Paloucek 2002). Administration of catecholamines should be avoided due to the risk of ventricular arrhythmia (Leikin and Paloucek 2002).

3.11.2 Reducing Body Burden

Following absorption into the blood, ethylbenzene is rapidly distributed throughout the body. The initial stage of ethylbenzene metabolism in humans is the formation of 1-phenylethanol via hydroxylation of the of the side chain. Further oxidation leads to the formation of mandelic acid and phenylglyoxylic acid, the major urinary metabolites of ethylbenzene in humans. Detoxication pathways generally involve the formation of glucuronide or sulfate conjugates of 1-phenylethanol or its subsequent metabolites. Urinary excretion is the primary route of elimination of metabolized ethylbenzene. Studies in humans and animals indicate that urinary excretion occurs in several phases, with half-lives of hours. Hence, ethylbenzene and its metabolites have relatively short half-lives in the body, and while some of these metabolites are clearly toxic, substantial body burdens are not expected.

No methods are currently used for reducing the body burden of ethylbenzene. It is possible that methods could be developed to enhance the detoxication and elimination pathways.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Treatments interfering with the mechanism of action for toxic effects have not been identified.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of ethylbenzene is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of ethylbenzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of Ethylbenzene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to ethylbenzene are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of ethylbenzene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 3-6 graphically describes the existing health effects information on ethylbenzene by route and duration of exposure. Little information concerning humans exposed via inhalation to ethylbenzene is available. Most of the information concerning health effects in humans is reported in occupational studies, which are difficult to interpret given the limitations of the studies (e.g., simultaneous exposure to other hazardous substances, unquantified exposure concentrations, and exposure probably occurring by a combination of routes). No data were available concerning human health effects following oral exposures to ethylbenzene. Dermal effects in humans exposed to ethylbenzene vapors include respiratory and ocular irritation.

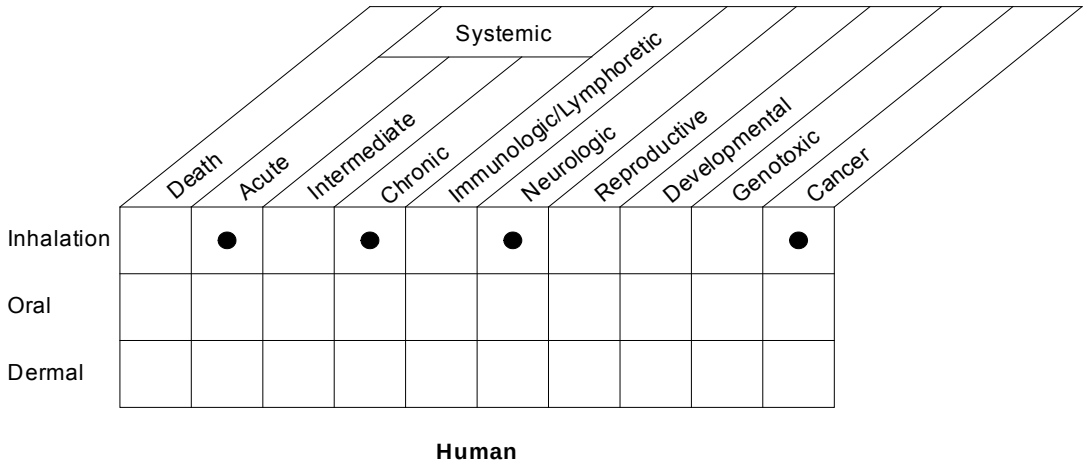
In animals, the lethality of ethylbenzene is documented for all routes of exposure. Systemic, immunologic, neurologic, developmental, and reproductive effects have been reported following acute-, intermediate-, or chronic-duration inhalation exposures to ethylbenzene. Limited data on the health effects resulting from oral or dermal exposure to ethylbenzene were located.

3.12.2 Identification of Data Needs

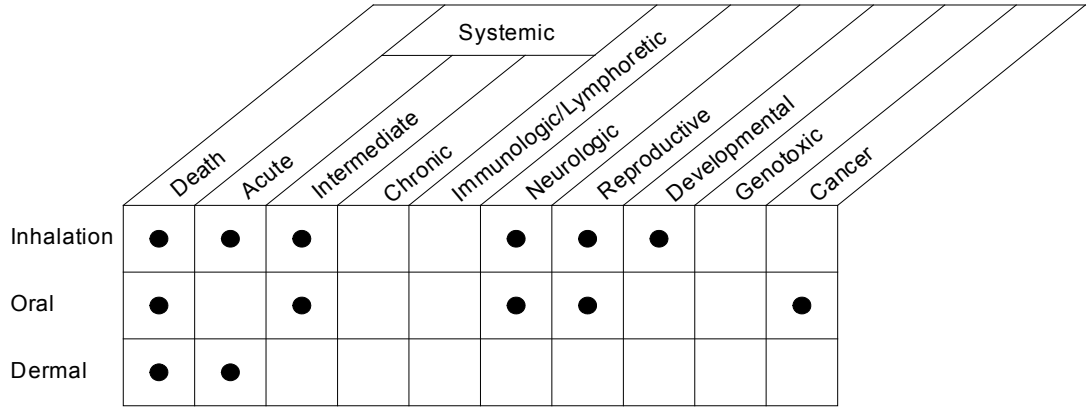
In general, data on the toxic effects of ethylbenzene in humans and animals are limited. In many areas for which studies have been conducted, the lack of reliable data precludes any definitive conclusions from being drawn.

Acute-Duration Exposure. The database for acute-duration inhalation exposure to ethylbenzene is largely composed of inhalation studies in laboratory animals with a limited number of human studies.

Figure 3-6. Existing Information on Health Effects of Ethylbenzene



Human



Animal

● Existing Studies

The available acute-duration studies in humans (Sliwinska-Kowalska et al. 2001; Yant et al. 1930) and animals (Andersson et al. 1981; Cappaert et al. 1999, 2000, 2001, 2002; Ethylbenzene Producers Association 1986a; Molnar et al. 1986; Tegeris and Balster 1994; Yant et al. 1930) indicate that the nervous system, and particularly the auditory system, is sensitive to the toxic effects of ethylbenzene. The available reports and studies in humans are of limited use for dose-response assessment because insufficient information was provided to clearly characterize the exposure to ethylbenzene or because exposures were for mixtures of solvents, not ethylbenzene alone. Ethylbenzene is an acute ocular and respiratory irritant in humans (Cometto-Muñiz and Cain 1995; Thienes and Haley 1972; Yant et al. 1930); however, animal studies suggest that acute sublethal inhalation exposures do not result in pulmonary histopathology (Ethylbenzene Producers Association 1986a). Developmental and reproductive effects were reported in animals exposed to ethylbenzene by inhalation during gestation (Ungváry and Tátrai 1985); however, the latter study is limited by incomplete description of the results and the absence of an analysis on a per litter basis. Increased organ weight (liver, kidneys) has been observed in a number of studies in animals (Ethylbenzene Producers Association 1986a; Toftgard and Nilsen 1982); however, no findings are made upon microscopic examination of the liver or kidneys (Ethylbenzene Producers Association 1986a). An acute-duration inhalation MRL was derived based on ototoxicity in animals. No studies describing acute-duration oral exposure of humans to ethylbenzene were found in the literature. Although two animal studies have examined the acute oral toxicity of ethylbenzene (Gagnaire and Langlais 2005; Ungvary 1986), they were not adequate for the derivation of an acute-duration oral MRL. Mild irritation (Smyth et al. 1962), reddening, exfoliation, and blistering (Wolf et al. 1956) have been reported in rabbits when ethylbenzene was applied directly on the skin. Slight irritation of the eye (Wolf et al. 1956) and corneal injuries (Smyth et al. 1962; Wolf et al. 1956) were observed in rabbits when ethylbenzene was instilled onto the eyes. There is a data need for additional acute-duration oral and dermal toxicity studies in animals exposed to ethylbenzene.

Intermediate-Duration Exposure. The database of intermediate-duration inhalation studies includes several studies in animals, but no studies in humans. It has been shown that the auditory system is sensitive to the toxic effects of ethylbenzene after intermediate-duration inhalation exposure (Gagnaire et al. 2007). Systemic effects have been observed at concentrations equal to or higher than those that elicited ototoxic effects in rats. Effects at even higher concentrations include neurological and hematological effects (Cragg et al. 1989; Wolf et al. 1956) and increased organ weight (Cragg et al. 1989; Elovaara et al. 1985; NIOSH 1981; NTP 1992; Wolf et al. 1956). Organs showing increased weight did not show treatment-related histopathological effects (Cragg et al. 1989; NTP 1992). Developmental and/or reproductive effects have been reported in offspring of animals following intermediate-duration

inhalation exposures to ethylbenzene during gestation (Faber et al. 2006, 2007; NIOSH 1981; Saillenfait et al. 2003, 2006, 2007) at concentrations that were higher than those that elicited ototoxic effects. An intermediate-duration inhalation MRL was derived based on the observed ototoxic effects in animals (Gagnaire et al. 2007). The intermediate-duration oral database for ethylbenzene is limited to the critical study by Mellert et al. (2007) evaluating the effects of oral exposure of rats to ethylbezene for 4 and 13 weeks, and a poorly reported 6-month exposure study in rats (Wolf et al. 1956). Results of the 13-week gavage study in rats observed effects indicative of liver toxicity, including increased activity of serum liver enzymes (alanine aminotransferase and γ-glutamyl transferase), increased absolute and relative liver weights, and a dose-related increase in the incidence of centrilobular hepatocyte hypertrophy. Results of the 4-week exposure study in rats are similar to those observed in the 13-week study, showing that the liver is the primary target organ for oral ethylbenzene; effects consistent with hepatotoxicity, including increased absolute and relative liver weights, increased incidence of hepatocyte centrilobular, and increased serum liver enzyme activity (alanine aminotransferase) (Mellert et al. 2007). Wolf et al. (1956) reported slight weight and histopathological effects in the liver and kidney of female rats. An intermediate-duration oral MRL was derived based on the observed liver effects in animals exposed for 13 weeks (Mellert et al. 2007).

Chronic-Duration Exposure and Cancer. The database of chronic-duration/carcinogenicity inhalation studies with ethylbenzene includes a chronic duration study in rats and mice (NTP 1999) and a limited number of studies in humans. Hematological effects were observed in workers exposed to solvents containing ethylbenzene (Angerer and Wulf 1985), but no hematological effects, liver lesions or effects on liver function, or cases of malignancy were reported in a study of workers exposed chronically (>20 years) to ethylbenzene (Bardodej and Cirek 1988). The severity of nephropathy was significantly increased in female and male rats exposed to ≥75 or 750 ppm ethylbenzene, respectively, for 2 years (NTP 1999). At higher concentrations rats showed significant increases in renal tubule hyperplasia; male rats showed an increased incidence of interstitial cell adenoma in the testis. Effects observed in male or female mice exposed to ethylbenzene for 2 years include an increased incidence of hyperplasia of the pituitary gland pars distalis, increased incidence of follicular cell hyperplasia in the thyroid gland, an increased incidence of syncytial alterations of the hepatocytes, hypertrophy, and hepatic necrosis, and an increased incidence of eosinophilic foci (NTP 1999). Ethylbenzene showed clear evidence of carcinogenic activity in male rats based on increased incidences of renal tubule neoplasms, some evidence of carcinogenic activity in female rats based on increased incidences of renal tubule adenomas, some evidence of carcinogenic activity in male mice based on increased incidences of alveolar/bronchiolar neoplasms, and some evidence of carcinogenic activity in female mice based on increased incidences of

hepatocellular neoplasms (NTP 1999). The 2-year inhalation study in rats suggests that an increase in the severity of nephropathy in female rats is the most sensitive end point of ethylbenzene exposure. This end point was selected to estimate a chronic-duration inhalation MRL. There is no need to conduct additional carcinogenicity studies of ethylbenzene via inhalation. A statistically significant increase in total malignant tumors was reported in female rats and in combined male and female groups exposed to 500 mg kg/day via gavage for 104 weeks (Maltoni et al. 1985). No data on specific tumor type were presented, only one dose was tested, and no information on survival was provided. It might be useful to conduct a toxicity and carcinogenicity study of ethylbenzene administered orally to at least two animal species. It might be useful to conduct chronic-duration studies in at least two species with an emphasis in ototoxic effects.

Genotoxicity. There are limited data on the genotoxicity of ethylbenzene in humans. Two studies examined potential genotoxic effects in workers (Holz et al. 1995; Sram et al. 2004); the interpretation of these results is limited by co-exposure to other chemicals (e.g., benzene, toluene, styrene). *In vivo* genotoxicity studies in laboratory animals did not cause significant alterations in micronuclei formation (Mohtashamipur et al. 1985; NTP 1992, 1999) or unscheduled DNA syntheseis (Clay 2001). Data are available regarding the genotoxic potential of ethylbenzene from *in vitro* assays in bacteria, yeast, and mammalian cell cultures (Dean et al. 1985; Degirmenci et al. 2000; Florin et al. 1980; Kubo et al. 2002; Nestmann and Lee 1983; Nestmann et al. 1980; NTP 1986, 1999; Zeiger et al. 1992). The results generally indicate that ethylbenzene is not mutagenic. Similarly, *in vitro* genotoxic assays in mammalian cells were negative when noncytotoxic concentrations were used (Dean et al. 1985; McGregor et al. 1988; NTP 1986, 1999; Seidel et al. 2006; Wollny 2000). However, some studies did not cause genotoxic alterations at concentrations not associated with cytotoxicity (Chen et al. 2008; Gibson et al. 1997); it is not known whether these positive results reflect a genotoxic response in a particular test system or whether they are outliers. Independent confirmation or refutation of these studies, as well as further genotoxicity studies, especially in mammalian systems, would help provide clarification of these conflicting results.

Reproductive Toxicity. No studies were located regarding reproductive effects in humans following inhalation exposure to ethylbenzene. Reproductive end points have been assessed after acute- (Ungváry and Tátrai 1985) and intermediate-duration exposures of animals exposed during gestation (Saillenfait et al. 2003, 2006, 2007) and in a two-generation reproductive toxicity study (Faber et al. 2006, 2007). Effects on reproductive organs (Cragg et al. 1989; NTP 1992, 1999; Wolf et al. 1956) in animals exposed to ethylbenzene have also been evaluated. The only available oral-exposure reproduction study reported

decreased hormone levels and blockage or delay of the estrus cycle in female rats (Ungvary 1986). Additional inhalation exposure reproductive toxicity studies are not deemed necessary at this time. It might be useful to conduct additional oral exposure reproductive toxicity studies in at least two species.

Developmental Toxicity. No studies of developmental effects in humans following inhalation exposure to ethylbenzene were located. The available studies have reported developmental effects in the offspring of animals exposed to ethylbenzene during gestation (NIOSH 1981; Saillenfait et al. 2003, 2006, 2007; Ungváry and Tátrai 1985). The report by Ungváry and Tátrai (1985) has many deficiencies, including poor reporting of the experimental conditions and poor description of the maternal and fetal observations. Maternal toxicity has been generally reported at the highest doses tested (Saillenfait et al. 2003, 2006, 2007; Ungváry and Tátrai 1985). Biologically or statistically significant developmental or neurodevelopmental effects were not observed in the offspring of animals in a two-generation inhalation study (Faber et al. 2006, 2007). No studies were located regarding developmental effects in humans and animals following oral or dermal exposure to ethylbenzene. A data need exists for oral and dermal developmental toxicity studies.

Immunotoxicity. No studies were found regarding immunological effects in humans following inhalation exposure to ethylbenzene. No effects on humoral immune function were observed in female rats exposed to ethylbenzene vapor for 28 days (Stump 2004). Studies also were located that examined organs of the immune system in animals after intermediate- (Cragg et al. 1989; NIOSH 1981; NTP 1992) or chronic-duration (NTP 1999) exposure to ethylbenzene. No studies were located regarding immunological effects in humans or animals following oral or dermal exposure to ethylbenzene. No additional immunological studies are deemed to be necessary at this time.

Neurotoxicity. Human studies have established that acute exposure to ethylbenzene may result in dizziness and vertigo (Yant et al. 1930) and that hearing loss is significantly increased in workers exposed to solvent mixtures that include ethylbenzene (Sliwinska-Kowalska et al. 2001). The role of ethylbenzene in the observed hearing losses cannot be ascertained from this study given that ethylbenzene was only one of several solvents.

Animal studies indicate that the auditory system is the most sensitive target of ethylbenzene toxicity after acute- (Cappaert et al. 1999, 2000, 2001, 2002) or intermediate-duration (Gagnaire et al. 2007) inhalation exposures and after acute-duration oral exposures to ethylbenzene (Gagnaire and Langlais 2005). The effects of ethylbenzene on the central nervous system have been assessed in animals after acute-duration

exposure to ethylbenzene (Ethylbenzene Producers Association 1986a; Molnar et al. 1986; Tegeris and Balster 1994; Yant et al. 1930). Neurobiochemical and electrical activity alterations have been reported in animals after acute-duration exposure to ethylbenzene (Andersson et al. 1981; Frantik et al. 1994; Mutti et al. 1988; Romanelli et al. 1986). No adverse histopathological findings were observed in brain tissue of animals after intermediate- (NTP 1992) or chronic-duration (NTP 1999) exposure to ethylbenzene. No studies were located regarding neurological effects in humans following oral exposure to ethylbenzene. Ototoxicity is also observed in animals after acute-duration oral exposure to ethylbenzene (Gagnaire and Langlais 2005). Additional studies are needed to establish the mechanism of action by which ethylbenzene elicits ototoxicity, as no information establishing the mechanism of ethylbenzene-induced ototoxicity was identified. Furthermore, due to the large differences between rats and guinea pigs in susceptibility to ethylbenzene-induced ototoxicity, additional studies on hearing and ear physiology in rodents are needed to evaluate which species is most similar to humans. No studies were located regarding neurological effects in humans or animals following dermal exposure to ethylbenzene. Additional inhalation studies are needed to establish an intermediate-duration NOAEL for ototoxic effects. Additional studies are also needed to characterize the concentration-response pattern for ototoxicity after acute- and intermediate-duration oral exposures to ethylbenzene.

Epidemiological and Human Dosimetry Studies. The few available epidemiological studies on the health effects of ethylbenzene were primarily limited to occupational studies in which quantitative estimates of exposure were lacking and other limitations (e.g., multiple exposure routes, simultaneous exposure to other hazardous chemicals) were present. Studies using volunteers exposed to low concentrations of ethylbenzene have provided useful information on effects of acute-duration inhalation exposure on the central nervous system (Yant et al. 1930). No studies were available in which humans were exposed orally or dermally to ethylbenzene. Epidemiological studies conducted in populations exposed to ethylbenzene alone may provide useful information on the effects of ethylbenzene in humans. Emphasis should be placed in the detection of ototoxic effects as this end point is the most sensitive effect observed in animal studies.

Biomarkers of Exposure and Effect. Sensitive methods are available for determining ethylbenzene and ethylbenzene metabolites in biological tissues and fluids. However, limited data are available associating levels of ethylbenzene in human tissues and fluids with adverse health effects. Additional animal or epidemiological studies evaluating the association between levels in tissue or fluids and adverse health effects would be useful to devise more sensitive and more specific early biomarkers of effect.

Exposure. Exposure to ethylbenzene can be monitored through levels of ethylbenzene in breath, blood, or tissue, or levels of its metabolites, mandelic or phenylglyoxylic acid in urine. A statistically significant correlation was observed between urinary excretion of mandelic acid and ethylbenzene exposure in workers exposed to mixed solvents (including an ethylbenzene TWA of 0.9 ppm) in a metal-coating factory (Kawai et al. 1991). However, neither one of the metabolites is specific to ethylbenzene. Additional studies to identify a biomarker or biomarkers of exposure specific to ethylbenzene are needed.

Effect. There are currently no known specific biomarkers of effect for ethylbenzene. Development of methods to identify biomarkers that would indicate toxic effects, and the extent of those toxic effects after exposure to ethylbenzene, would be helpful in managing health effects that occur after significant exposure to ethylbenzene.

Absorption, Distribution, Metabolism, and Excretion. Ethylbenzene is absorbed by humans following inhalation (Bardodej and Bardodejova 1970; Gromiec and Piotrowski 1984) and dermal (Dutkiewicz and Tyras 1967) exposures. Absorption rates were 49–64% by inhalation. Dermal absorption rates were in the range of 24–33 and 0.11–0.23 mg/cm^2/hour for male subjects exposed to liquid ethylbenzene and ethylbenzene from aqueous solutions, respectively (Dutkiewicz and Tyras 1967). Animal data support these findings and indicate that absorption rates are high following oral exposures as well (Climie et al. 1983; El Masry et al. 1956).

Only one study (Engstrom and Bjurstrom 1978) is available that outlines the distribution of ethylbenzene in humans following inhalation exposure. This study indicates rapid distribution to adipose tissues throughout the body. Inhalation studies in animals support these results (Elovaara et al. 1982; Engstrom et al. 1985). Ethylbenzene is accumulated primarily in the intestine, liver, kidney, and fat, which provides some basis for ethylbenzene-induced effects observed in the liver and kidney. No data on distribution of ethylbenzene following dermal exposure were located. Such information would be useful because absorption of liquid ethylbenzene via this route is rapid in humans and because the potential exists in humans for dermal exposure.

The metabolism of ethylbenzene in humans and animals has been studied. Although some differences in the metabolic pattern according to route of exposure, sex, nutritional status (Nakajima and Sato 1979), and species (Bakke and Scheline 1970; Climie et al. 1983; El Masry et al. 1956; Engstrom et al. 1984, 1985; Smith et al. 1954a, 1954b; Sollenberg et al. 1985) have been documented, pharmacokinetic data

show no significant differences in metabolism between oral and inhalation routes in either humans or animals (Climie et al. 1983; Engstrom et al. 1984, 1985). Further studies that correlate these differences in metabolism with differences in health effects would be useful. Data on metabolism following dermal exposure are sparse, because it is difficult to accurately measure absorption of volatile compounds. Additional data on metabolism following dermal exposure would be useful as these exposures could occur both from contaminated soil or groundwater.

Ethylbenzene has been shown to be rapidly eliminated from the body following inhalation exposure (primarily in the urine) in both humans and animals. These studies (Gromiec and Piotrowski 1984; Yamasaki 1984) are sufficient to characterize the elimination of ethylbenzene following inhalation exposure. A small number of studies in animals exposed orally and humans exposed dermally support these findings. Further studies on elimination of ethylbenzene via these exposure routes would be useful, especially because differences in the excretion patterns have been observed with different routes of exposure.

PBPK models have been developed for predicting ethylbenzene kinetics (including metabolism) in rats and humans exposed by inhalation (Dennison et al. 2003, 2004; Tardif et al. 1997) and in rats exposed to gavage doses of ethylbenzene in corn oil (Faber et al. 2006).

Comparative Toxicokinetics. Quantitative and qualitative variations in the absorption, distribution, metabolism, and excretion of ethylbenzene were observed depending on exposure routes, sex, nutritional status, and species, as previously outlined. Further studies that focus on these differences and their implications for human health would be useful. Additionally, *in vitro* studies using human tissue and PBPK modeling would contribute significantly to the understanding of the kinetics of ethylbenzene, since they would provide information on half-lives and saturation kinetics associated with the metabolism of ethylbenzene.

Methods for Reducing Toxic Effects. No information was found that specifically addressed the reduction of toxic effects after absorption of ethylbenzene. Development of clinical procedures for minimizing the effects of ethylbenzene on the respiratory, hepatic, and renal systems, and the central nervous system would be useful in situations where significant exposure had occurred.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

There are no data describing the effects of ethylbenzene exposure in children or developing postnatal animals. Data needs relating to development are discussed in more detail above under developmental effects. In order to evaluate whether ethylbenzene presents a unique hazard to children, additional information on the health effects, pharmacokinetics, metabolism, and mechanism of action in children is needed. It is unknown whether children differ from adults in their susceptibility to health effects from exposure to ethylbenzene. Pharmacokinetic studies investigating whether ethylbenzene or its active metabolites cross the placenta or are transferred into breast milk would be useful. Studies to determine whether there are specific biomarkers of exposure in children would be helpful in monitoring the exposure of children to this chemical. In addition, information describing methods of reducing toxic effects and decreasing body burden in children might be helpful.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Ongoing studies pertaining to ethylbenzene have been identified and are shown in Table 3-10 (FEDRIP 2007). In addition, the American Chemistry Council is conducting investigations on the mode of action of ethylbenzene-induced mouse lung tumors, including the role of ethylbenzene ring-oxidation to cytotoxic quinine metabolites.

Table 3-10. Ongoing Studies on Ethylbenzene

Investigator	Affiliation	Research description	Sponsor
Thrall KD	Battelle Pacific Northwest Laboratories	Dermatopharmacokinetics of paint solvents.	National Institute for Occupational safety and Health
Backes WL	Louisiana State University	Identify conditions under which individuals may be susceptible to alkylbenzene-induced toxicity.	National Institute of environmental Health Sciences
Burke J	Environmental Protection Agency	A critical review and probabilistic model input distribution development for microenvironmental exposures to benzene.	Environmental Research Laboratories
Davis-Hoover W	Environmental Protection Agency	Determining if biologically active *in situ* BioNets could bioremediate methyl-*tert*-butyl ether and BTEX contaminated groundwater.	Risk Management Resource Library
Venosa A	Environmental Protection Agency	Treatability of co-mingled groundwater plume contaminated with polycyclic aromatic hydrocarbons.	Risk Management Resource Library
Vroblesky DA	Department of the Interior, U.S. Geological Survey, Water Resources Division	Remediation of JP-4 contamination using hydraulic containment and in situ biodegradation at the Defense Fuel Supply Center, Charleston, South Carolina.	Department of the Interior, U.S. Geological Survey, Water Resources Division
Jayarao BM	Pennsylvania State University, Veterinary Science	Bioreporter-based technology for detection of organic toxicants directly from milk and milk products.	Pennsylvania State University, Veterinary Science
Sylva TY	University of Hawaii, Molecular Biosciences and Biosystems	Application of bioremediation to hydrocarbon contaminated soils.	University of Hawaii, Molecular Biosciences and Biosystems
Holm RH	Harvard University, Department of Chemistry	Transformations catalyzed by the molybdenum and tungsten oxotransferases and hydroxylases.	National Science Foundation
Miknis F	FETC-MGN	Noncatalytic concept for the direct conversion of fossil fuels and hydrocarbon-containing materials to transportation fuels with simultaneous reduction in the heteroatom content.	Office of Fossil Energy
Starr RC	Idaho National Engineering and Environmental Laboratory	Development and testing of an in situ system for remediating groundwater contaminated with BTEX.	Office of Fossil Energy
Friesen DT	Bend Research, Inc.	High-performance membranes for gas, vapor, and liquid separations.	Office of Energy Research

Table 3-10. Ongoing Studies on Ethylbenzene

Investigator	Affiliation	Research description	Sponsor
Sibold J	Golden Technologies Company, Inc.	Investigating the use of ceramic membranes in dehydrogenation reactions.	Energy Efficiency, Department of Energy
Ittrell J	SE, Inc.	Advanced low temperature emissions control technology for MTB destruction.	SE, Inc.
aPierre R	Recision Combustion, Inc	Investigating ano-zeolite coatings on microlith substrates for high selectivity chemical reactions.	Recision Combustion, Inc.

BTEX = benzene, toluene, ethylbenzene, and xylenes

Source: FEDRIP 2007