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 styrene. 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

Most information on the effects of inhalation exposure to styrene in humans comes from studies of workers exposed to styrene vapors in the production and use of plastics and resins, especially polyester resins dissolved in styrene. In most cases, the studies involve workplace exposures such as fiberglass boat building factories where the actual levels of styrene are reported as a range of styrene air concentrations. However, there are a few human clinical studies in which exposures are better quantified. A common limitation of many of the occupational exposure studies is the phenomenon of the healthy worker effect. The selection of healthy individuals for employment and the likelihood that more susceptible workers are more likely to leave the workforce can result in workers who are healthier than the general population. This type of bias typically affects comparisons with the general population and is less likely to influence comparisons with other groups of workers. Provided below are descriptions of the known effects of inhalation exposure of humans and animals to styrene.

3.2.1.1 Death

There have been no reports of deaths in humans directly associated with exposure to styrene in the workplace (EPA 1985a; Gosselin et al. 1984; NIOSH 1983).

In animals, inhalation studies indicate that the acute toxicity of styrene is low to moderate. An LC_{50} of 2,770 ppm after 2 hours of exposure was reported in rats, and the LC_{50} for mice after exposure for 4 hours was 4,940 ppm (Shugaev 1969). All rats and guinea pigs survived after exposure to 1,300 ppm styrene

for 30 hours and 16 hours, respectively (Spencer et al. 1942). However, all animals died after 40 hours of exposure. Gender differences in mortality were observed in repeated-exposure studies (6 hours/day, 5 days/week for 2 weeks) (Cruzan et al. 1997). Increases in mortality were observed in female CD-1 and B6C3F1 female mice exposed to 250 ppm; no deaths were observed at 500 ppm. In the CD-1 and B6C3F1 males, very few deaths were observed at 250 ppm, but increases in deaths were observed at 500 ppm. A similar finding was reported by Morgan et al. (1993a): increases in mortality were observed in female B6C3F1 mice exposed to 250 ppm and no deaths were observed at 500 ppm; in males, deaths were observed at 250 and 500 ppm. In contrast to these findings, no deaths were observed in Sprague Dawley rats exposed to concentrations as high as 1,500 ppm 6 hours/day, 5 days/week for 13 weeks (Cruzan et al. 1997).

All reliable LOAEL values and LC_{50} values for lethality in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No studies were located regarding dermal or metabolic effects in humans or animals after inhalation exposure to styrene.

For the following systemic effects resulting from inhalation exposure to styrene, the highest NOAEL values and all reliable LOAEL values in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Several human studies have examined the respiratory effects caused by inhalation exposure to styrene. The most commonly reported general symptom is mucous membrane irritation. Irritation of the upper respiratory tract (i.e., nose and throat) has been reported by volunteers (Carpenter et al. 1944; Stewart et al. 1968) and workers (NIOSH 1983). Throat irritation and increased nasal secretion occurred following exposure of two male subjects to 800 ppm for 4 hours (Carpenter et al. 1944). Nasal irritation was observed in all volunteers after exposure to 376 ppm styrene for 60 minutes (Stewart et al. 1968). Obstructive lung changes were observed in 4 of 21 workers exposed to styrene for about 10 years (Chmielewski and Renke 1975). However, exposure levels were not defined. No histological alterations were observed in nasal biopsies from styrene workers exposed to 50–60 ppm styrene for 7 years (Ödkvist et al. 1985).

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Table 3-1 Levels of Significant Exposure to Styrene - 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	3-40 hr				1300 (100% mortality after >40 hours exposure)	Spencer et al. 1942 Styrene	
2	Mouse (CD-1 and B6C3F1)	6 hr/d 5 d/wk 2 wk				250 (increased mortality)	Cruzan et al. 1997 Styrene	
3	Mouse (B6C3F1)	6 hr/d 14 d				250 M (44% mortality)	Morgan et al. 1993a Styrene	
4	Gn Pig	3-40 hr				1300 (100% mortality after 40 hours exposure)	Spencer et al. 1942 Styrene	
Systemic								
5	Human	7 hr	Ocular		99 M (mild, transient eye irritation)		Stewart et al. 1968 Styrene	
6	Human	1 or 2 hr	Resp	216 M	376 M (nasal irritation)		Stewart et al. 1968 Styrene	
			Ocular	216 M	376 M (eye irritation)			

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Table 3-1 Levels of Significant Exposure to Styrene - 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
7	Mouse (CD-1 and B6C3F1)	6 hr/d 5 d/wk 2 wk	Resp		250 (shallow breathing)		Cruzan et al. 1997 Styrene	
			Hepatic	60	250 (increased liver weight and centrilobular hepatocyte necrosis)			
8	Mouse (CD-1)	6 hr/d 3 d	Resp		80 M (single cell necrosis in nasal olfactory epithelium)		Cruzan et al. 2001 Styrene	
9	Mouse (CD-1)	6 hours/day 3 days	Resp	40 M	160 M (moderate to marked degenerative changes in olfactory epithelium)		Green et al. 2001a Styrene	
10	Mouse (B6C3F1)	6 hr/d 14 d	Hepatic	125	250 M (pigmented macrophages and focal necrosis)		Morgan et al. 1993a Styrene	
			Renal	500				
			Bd Wt	500				
11	Mouse (B6C3F1)	6 hr/d 1-3 d	Hepatic			250 M (mild to marked necrosis)	Morgan et al. 1993a Styrene	

Table 3-1 Levels of Significant Exposure to Styrene - 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
12	Mouse (B6C3F1)	6 hr/d 3 d	Hepatic	125		250 (severe hepatocellular degeneration/necrosis)	Morgan et al. 1993b Styrene	
13	Mouse	6 hr/d 4 d	Hepatic	125		250 (marked degeneration and/or coagulative necrosis of centrilobular hepatocytes)	Morgan et al. 1993c Styrene	
Immuno/ Lymphoret								
14	Mouse (BALB/c)	6 hr/d 4 d			100 F (exacerbated inflammatory reaction after ovalbumin challenge)		Ban et al. 2006 Styrene	
Neurological								
15	Human	1 hr			87 (inhibition of vestibular-oculomotor system)		Odkvist et al. 1982 Styrene	
16	Human	3 or 4 hr		20[b]			Seeber et al. 2004 Styrene	
17	Human	1 or 2 hr		216 M	376 M (impaired performance on balance and coordination tests)		Stewart et al. 1968 Styrene	

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Table 3-1 Levels of Significant Exposure to Styrene - 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
18	Human	7 hr		99 M			Stewart et al. 1968 Styrene	
19	Rat (Long- Evans)	6 hr/d 5 d/wk 1 or 2 wk				1000 M (hearing loss and loss of OHC)	Campo et al. 2001 Styrene	
20	Rat (Long- Evans)	8 hr/d 5 d				1600 M (hearing loss at 8 and 16 kHz)	Crofton et al. 1994 Styrene	
21	Rat (Long- Evans)	6 hr/d 5 d				1000 M (hearing loss, loss of OHC)	Lataye et al. 2003 Styrene	
22	Mouse (CD-1 and B6C3F1)	6 hr/d 5 d/wk 2 wk		60		250 (lethargy and unsteady gait)	Cruzan et al. 1997 Styrene	
23	Mouse (Swiss OF1)	4 hr		413 M	610 M (impaired performance on a swimming test)		De Ceaurriz et al. 1983 Styrene	
24	Gn Pig (NS)	6 hr/d 5 d		1000 M			Lataye et al. 2003 Styrene	
Reproductive								
25	Mouse	5 d 5 hr/d		300 M			Salomaa et al. 1985 Styrene	

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Table 3-1 Levels of Significant Exposure to Styrene - 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
Developmental								
26	Rat (Sprague-Dawley)	7 hr/d Gd 6-15		600 F			Murray et al. 1978 Styrene	
27	Mouse (BMR/T6T6)	6 hr/d Gd 6-16		250 F			Kankaanpaa et al. 1980 Styrene	
28	Hamster (Chinese)	6 hr/d Gd 6-18		750 F		1000 F (fetal deaths or resorptions)	Kankaanpaa et al. 1980 Styrene	
29	Rabbit (New Zealand)	7 hr/d Gd 6-18		600 F			Murray et al. 1978 Styrene	
INTERMEDIATE EXPOSURE								
Systemic								
30	Rat (Sprague-Dawley)	6 hr/d 5 d/wk 13 wk	Resp	500	1000 (focal hyperplasia in nasal olfactory epithelium)		Cruzan et al. 1997 Styrene	
			Hemato	1500				
			Hepatic	1500				
			Renal	1500				
			Ocular		200 (eye irritation)			
			Bd Wt	1000 M	1500 M (10% decrease in body weight gain)			

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Table 3-1 Levels of Significant Exposure to Styrene - 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	Rat (Long- Evans)	6 hr/d 5 d/wk 4 wk	Hepatic	750 M			Loquet et al. 2000 Styrene	Urinary markers of renal toxicity and serum markers of liver toxicity.
			Renal	750 M				
32	Rat	21 d 5 d/wk 4 hr/d	Resp		150 M (decreased nasal cilia activity)	1000 M (disabled nasal cilia activity)	Ohashi et al. 1986 Styrene	
33	Rat (Sprague-Dawley)	13 wk 5 d/wk 7 hr/d	Renal	133			Viau et al. 1987 Styrene	
34	Rat (Wistar)	8 hr/d 5 d/wk 32 wk	Bd Wt	200 M	2000 M (>15% decrease in body weight gain)		Yamamoto et al. 1997 Styrene	
35	Rat (Fischer- 344)	14 hr/d 5 d/wk 3 wk	Bd Wt		800 M (10-13.5% decrease in body weight)		Yano et al. 1992 Styrene	

Table 3-1 Levels of Significant Exposure to Styrene - 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
36	Mouse (CD-1)	6 hr/d 5 d/wk 13 wk	Resp		50 (atrophy of olfactory epithelium, dilatation, hypertrophy, hyperplasia of Bowman's gland; decreased eosinophilia of bronchiolar epithelial cells)		Cruzan et al. 1997 Styrene	
			Hemato	200				
			Hepatic	100 F	150 F (centrilobular aggregates of siderphages)			
			Bd Wt	150 M	200 M (decreased body weight gain)			
37	Pig	3 wk 5 d/wk 6 hr/d	Hemato	360			Johnston et al. 1983 Styrene	
Neurological								
38	Rat (Long- Evans)	6 hr/d 5 d/wk 3 or 4 wk				1000 M (hearing loss, loss of OHC in organ of Corti)	Campo et al. 2001 Styrene	
39	Rat (Long- Evans)	6 hr/d 5 d/wk 4 wk				750 M (hearing loss and loss of OHC)	Lataye et al. 2000 Styrene	

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Table 3-1 Levels of Significant Exposure to Styrene - 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	Rat (Long- Evans)	6 hr/d 5 d/wk 4 wk				1000 M (loss of OHC and spiral ganglion cell density in organ of Corti)	Lataye et al. 2001 Styrene	
41	Rat (Long- Evans)	6 hr/d 5 d/wk 4 wk			650 M (OHC loss)	850 M (hearing loss and loss of OHC)	Loquet et al. 1999 Styrene	
42	Rat (Long- Evans)	6 hr/d 5 d/wk 4 wk				750 M (hearing loss and loss of OHC)	Loquet et al. 2000 Styrene	
43	Rat (Wistar)	12 hr/d 5 d/wk 4 wk		300 M	600 M (hearing impairment and loss of OHC)		Makitie et al. 2002 Styrene	
44	Rat (Long- Evans)	6 hr/d 5 d/wk 4 wk			650 M (OHC loss)	750 M (hearing loss and OHC loss)	Pouyatos et al. 2002 Styrene	
45	Rat (Fischer- 344)	3 wk 14 hr/d				800 M (hearing loss)	Pryor et al. 1987 Styrene	
46	Rat (Sprague-Dawley)	3 mo continuous		90 M	320 M (astroglial alterations)		Rosengren and Haglid 1989 Styrene	

Table 3-1 Levels of Significant Exposure to Styrene - 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
47	Rat (Wistar)	8 hr/d 5 d/wk 32 wk		200 M	2000 M (decreased sensory nerve conduction velocity)		Yamamoto et al. 1997 Styrene	
48	Rat (Fischer- 344)	14 hr/d 5 d/wk 3 wk				800 M (hearing loss and loss of OHC in organ of Corti)	Yano et al. 1992 Styrene	
49	Mouse (CD-1)	6 hr/d 5 d/wk 13 wk		50	100 (atrophy of olfactory nerve fibers)	200 F (transient lethargy, cold to touch, and slow respiration)	Cruzan et al. 1997 Styrene	
Reproductive								
50	Rat (CD)	6 hr/d 70 pmd 14 d mating Gd 0-21, Ld 5-21		500			Cruzan et al. 2005a, 2005b Styrene	
Developmental								
51	Rat (CD)	6 hr/d 70 pmd 14 d mating Gd 0-21, Ld 5-21		500			Cruzan et al. 2005a, 2005b Styrene	
52	Rat (Wistar)	6 hr/d Gd 6-20				300 (increased neonatal deaths, delays in righting reflex and incisor eruption, alterations in neurochemical levels)	Katakura et al. 1999, 2001 Styrene	

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Table 3-1 Levels of Significant Exposure to Styrene - 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								
Systemic								
53	Human	5.1 yr (Occup)	Hepatic	40 F			Harkonen et al. 1984 Styrene	
54	Human	occupational exposure 7 years	Resp	46 M			Odkvist et al. 1985 Styrene	
55	Human	12.6 yr (Occup)	Renal	26			Verplanke et al. 1998 Styrene	
56	Rat (Sprague-Dawley)	6 hr/d 5 d/wk 104 wk	Resp		50 (atrophic and/or degenerative changes in nasal olfactory epithelium)		Cruzan et al. 1998 Styrene	
			Cardio	1000				
			Gastro	1000				
			Hemato	1000				
			Hepatic	1000				
			Renal	1000				
			Ocular	1000				
			Bd Wt	200 F	500 F (decreased body weight gain)			

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Table 3-1 Levels of Significant Exposure to Styrene - 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
57	Mouse (CD-1)	6 hr/d 5 d/wk 98-104 wk	Resp		20 (respiratory metaplasia in nasal olfactory epithelium, bronchiolar epithelial hyperplasia)		Cruzan et al. 2001 Styrene	
			Cardio	160				
			Gastro	160				
			Hemato	160				
			Hepatic	160				
			Renal	160				
			Ocular	160				
			Bd Wt	80 M	160 M (11% decrease in body weight gain)			
Immuno/ Lymphoret								
58	Human	7 yr (Occup)			30 (alterations in lymphocyte subsets)		Bergamaschi et al. 1995b Styrene	
59	Human	13 yr (Occup)			26 (impaired immune response to concanavalin A)		Tulinska et al. 2000 Styrene	
Neurological								
60	Human	(Occup)			20[c] (decreased color discrimination and reaction time)		Benignus et al. 2005 Styrene	

Table 3-1 Levels of Significant Exposure to Styrene - 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
61	Human	7.6 yr (Occup)			36 (altered performance vestibular tests)		Calabrese et al. 1996 Styrene	
62	Human	62.5 or 79.3 mo (Occup)			26 (decreased color discrimination)		Campagna et al. 1996 Styrene	
63	Human	4-6.4 yr (Occup)		10.8	18.9 (increased prevalence of neurological symptoms)		Checkoway et al. 1992 Styrene	
64	Human	(Occup)			92 M (tiredness, slow reaction times, mood changes)		Cherry et al. 1980 Styrene	
65	Human	18.8 yr (Occup)			6 M (decreased color discrimination and performance on neurobehavioral tests)		Chia et al. 1994 Styrene	
66	Human	12.5 yr (Occup)		24.6			Dalton et al. 2003 Styrene	
67	Human	9 yr (Occup)			8.6 M (increased symptoms of neurotoxicity)		Edling et al. 1993 Styrene	

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Table 3-1 Levels of Significant Exposure to Styrene - 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
68	Human	7.0 yr (Occup)		8 M	93 M (decreased color discrimination)		Eguchi et al. 1995 Styrene	
69	Human	6.5 yr (Occup)			24.3 M (decreased color discrimination)		Fallas et al. 1992 Styrene	
70	Human	2.7 yr (Occup)			47 M (slowed reaction time)		Gamberale et al. 1976 Styrene	
71	Human	(Occup)			16 (decreased color discrimination)		Gobba et al. 1991 Styrene	
72	Human	76.6 mo (Occup)			10 M (decreased color discrimination)		Gong et al. 2002 Styrene	
73	Human	12.9-17.8 yr (Occup)			22 M (decreased color discrimination)		Iregren et al. 2005 Styrene	
74	Human	5 yr (Occup)			22.68 (impaired performance on neurobehavioral tests of visual reaction and memory)		Jegaden et al. 1993 Styrene	

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Table 3-1 Levels of Significant Exposure to Styrene - 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
75	Human	6.2 yr (Occup)		4	10 (decreased color discrimination)		Kishi et al. 2001 Styrene	
76	Human	4.9 yr (Occup)			75 M (impaired performance on visuomotor accuracy and psychomotor performance tests)		Lindstrom et al. 1976 Styrene	
77	Human	10.8 yr (Occup)			18 M (impaired vestibular function)		Moller et al. 1990 Styrene	
78	Human	17 yr (Occup)		3.68			Morata et al. 2002 Styrene	
79	Human	9.4 yr (Occup)			16 M (reduction in upper limit of hearing)		Morioka et al. 1999 Styrene	
80	Human	5 yr (Occup)			22 M (slowed distribution of nerve conduction velocities and ECG R-R interval)		Murata et al. 1991 Styrene	
81	Human	8.6 yr (Occup)			25 (decreased verbal learning skills)		Mutti et al. 1984a Styrene	

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Table 3-1 Levels of Significant Exposure to Styrene - 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
82	Human	5 yr (Occup)			30 M (EEG abnormalities)		Seppalainen and Harkonen 1976 Styrene	
83	Human	(Occup)			15.6 (hearing loss)		Sliwinska-Kowalska et al. 2003 Styrene	
84	Human	11 yr (Occup)			50 F (decreased peripheral nerve conduction velocity and prolonged latency of somatosensory evoked potentials)		Stetkarova et al. 1993 Styrene	
85	Human	(Occup)			24.8 M (impaired postural stability)		Toppila et al. 2006 Styrene	
86	Human	4 yr		100 M			Triebig et al. 1985 Styrene	Measured nerve conduction velocity.
87	Human	8.3 yr (Occup)			21.9 (impaired performance on neurobehavioral tests)		Tsai and Chen 1996 Styrene	
Developmental								
88	Human	>1 yr 7 d/wk 8 hr/d		82 F			Lemasters et al. 1989 Styrene	

Table 3-1 Levels of Significant Exposure to Styrene - 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
Cancer								
89	Mouse (CD-1)	6 hr/d 5 d/wk 98-104 wk				160 F (CEL: bronchioloalveolar carcinoma)	Cruzan et al. 2001 Styrene	

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

b The acute-duration inhalation MRL of 2 ppm; concentration divided by an uncertainty factor of 10 to account for human variability.

c The chronic-duration inhalation MRL of 0.2 ppm was calculated from a LOAEL of 20 ppm identified in meta-analysis (Benignus et al. 2005) of occupational exposure studies reporting significant alterations in color vision and choice reaction time and an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); ECG = electrocardiographic; F = Female; Gastro = gastrointestinal; Gd = gestational day; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); kHz = kilohertz; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; OHC = outer hair cell(s); pmd = pre-mating day; ppm = parts per million; Resp = respiratory; x = time(s); wk = week(s); yr = year(s)

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

Acute (≤14 days)

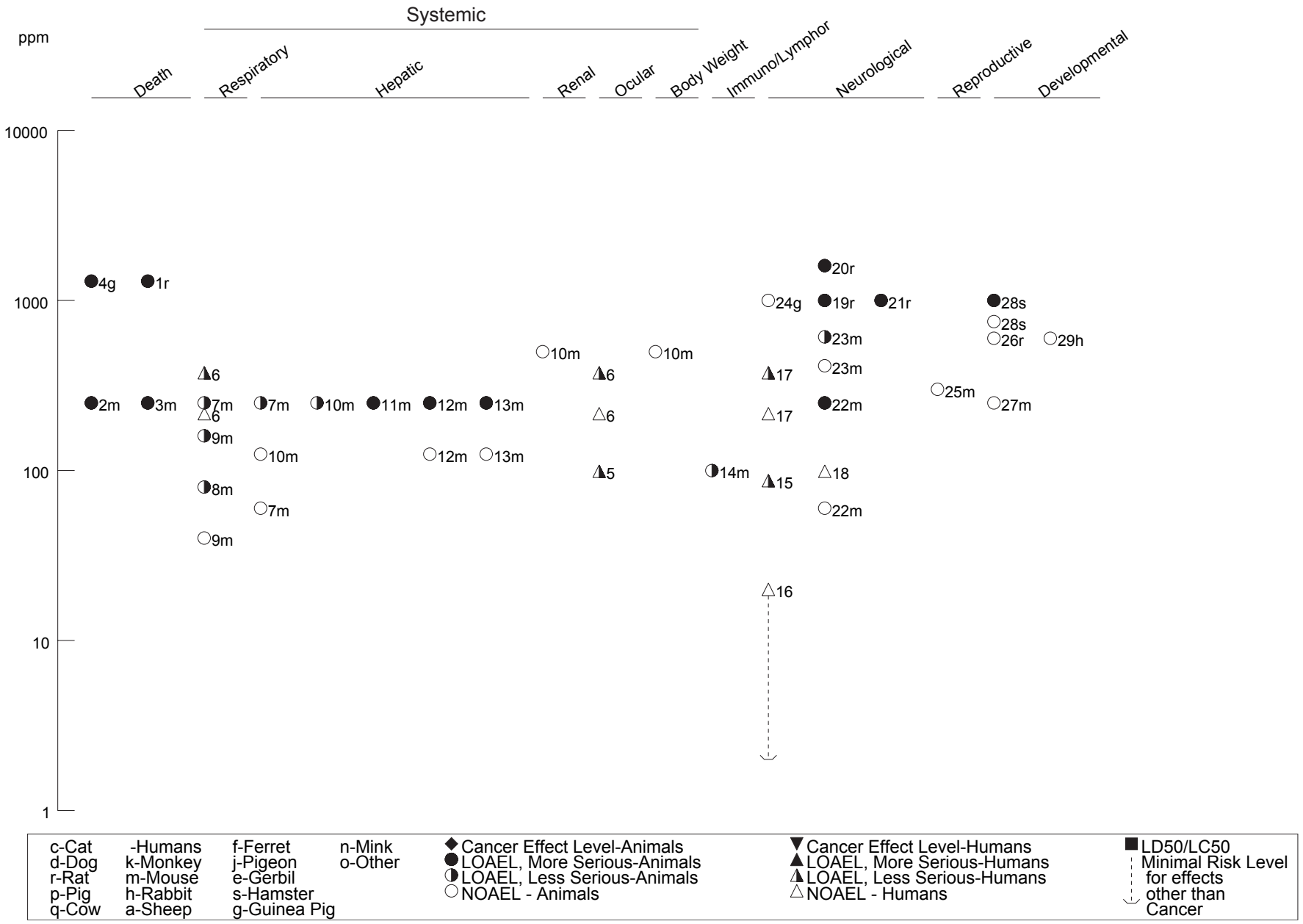

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Figure 3-1 Levels of Significant Exposure to Styrene - Inhalation *(Continued)*

Intermediate (15-364 days)

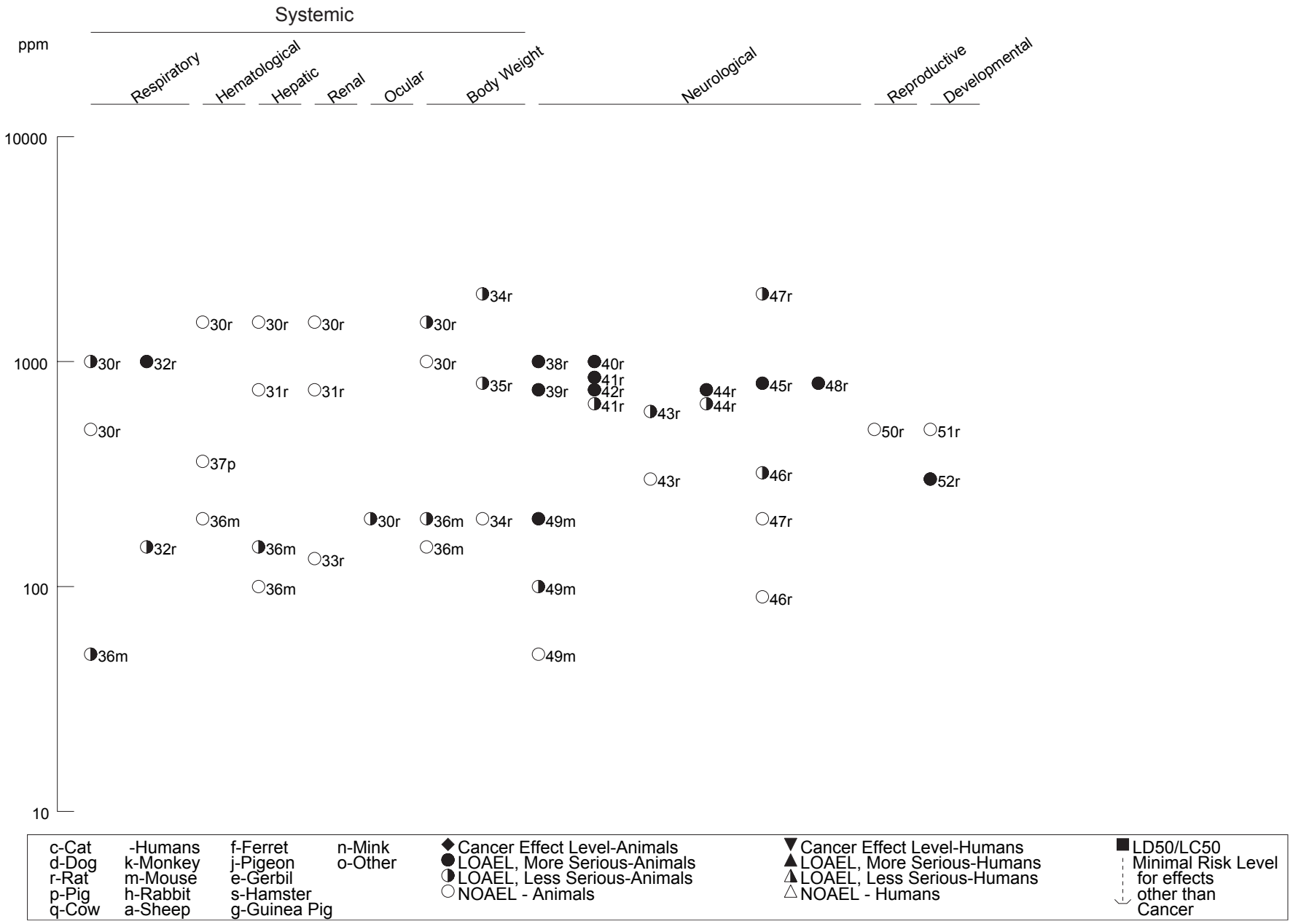

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Figure 3-1 Levels of Significant Exposure to Styrene - Inhalation *(Continued)*

Chronic (≥365 days)

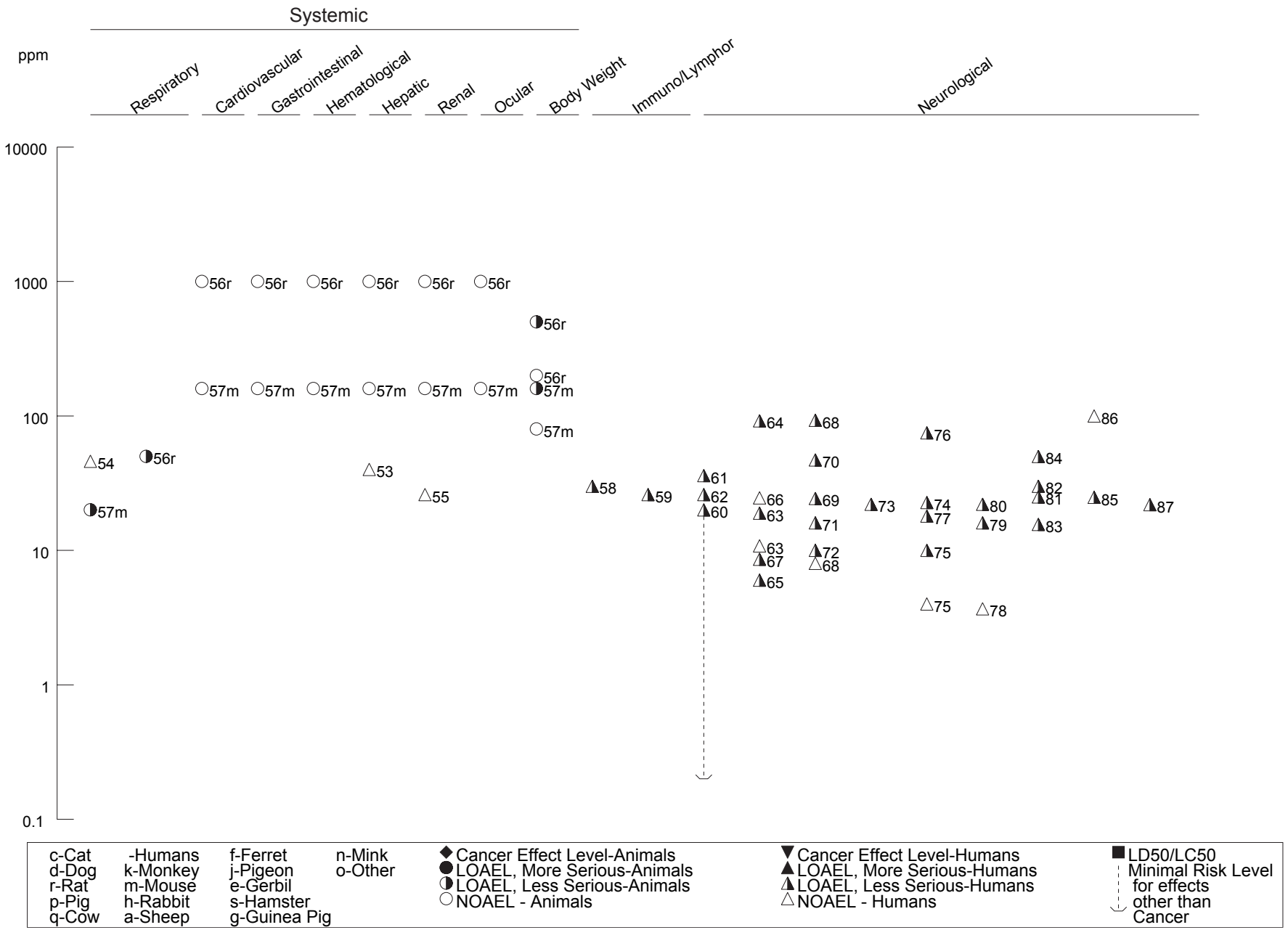
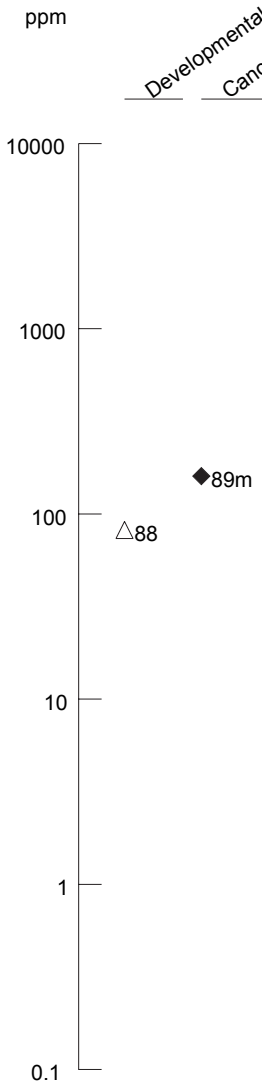


Figure 3-1 Levels of Significant Exposure to Styrene - Inhalation *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

In rats exposed to 150 ppm styrene 4 hours/day, 5 days/week for 3 weeks a decrease in nasal ciliar activity was observed; at 1,000 ppm, the nasal cilia activity was considered disabled (Ohashi et al. 1986). Electron microscopic examination of the nasal cavity of rats exposed to 1,000 ppm revealed very few ciliated cells and severe degeneration with marked vacuolization. Decreases in cilia activity were also observed in the trachea at 150 and 1,000 ppm. After a 12-week recovery period, nasal and tracheal cilia activity in the 150 ppm group was similar to controls; decreases in nasal cilia activity in the 1,000 ppm group was still lower than controls but was increased compared to rats killed at the end of the exposure period (Ohashi et al. 1986). In a longer-term study, focal hyperplasia was observed in the nasal olfactory epithelium of rats exposed to 1,000 ppm for 13 weeks (Cruzan et al. 1997); at 500 ppm, no histological alterations were observed in the respiratory tract. Chronic exposure to 50 ppm resulted in atrophic and/or degenerative changes in the nasal olfactory epithelium (Cruzan et al. 1998).

Mice appear to be more sensitive than rats to the respiratory toxicity of styrene. Exposure to 50 ppm styrene for 13 weeks resulted in atrophy of the nasal olfactory epithelium and dilatation, hypertrophy and hyperplasia of Bowman's gland (Cruzan et al. 1997). At 100 ppm, atrophy of the nasal olfactory nerve fibers was observed; focal crowding of nonciliated epithelial cells in the bronchioles were observed at 150 ppm. Chronic exposure resulted in respiratory metaplasia of the nasal olfactory epithelium and dilatation, respiratory metaplasia, epithelial hyperplasia of the Bowman's gland in mice exposed to 20 ppm and higher for 2 years (Cruzan et al. 2001). Decreased eosinohilia of epithelial cells and bronchiolar epithelial hyperplasia were observed in the lungs of mice exposed to 20 ppm and higher.

A study by Spencer et al. (1942) also provides some information on species differences. Rats and guinea pigs exposed 1,300 ppm for 7–8 hours/day, 5 days/week for 6 months showed nasal irritation, but rabbits and monkeys did not (Spencer et al. 1942). Histopathological examinations revealed no changes between test and control rats, but pronounced lung irritation was seen in guinea pigs that died after a few exposures. The irritation, which included congestion, hemorrhages, edema, exudation, and a general acute inflammatory reaction, was not seen in the guinea pigs, rabbits, and monkeys that survived the 6-month exposure period (Spencer et al. 1942).

Green et al. (2001a) suggest that the observed species differences between mice and rats are due to differences in styrene metabolism in the nasal epithelium. The rates of metabolism of styrene by cytochromes P-450 CYP2E1 and CYP2F2 to styrene oxide was similar for the two species. However, styrene oxide is more efficiently metabolized by epoxide hydrolases and glutathione S-transferases in rats than in mice. Thus, the higher levels of the reactive epoxide styrene oxide in mice is the likely cause of

the increased sensitivity in this species. In *in vitro* assays in fresh human nasal tissues, styrene oxide was not detected and high levels of epoxide hydrolases were detected, suggesting that humans have limited capacity to metabolize styrene in the nasal cavity and a high potential to detoxify styrene oxide. These data suggest that rodents may not be a good model for nasal toxicity in humans.

These well-conducted human and animal studies demonstrate the characteristic irritant properties of styrene on the upper respiratory tract.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation exposure to styrene.

No cardiovascular effects were observed in rats or mice exposed to concentrations as high as 1,000 ppm or 160 ppm, respectively, for 2 years (Cruzan et al. 1998, 2001).

Gastrointestinal Effects. Nausea was observed in humans exposed to 376 ppm styrene after 1 hour of exposure (Stewart et al. 1968). This effect is probably secondary to effects on the central nervous system, although mucociliary transport of styrene aerosol droplets from the upper respiratory tract to the gastrointestinal tract might also contribute to gastrointestinal irritation. A Russian study (Basirov 1975) reviewed by the World Health Organization (WHO 1983) investigated the effects of styrene on digestive function by testing the secretory, excretory, motor, and pepsinogen-generating functions of the stomach in 20 unexposed and 80 exposed workers. The authors reported that some workers in the styrene-butadiene synthetic rubber manufacture exposed to 60–130 mg/m^3 (14–31 ppm) styrene had decreased digestive function and decreased stomach acidity.

No histological alterations were observed in the stomach or intestines of rats exposed to 1,000 ppm (Cruzan et al. 1998) or mice exposed to 160 ppm (Cruzan et al. 2001) styrene for 2 years.

Hematological Effects. Several studies indicate that inhalation exposure of humans to styrene cause mild or no effects on the blood. In one study, the incidence of abnormal values for hematological parameters including erythrocyte, leukocyte, and platelet counts, and hemoglobin levels for 84 styrene workers generally exposed to <1 ppm styrene for 1–36 years was investigated. However, these workers were also exposed to intermittent high levels of styrene as well as to other chemicals. The percentages of the exposed group with abnormally low hemoglobin and erythrocyte values or abnormally high leukocyte values were less than those percentages in the 62-person control group. There were no abnormal

thrombocyte values reported in either the exposed or control groups (Thiess and Friedheim 1978). Findings from a group of 93 workers engaged in the manufacture of styrene polymers and exposed to generally <1 ppm styrene for 1–38 years were also presented in this study; only the incidence of abnormally low erythrocyte counts (in the group exposed to styrene) was found to be statistically significant ($p \leq 0.05$). However, because exposures could not be determined accurately and because there were concomitant exposures to other chemicals, the results of these studies are difficult to interpret.

Lowered erythrocyte counts, hemoglobin, platelets, and neutrophils and slightly higher mean corpuscular red cell volumes and neutrophil band counts were observed in workers in a styrene-butadiene rubber manufacturing plant (Checkoway and Williams 1982). The highest mean styrene level was 13.67 ppm. However, interpretation of this study is limited because multiple-chemical exposures were involved and exposure and clinical signs were measured at the same time and only once. An earlier study of styrene workers showed no definite pattern of hematological changes (Lorimer et al. 1978). In these studies, exposure levels were uncertain and multiple chemicals were involved.

In rats exposed to 49 ppm styrene, erythrocyte-aminolevulinate dehydratase (ALA-D) was depressed markedly. The decrease in enzyme activity was accompanied by a decrease in the enzyme content in bone marrow cells (Fujita et al. 1987). The investigators suggested that the changes may have been a result of styrene oxide reducing the enzyme protein is based on *in vitro* data. No hematological alterations were observed in 2-year studies in rats (Cruzan et al. 1998) and mice (Cruzan et al. 2001) exposed to concentrations as high as 1,000 or 160 ppm, respectively.

The well-conducted Thiess and Friedheim (1978) study as well as the more limited studies indicate that few adverse hematological effects occurred in styrene-exposed workers. However, the full meaning of the findings is not clear because of poor characterization of the exposure level and concurrent exposures to other chemicals.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after inhalation exposure to styrene.

No histological alterations were observed in skeletal muscle or bone of rats exposed to 1,000 ppm (Cruzan et al. 1998) or mice exposed to 160 ppm (Cruzan et al. 2001) for 2 years.

Hepatic Effects. Human studies on the hepatic effects of styrene inhalation frequently used serum levels of enzymes as indicators of liver dysfunction. In general, human studies have resulted in negative or equivocal results (Härkönen et al. 1984; Hotz et al. 1980; Lorimer et al. 1978; Thiess and Friedheim 1978). No significant alterations in alanine aminotransferase, aspartate aminotransferase, or γ-glutamyl transferase levels were observed in workers exposed to generally <1 ppm for 1–36 years (Thiess and Friedheim 1978) or 50–120 ppm for 5.1 years (Härkönen et al. 1984). A significant increase in γ-glutamyl transferase levels was observed in workers exposed to 5–20 ppm for up to 20 years; however, no alterations in alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase levels were observed (Lorimer et al. 1978). Another study of workers (Hotz et al. 1980) found significant correlations between the exposure level (as measured by styrene metabolite concentrations in morning urine) and ornithine carbamoyl transferase, alanine aminotransferase, and γ-glutamyl transferase levels. Among workers exposed to 50–100 ppm, the increases in these enzymes were modest, 67.8, 55, and 64.9% of reference levels.

Animal studies provide evidence that the liver is a target tissue for styrene; however, the hepatotoxicity of styrene in mice is inversely related to the duration of exposure. Hepatic effects have been observed following acute- and intermediate-duration exposure, but not after chronic exposure and the severity of the effects decreases with continuing exposure. Exposure to 250 or 500 ppm for 1–4 days resulted in marked to severe hepatocellular necrosis and degeneration in mice (Morgan et al. 1993a, 1993b, 1993c). The necrosis was characterized as centrilobular coagulative necrosis and was accompanied by pooling of erythrocytes in dilated sinusoids (Morgan et al. 1993a). The necrosis was often observed after a single exposure to 500 ppm or a 2-day exposure to 250 ppm and the severity did not increase with increasing duration (Morgan et al. 1993a). However, continued exposure resulted in regeneration and repair of the initial hepatic damage. After 14 days of exposure, minimal to mild focal necrosis was observed in female mice exposed to 250 ppm and no hepatic effects were observed in male mice exposed to 250 ppm or male and female mice exposed to 500 ppm (Morgan et al. 1993a). Similarly, a 13-week exposure to 200 ppm resulted in focal loss of hepatocytes with siderosis and centrilobular aggregates of siderophages in female mice (Cruzan et al. 1997). No histological alterations were observed in the livers of mice exposed to 160 ppm for 2 years (Cruzan et al. 2001). Strain differences have also been detected in mice. Morgan et al. (1993c) found that B6C3F1 and C57BL/6 mice were more sensitive than DBA/2 mice, which were more sensitive than Swiss mice. The severity scores for hepatocellular degeneration/necrosis following a 4-day exposure to 250 ppm were 3.2–3.5 in B6C3F1 mice, 3.6 in C57BL/6 mice, 2.4–2.9 in DBA/2 mice, and 2.0 in Swiss mice.

Rats appear to be less sensitive than mice to styrene-induced hepatoxicity. No histological alterations were observed in the livers of Sprague Dawley rats exposed to 1,000 ppm styrene for 13 weeks (Cruzan et al. 1997) or 2 years (Cruzan et al. 1998; Jersey et al. 1978). Parenchymal hydropic degeneration, steatosis, and congestion were observed in rats exposed to 300 ppm for 2 weeks (Vainio et al. 1979); the lack of incidence data limits the interpretation of these results.

Renal Effects. Based on the results of occupational exposure studies and animal toxicity studies, the kidney does not appear to be a sensitive target of styrene toxicity. Occupational exposure studies of workers exposed to 24 ppm (Viau et al. 1987), 53 ppm (Vyskocil et al. 1989), or 26 ppm styrene (Verplanke and Herber 1998) did not find significant alterations in urinary levels of β-microglobulin (not examined in Verplanke and Herber 1998 study), retinol-binding protein, or albumin. The Vyskocil et al. (1989) study also found no significant alterations in total protein, glucose, lysozyme, lactate dehydrogenase, or β-N-acetyl-D-glucosaminidase levels and Verplanke and Herber (1998) did not find alterations in β-galactosidase, N-acetyl-β-D-glucosaminidase, or alanine aminopeptidase. No histological alterations were observed in the kidneys following acute exposure of rats to 300 ppm (Vainio et al. 1979) or mice to 500 ppm (Morgan et al. 1993a), intermediate exposure of rats to 133–1,500 ppm (Cruzan et al. 1997; Spencer et al. 1942; Viau et al. 1987), or chronic exposure of rats to 1,000 ppm (Cruzan et al. 1998) or mice to 160 ppm (Cruzan et al. 2001). Additionally, no alterations in urinary levels of N-acetyl-D-glucosaminidase, γ-glutamyl transpeptidase, protein, or urea were observed in rats exposed to 500 ppm for 4 weeks (Loquet et al. 2000).

Endocrine Effects. Several occupational studies have examined potential endocrine effects in reinforced plastics industry workers exposed to styrene. Significant increases in serum prolactin levels were observed in male and female workers (Bergamaschi et al. 1996, 1997; Luderer et al. 2004; Mutti et al. 1984b). The serum prolactin levels significantly correlated with urinary metabolite (mandelic acid plus phenylglyoxylic acid) levels (Mutti et al. 1984b) and blood styrene levels (Luderer et al. 2004). Based on a logistic regression model, Luderer et al. (2004) estimated that workers exposed to styrene exposures >20 ppm would be more likely to have elevated serum prolactin levels than workers exposed to lower levels; a 10-fold increase in blood styrene concentrations would result in a 2.06-fold increase in serum prolactin levels. Similarly, Arfini et al. (1987) found that female styrene workers had an abnormal response to an intravenous dose of thyrotropin-releasing hormone; the levels of serum prolactin were significantly higher following exposure to thyrotropin-releasing hormone, as compared to referents. Two of these workers were re-examined after a 3-month period without styrene exposure; the serum prolactin response following thyrotrophin-releasing hormone exposure was similar to that in the referent group. No

significant alterations in the levels of thyroid stimulating hormone, follicle stimulating hormone, or luteinizing hormone were found in this study (Arfini et al. 1987). However, thyroid stimulating hormone levels were significantly correlated with urinary metabolite (mandelic acid plus phenylglyoxylic acid) levels.

Significant increases in serum prolactin levels have also been observed in female rats exposed to 150 ppm, 8 hours/day for 10 days (Umemura et al. 2005). No significant alterations in serum prolactin levels were observed in similarly exposed male rats (Umemura et al. 2005) or in male rats exposed to approximately 150, 500, or 1,500 ppm 6 hours/day for 5 days (Jarry et al. 2002). Acute exposure to styrene did not result in significant alterations in thyroid stimulating hormone levels in male or female rats (Umemura et al. 2005).

Ocular Effects. Eye irritation in humans has been reported at high styrene concentrations (Carpenter et al. 1944; Stewart et al. 1968). Immediate eye irritation was reported in two human subjects exposed to 800 ppm styrene for 4 hours (Carpenter et al. 1944). Eye irritation was also noted by Stewart et al. (1968) in two of five volunteers exposed to 376 ppm styrene for 1 hour. Also, 345 styrene-exposed workers 98% male) were evaluated for ocular toxicity due to exposure to styrene (5–200 ppm) for 7–20 years. No evidence of optic neuritis, central retinal vein occlusion, or retrobulbar neuritis was found. Conjunctival irritation was a complaint of 22% of the 345 workers exposed to styrene levels above 50 ppm (Kohn 1978).

Eye and nasal irritation was observed in rats and guinea pigs exposed to 1,300 or 2,000 ppm styrene, 7–8 hours/day, 5 days/week for durations ranging from 21 to 30 weeks (Wolf et al. 1956). Rabbits and monkeys were exposed for up to 360 days with no effects.

3.2.1.3 Immunological and Lymphoreticular Effects

Two occupational studies have found significant alterations in lymphocyte subsets in styrene workers. Increase in percentage of CD4+/CD3CD4 T-lymphocytes and decrease in CD35+CD+ peripheral lymphocytes were observed in oil industry workers exposed to styrene, as compared to unexposed controls (Biró et al. 2002). However, these results should be interpreted cautiously because there were marked differences in smoking habits between the styrene workers and controls (80% styrene workers smoked compared to 20% of controls) and the two groups were not matched for gender. Bergamaschi et al. (1995b) found an altered distribution of lymphocyte subsets in styrene workers exposed to an 8-hour

time-weighted average (TWA) of 10–50 ppm. Some of these alterations, particularly the reduction in total T-lymphocytes (CD3+) and T-helper cells (CD4+), may be indicative of reduced cell-mediated immunity. This is supported by the finding of an impaired response to concanavalin in styrene workers exposed to a median styrene concentration of 26 ppm (Tulinska et al. 2000), 187–256 ppm (Somorovská et al. 1999) or 54–56 ppm (Somorovská et al. 1999). No alterations in the response to pokeweed mitogen were observed (Somorovská et al. 1999; Tulinska et al. 2000).

In patch-testing studies of cross-reactors to styrene, styrene 7,8-oxide was more sensitizing than styrene itself (Sjöborg et al. 1984). The authors interpreted this as evidence that styrene requires metabolism by skin aryl hydrocarbon hydroxylase to styrene epoxide for its sensitizing activity.

In animals, styrene exacerbated the inflammatory reaction in mice challenged with ovalbumin (Ban et al. 2006). Styrene-only exposure resulted in slight increases in Th2 cytokine (IL-4, IL-5, IL-13) and Th1 cytokine (interferon-γ) levels; however, the statistical significance of these alterations were not reported.

3.2.1.4 Neurological Effects

The available human data suggest that the nervous system is the most sensitive target following chronic-duration inhalation exposure. It is likely the most sensitive target following shorter-term durations, but this has not been as extensively investigated. In studies examining the acute neurotoxicity of styrene, impairment of the vestibular-oculomotor system was observed in experimental subjects exposed to 87 ppm for 1 hour (Ödkvist et al. 1982) or 376 ppm for 1 hour (Stewart et al. 1968). No alterations in the performance of balance tests were observed at 216 ppm for 1 hour (Stewart et al. 1968), 117 ppm for 2 hours (Stewart et al. 1968), or 99 ppm for 7 hours (Stewart et al. 1968). Although these NOAELs are higher than the LOAEL identified in the Ödkvist et al. (1982) study, the studies are not comparable. The Ödkvist et al. (1982) study used sensitive tests of vestibular-oculomotor function compared to the modified Romberg test (subjects stand on one foot with eyes closed, walk heel to toe, touch finger to nose) used in the Stewart et al. (1968) studies. An increase in the reporting of “feeling inebriated” was found in subjects exposed to 376 ppm for 1 hour (Stewart et al. 1968); no increases in subjective symptoms were observed in subjects exposed to 20 ppm for 3–4 hours (Seeber et al. 2004) or 216 ppm for 1 hour (Stewart et al. 1968). Additionally, no alterations in reaction time were observed in subjects exposed to 20 ppm for 3 or 4 hours (Seeber et al. 2004). No human studies examined neurotoxicity following intermediate-duration exposure.

In an international cohort of styrene workers, a significant association between mortality from central nervous system disease and cumulative styrene exposure was found (Welp et al. 1996c). The rate ratio was 3.29 (95% confidence interval [CI] of 0.48–22.65) for workers exposed to 25–49 ppm-years and 16.32 (95% CI 3.47–76.73) for those exposed for 200–349 ppm-years. A similar relationship was found for shorter durations of styrene exposure. The rate ratio was 2.33 (95% CI 0.40–13.56) for workers exposed for 6–11 months and 8.80 (95% CI 1.87–41.33) for workers exposed for 7–9 months. A significant association between mortality from epilepsy and duration of styrene exposure was found; the rate ratio in workers exposed for ≥10 years was 28.4 (95% CI 2.11–381.5). Time since first exposure was also significantly associated with mortality from epilepsy. Significant associations between mental disorders and duration of exposure and between suicide and duration of exposure were also found; however, for both of these causes of death, the rate ratio decreased with increasing duration of exposure and the investigators noted that lifestyle factors, rather than a direct effect of styrene, appear to be the most likely cause of the higher mortality.

A variety of neurological effects have been reported in workers chronically exposed to styrene including altered vestibular function, impaired hearing, decreased color discrimination, altered performance on neurobehavioral tests, and increased clinical symptoms. In general, these occupational exposure studies have several limitations. In most cases, the exposure levels reflect current exposure conditions and do not take into consideration past exposure to higher styrene levels that may have resulted in permanent damage. Some workers, particularly laminators, wore respiratory masks with or without canisters; many investigators estimated exposure based on biomarker levels, particularly urinary mandelic acid levels, while others did not. As discussed in greater detail in Sections 3.4.3, 3.4.3.1, and 3.8.1, urinary levels of styrene metabolites mandelic acid and phenoxyglycolic acid have been shown to correlate with time-weighted average styrene exposure levels and may be a more reliable biological indicator of styrene exposure in workplaces with highly variable styrene exposure levels. Significant differences between workers and referents were reported as LOAELs; however, the magnitude of the alteration may have been subclinical. A summary of the neurological effects observed in styrene workers is presented in Table 3-2.

Color vision appears to be one of the more sensitive targets of styrene toxicity, with many studies reporting alterations. Color vision was typically measured using the Lanthony desaturated panel D-15 test in which the subjects were asked to arrange 15 painted caps in a line with definite chromatic

Table 3-2. Summary of Occupational Exposure Studies Examining Neurological End Points

Reference	Number of workers	Mean duration of exposure (years)	NOAEL (ppm)	LOAEL (ppm)	Comments
Decreased color discrimination					
Chia et al. 1994	21	18.8 (range: 5–23)		6	Styrene exposure was estimated from mean urinary levels of mandelic acid (84.0 mg/g creatinine; range of 1.3–504.1 mg/g creatinine). Significant decreases in color discrimination, as expressed as total color difference score; no concentration-response relationship was found.
Kishi et al. 2001	21–42	6.2	4	10	Workers divided into three groups based on urinary mandelic acid levels. Significant differences in CCI, compared to age-matched controls, were found in the two highest groups. Mean CCI in 4, 10, and 46 ppm groups (CCI levels in age-paired controls): 1.21 (1.17), 1.23 (1.12), and 1.27 (1.13). Significant difference in CCI also found in analysis using 87 age-matched workers/ controls.
Gong et al. 2002	43	6.4		10	Workers divided into two groups based on combined urinary mandelic acid and phenylglyoxylic acid level dividing line of 0.24 g/g creatinine (approximately 10 ppm); both groups were significantly different from controls. Mean CCI (mean of right and left eyes) in controls, low exposure, and high exposure groups: 1.02, 1.09, and 1.14.
Gobba et al. 1991	41			16	Significant differences found when compared to age-matched controls (41 workers/controls) and in older workers (≥40 years of age). CCI was significantly different in workers exposed to ≥50 ppm compared to workers exposed to <50 ppm.
Iregren et al. 2005	53–55	12.9–17.9 (range: 2–39)		22	Lifetime weighted average exposure calculated for each worker using historical exposure data. Total error score was significantly different from workers in the low exposure group (9 ppm).

Table 3-2. Summary of Occupational Exposure Studies Examining Neurological End Points

Reference	Number of workers	Mean duration of exposure (years)	NOAEL (ppm)	LOAEL (ppm)	Comments
Fallas et al. 1992	60	6.5 (range: >1–29)		24.3	Significant difference in the number of subjects with error axis in the red-green or blue-yellow ranges; no significant difference in calculated error scores.
Campagna et al. 1996	118	5.2 or 6.6		26	Mathematical threshold of impaired CCI was 4 ppm; the upper limit of the CI was 25.7 ppm.
Eguchi et al. 1995	57	7.0 (range: 0.2–26.8)	8	93	Significant difference in CCI between age-matched workers and controls. Workers divided into two groups—significant difference in CCI between high-concentration workers (urinary mandelic acid level of 1.06 g/L [range: 0.46–3.98 g/L] equivalent to 93 ppm) and age-matched controls. No difference in low concentration workers (urinary mandelic acid levels—mean of 0.02 g/L; range of 0.04–0.41 g/L; equivalent to 8 ppm). CCI scores in low and high exposure groups (CCI in age-matched controls) of 1.173 (1.118) and 1.332 (1.125).
Reaction time					
Edling et al. 1993	20	9 (range: 1–25)	8.6		Simple and choice reaction time (measured before and after work).
Tsai and Chen 1996	45	8.3		21.9	Directly exposed workers compared to workers without direct styrene exposure (range of styrene levels was 0–6.4 ppm). Complex reaction time (continuous performance test): 532.8 and 495.6 ms in directly and indirectly exposed groups.
Jegaden et al. 1993	30	5		22.68	Reaction times (measured in morning before work) in workers and controls were 0.29 and 0.27 seconds for simple reaction time and 0.37 and 0.32 seconds for complex reaction time.
Fallas et al. 1992	60	6.5 (range: >1–29)	24.3		No significant difference in simple reaction time (23.7 seconds versus 22.7 seconds in controls).

Table 3-2. Summary of Occupational Exposure Studies Examining Neurological End Points

Reference	Number of workers	Mean duration of exposure (years)	NOAEL (ppm)	LOAEL (ppm)	Comments
Mutti et al. 1984a	50			25	Workers divided into four groups based on combined levels of urinary mandelic acid and phenylglyoxylic acid of <150, 150–299, 300–350, and >450 mmole/mole creatinine; 150 mmole/mole creatinine equivalent to 25 ppm. Complex reaction time in all workers and controls: 623.6 and 488.3 ms; the percent variations from matched controls were 120 and 165% in the 25 and 50 ppm groups.
Gamberale et al. 1976	106	2.7 (range: 0.1–11)		47	Mean styrene levels in each location were 16.6, 59.3, 41.6, and 101.4 ppm for resin appliers and 13.6 and 49.3 ppm for assemblers. The average styrene level for the six sites was 47 ppm. Simple reaction times (measured in morning before work) were 274 ms in workers and 260 ms in controls.
Cherry et al. 1980	27	NR		92	Simple reaction times (measured in morning before work) were 252 and 230 ms in workers and controls. A slower reaction time was also observed during the workshift.
Other neurobehavioral tests					
Chia et al. 1994	21	18.8 (range: 5–23)		6	Styrene exposure was estimated from mean urinary levels of mandelic acid (84.0 mg/g creatinine; range of 1.3–504.1 mg/g creatinine). Several tests of memory—Benton Visual Retention test (score 6.0 in workers vs. 7.7 in controls), digit symbol test (26.3 in workers vs. 38.0 in controls), and digit span (11.7 in workers vs. 15.6 in controls) were significantly affected. No significant relations between test score and urinary mandelic acid or phenylglyoxylic acid levels. No alteration in Santa Ana dexterity test or pursuit aiming.
Edling et al. 1993	20	9 (range: 1–25)	8.6		No effect on performance of symbol digit test.
Jegaden et al. 1993	30	5		22.68	Significant impairment in performance on digit span test.

Table 3-2. Summary of Occupational Exposure Studies Examining Neurological End Points

Reference	Number of workers	Mean duration of exposure (years)	NOAEL (ppm)	LOAEL (ppm)	Comments
Fallas et al. 1992	60	6.5 (range: >1–29)		24.3	Significant alteration in aiming test. No significant alteration in Santa Ana test of dexterity, digit span, digit symbol, or Benton visual retention tests. However, there were significantly more workers with digit span test scores differing by more than one standard deviation from the mean, particularly in workers exposed to styrene for >10 years.
Mutti et al. 1984a	50			25	Workers divided into four groups based on combined levels of urinary mandelic acid and phenylglyoxylic acid of <150, 150–299, 300–350, and >450 mmole/mole creatinine; 150 mmole/mole creatinine equivalent to 25 ppm. Impaired performance on verbal learning test; no significant relationship with test scores in duration of exposure or exposure level.
Clinical symptoms					
Flodin et al. 1989	8 or 9	11.6 (range: 6–21)		6	High prevalence of clinical symptoms—abnormal tiredness (7/8 subjects) and short memory (8/8 subjects) in workers exposed to 6 ppm styrene; high prevalences of problems concentrating (7/9 subjects) and irritation (8/9 subjects) were also observed in workers exposed to 12 ppm. Prevalence was not compared to a referent group.
Edling et al. 1993	20	9 (range: 1–25)		8.6	Reported more acute symptoms than controls (mean of 2.9 in workers versus 1.8 in controls). More frequently responded positively to the following questions: are you abnormally tired, do you often have painful tingling in some parts of your body, do you have a headache at least once a week.
Checkoway et al. 1992	16–27	3.4–4.1	10.8	18.9	Higher prevalence of headache, dizziness, light headedness, fatigue, irritability, feeling "drunk" at work, and memory loss.
Cherry et al. 1980	27	NR		92	At the end of the workshift, changes in self-reported physical and mental tiredness and general health scores were correlated with blood styrene concentrations.

Table 3-2. Summary of Occupational Exposure Studies Examining Neurological End Points

Reference	Number of workers	Mean duration of exposure (years)	NOAEL (ppm)	LOAEL (ppm)	Comments
Hearing					
Morata et al. 2002	65	17 (range: 1–39)		3.68	Noise level 82 dBA. A higher prevalence of high frequency hearing loss (47%) compared to controls (33%), but difference was not statistically significant. Significantly poorer thresholds were observed at 2, 3, 4, 6, and 8 kHz in the styrene group. The OR of 1.19 (95% CI, 1.11–1.28) times greater for each 1 year of age was calculated and 2.44 times greater (95% CI, 1.01–5.89) for each increment of 1 mmol mandelic acid/g creatinine in urine.
Śliwińska-Kowalska et al. 2003	194	NR		15.6	Current styrene concentrations ranged from 0.05 to 46 ppm; average worklife mean styrene concentration was 15.6 ppm. Average noise level was 80.3 dBA. Abnormal audiograms were found in 63.3% of styrene workers, compared to 33.8% in the unexposed controls. The OR of hearing loss was 5.2 (95% CI, 2.9–8.9). A positive linear relationship between styrene working life exposure levels and hearing thresholds.
Morioka et al. 1999	93	9.4		16	Sound levels in the workplace ranged from 53 to 95 dBA. Significant correlation between individual percentiles of the upper limit of hearing and styrene concentrations in workers exposed for ≥5 years; the prevalence rates below the 75th percentile were significantly higher than 25% in workers exposed to >16 ppm.
Möller et al. 1990	18	10.8 (range: 6–15)	18		No styrene-related alterations in the pure-tone audiometry and speech discrimination tests were found. Seven workers displayed abnormal results in distorted speech and/or cortical response audiometry tests.
Calabrese et al. 1996	20	7.6 (range: 2–23)	36		All workers had normal hearing thresholds and no abnormalities of stapedial reflex were found. No significant effect on ABRs (as compared to 10 control subjects) were observed; additionally, when nine subjects were re-tested after 3 weeks without exposure, no significant difference between pre- and postrecovery values were found.

Table 3-2. Summary of Occupational Exposure Studies Examining Neurological End Points

Reference	Number of workers	Mean duration of exposure (years)	NOAEL (ppm)	LOAEL (ppm)	Comments
Vestibular					
Möller et al. 1990	18	10.8 (range: 6–15)		18	Significantly higher sway with eyes open or closed in the static posturography test, increased latency in saccade test, a phase lag and depressed gain in unpredictable and predictable stimulation in the smooth eye pursuit test, and impaired ability to suppress vestibuloccular reflex in sinusoidal and pseudorandomized tests.
Toppila et al. 2006	88	NR		24.8	Nonlaminators used as the comparison group; 88 pairs of age-matched workers were used for postural stability tests; the mean styrene and mandelic acid phenylglycoxylic acid concentrations for these pairs were 24.8 ppm and 1.4 mmol/L for the laminators and 4.8 ppm and 0.3 mmol/L for the nonlaminators. Poorer performance in dynamic tests on the static platform. In the tilting platform and virtual reality tests, sway velocity was greater and workers became unstable and displayed large correctional movements.
Calabrese et al. 1996	20	7.6 (range: 2–23)		36	Abnormal results in the vestibulocular tests, these alterations persisted after the 3-week recovery period. No alterations in visual suppression test or postural performance were found.
Nerve conduction velocity					
Seppäläinen and Härkönen 1976	96	5	30		Styrene exposure was estimated using end-of-shift urinary mandelic acid levels collected weekly for 5 consecutive weeks. No significant differences in median, ulnar, deep peroneal, or posterior tibial nerve motor or sensory conduction velocities were observed, as compared to a referent group with a similar age distribution.
Štětkářová et al. 1993	15	11		50	Decreased peripheral conduction velocities in median and tibial nerves were observed in female styrene workers; prolonged latencies of peripheral and cortical somatosensory evoked potentials were also observed in the female workers exposed to styrene.

Table 3-2. Summary of Occupational Exposure Studies Examining Neurological End Points

Reference	Number of workers	Mean duration of exposure (years)	NOAEL (ppm)	LOAEL (ppm)	Comments
Triebig et al. 1985	11	4	100		No significant alterations in maximum conduction velocity in the ulnar nerve or distal conduction velocity of the sensory fibers of the ulnar and median nerves.

ABR = auditory brainstem response; CCI = color confusion index; CI = confidence interval; LOAEL = lowest-observed-adverse-effect level; MS = millisecond; NOAEL = no-observed-adverse-effect level; NR = not reported; OR = odds ratio

sequence; the color confusion index (CCI) quantifies the number of types of mistake. A significant correlation between CCI and urinary mandelic acid concentration (after correction for age) was observed in workers at fiberglass reinforced plastic facilities (Kishi et al. 2001). When workers were divided into three groups based on end-of-shift urinary mandelic acid levels, there were significant differences between CCI in workers with a mean a mandelic acid level of 0.14 or 0.65 g/L and age-matched referents; no difference was found for the third group with a mean mandelic acid level of 0.05 g/L. The investigators estimated that these urinary mandelic acid levels were equivalent to styrene exposure levels of 4, 10, and 46 ppm. Thus, this study identifies a NOAEL of 4 ppm and a LOAEL of 10 ppm for decreased color discrimination. Similarly, Gong et al. (2002) found significantly higher CCI values in workers at a fiberglass reinforced plastic boat facility with end-of-shift urinary mandelic acid and phenylglyoxylic acid levels of ≥0.24 g/g creatinine or <0.24 g/g creatinine; a mandelic acid plus phenylglyoxylic acid urine level of 0.24 g/g creatinine is equivalent to a styrene exposure level of 10 ppm. A significant increase in CCI was also observed in workers at fiberglass reinforced plastic facilities exposed to a geometric man concentration of 16 ppm, as compared to age-matched controls (Gobba et al. 1991). In contrast to other studies, Gobba et al. (1991) did not find a significant relationship between end-of-shift urinary mandelic acid levels and CCI; however, urinary styrene levels correlated with CCI values. Significantly higher CCI values were observed in fiberglass reinforced workers with a mean urinary mandelic acid levels of 1.06 g/L, which is roughly equivalent to a styrene exposure level of 93 ppm (Eguchi et al. 1995). This study did not find significant alteration in workers with a mean urinary mandelic acid level of 0.02 g/L, equivalent to 8 ppm. Another study of fiberglass reinforced plastic workers (some of this cohort was examined by Gobba et al. 1991 and Campagna et al. 1995) found a significant association between CCI and styrene exposure levels (Campagna et al. 1996). The investigators concluded that color vision impairment could be detected at styrene levels of 4 ppm with a 95% upper confidence limit of 26 ppm. Two other occupational exposure studies using different measures of color vision impairment also found significant alterations. Chia et al. (1994) found significantly poorer color discrimination, after adjusting for age, education, and alcohol consumption, in 21 workers at a fiber-reinforced plastic boat manufacturing facility; the styrene exposure level of 6 ppm was estimated from a mean end-of-shift urinary mandelic acid level of 84.0 mg/g creatinine. No relationship between the total color difference score and the urinary mandelic acid level was found. In 60 workers in the shipbuilding industry with a mean styrene exposure level of 24.3 ppm, a significantly higher incidence of workers with errors in the blue-yellow or red-green ranges, compared to a referent group, was found (Fallas et al. 1992). Total error score was significantly different in workers, with a lifetime weighted average exposure level of 22 ppm styrene, as compared to workers in a low exposure group (9 ppm) (Iregren et al. 2005). Several studies found improvements in color vision following an

extended period of no styrene exposure or lower exposure. Triebig et al. (2001) reported a significant improvement in CCI scores following a 4-week period with no styrene exposure; in contrast, no improvement in CCI scores was found in another group of styrene workers following a 1-month period without styrene exposure (Gobba et al. 1991). Two studies found significant improvements in color vision (age-adjusted color confusion score or CCI score) were observed in styrene workers following a decrease in styrene air level (Castillo et al. 2001; Triebig et al. 2001). However, one study found no change in age-adjusted near visual contrast sensitivity following a decrease in styrene exposure levels (Castillo et al. 2001).

A number of studies have found significant alterations in performance on a variety of neurobehavioral tests; among these studies, reaction time appears to be the most frequently examined end point. Significant increases in simple reaction time have been observed in styrene workers exposed to concentrations of 21.9, 22.68, 47, or 92 ppm (Cherry et al. 1980; Gamberale et al. 1976; Jegaden et al. 1993; Tsai and Chen 1996); tests for reaction time were measured in the morning before the work shift, suggesting that the effect was not due to acute exposure to styrene. The reaction times were 4–10% slower in the styrene workers as compared to the referent groups. No significant alterations in simple reaction time were observed in workers exposed to 8.6 ppm (Edling et al. 1993) or 24.3 ppm (Fallas et al. 1992). Similarly, complex reaction time was significantly increased among styrene workers exposed to 21.9, 22.68, or 25 ppm (Fallas et al. 1992; Jegaden et al. 1993; Mutti et al. 1984a); the variance from controls ranged from 7.5 to 20%. No alterations in complex reaction time were observed in workers exposed to 8.6 ppm (Edling et al. 1993). Impaired performance on the digit span test, which measures attention/concentration, was observed in workers exposed to 6 ppm (Chia et al. 1994), 22.68 ppm (Jegaden et al. 1993), or 24.3 ppm (Fallas et al. 1992). Other neurobehavioral performance tests that may be altered by chronic exposure to styrene included digit symbol or symbol digit tests at 6 ppm (Chia et al. 1994), visuomotor at 50 ppm (Mutti et al. 1984a) or 75 ppm (Lindstrom et al. 1976), and memory at 25 ppm (Mutti et al. 1984a). However, other studies have not found significant alterations in digit symbol at 8.6 ppm (Edling et al. 1993), 24.3 ppm (Fallas et al. 1992), or 25 ppm (Mutti et al. 1984a), or memory at 75 ppm (Lindstrom et al. 1976). By pooling response data for several tests of neurobehavioral performance, Mutti et al. (1984a) were able to analyze exposure-response relationships. When workers were divided into four groups based on morning urinary mandelic acid and phenylglycoxylic acid levels, the number of subjects with abnormal scores on greater than one, two, or three tests increased with increasing exposure concentration. Additional analyses demonstrated that the exposure intensity and duration of exposure affected a worker’s performance on neurobehavioral tests and the duration of exposure appeared to affect performance more than exposure intensity.

Clinical symptoms of neurotoxicity have been reported by styrene workers; commonly reported symptoms included headaches, dizziness, impaired memory, and feeling "drunk". At 6 or 12 ppm, abnormal tiredness and short memory were reported by most of the styrene workers examined by Flodin et al. (1989); problems concentrating and irritation were also reported by most workers exposed to 12 ppm. After a 7-month period without styrene exposure, there was a marked improvement in symptoms and the mean number of symptoms reported was 1.9, compared to 5.3 reported 7 months earlier. Fiberglass reinforced plastic industry workers exposed to 18.9 or 50.0 ppm reported a higher prevalence of headaches, dizziness, light headedness, fatigue, irritability, feeling "drunk", and memory loss (Checkoway et al. 1992); the prevalence of clinical signs was not significantly increased in workers exposed to 10.8 ppm. Increases in the incidence of headache, memory disturbances, forgetfulness, dizziness, and sensory symptoms in the upper and lower extremities were observed in workers with high exposure to styrene compared to those with low styrene exposure (Matikainen et al. 1993a); exposure levels were not reported. A significantly higher incidence of subjective symptoms (nausea, feeling of drunkenness, dizziness, and disturbance) was observed in styrene workers exposed to 4–164 ppm, as compared to controls (Geuskens et al. 1992). No significant difference in the incidence of symptoms related to cognitive motor disturbances (lack of concentration, understanding, trouble with movements) was found. Fiberglass boat manufacturing workers exposed to 92 ppm reported a higher prevalence of physical and mental tiredness at the end of the work shift than controls (Cherry et al. 1980). No alterations in the reporting of clinical symptoms were observed in plastic industry workers exposed to a mean concentration of 8.6 ppm (Edling et al. 1993). One study examined styrene's potential to affect olfactory function. No alterations in three clinical tests of olfactory function were observed in styrene workers exposed to 24.6 ppm styrene for minimum of 4 years (Dalton et al. 2003).

Styrene-induced damage to hearing and the vestibular system have been examined in a number of studies of chronically exposed workers. Several studies have reported significant associations between styrene exposure and hearing impairment; however, interpretation of the findings is limited by confounding exposure to noise or other solvents. Noise studies have found that exposure to >85 dB for over 10 years can result in a 10% hearing loss (Prince et al. 1997). Morioka et al. (1999) found an increased prevalence of workers with a urinary mandelic acid level of >0.3 g/L (approximately 16 ppm) with an upper frequency of hearing below the 75th percentile for normal. However, interpretation of the results is limited by confounding exposure to noise and exposure to other solvents, particularly toluene, which has been shown to be ototoxic. The noise levels ranged from 53.0 to 95.0 dBA with 14% of the measurements exceeding 85 dBA. Another study (Muijser et al. 1988) of styrene workers found a

significant difference in hearing threshold at 8 kHz between indirectly exposed workers (mean styrene level of 14 ppm) and directly exposed workers (mean styrene level of 32 ppm); however, no differences were found in comparisons of indirectly and directly exposed workers with referent workers. The noise level for both groups of styrene workers was 80–85 dBA for most of the day. Śliwińska-Kowalska et al. (2003) found a significantly elevated risks of hearing loss among styrene workers exposed to a mean styrene concentration of 15.6 ppm (average noise level of 80.3 dBA). The odds ratio (adjusted for noise and gender) in workers only exposed to styrene was 5.2 (95% CI 2.9–8.9). The hearing losses were found within the range of 2–8 kHz. Morata et al. (2002) found significant decreases in hearing thresholds at 2, 3, 4, and 6 kHz in workers exposed to 0.05–22 ppm (mean of 4 ppm); no difference in the prevalence of high frequency hearing loss, as compared to referent workers, was found. The fairly wide range of exposure levels adds a great deal of uncertainty to estimating the LOAEL from this study; although the mean exposure is reported as the LOAEL in Figure 3-1, this value may be an overly conservative estimate of the true LOAEL. Other studies have not found significant alterations in hearing. Möller et al. (1990) found no indications of hearing loss in workers exposed to 18 ppm styrene. Sass-Korstak et al. (1995) did not find significant relationships between lifetime styrene exposure and hearing loss in workers at fiber-reinforced plastics manufacturing facilities. The cumulative styrene exposure level was calculated using data for current exposure (25 ppm for directly exposed workers and 8 ppm for indirectly exposed workers), length of time in each job category, and a downward adjustment for self-reported respirator use. The average noise levels (L_{eq}) were 88.1 and 89.2 dBA for the directly and indirectly exposed workers, for nonexposed workers, a sound level of 80 dBA was assumed. In another study of fiberglass workers (Calabrese et al. 1996), no significant alterations in audiometric tests or auditory brainstem response were observed in workers exposed to a mean styrene level of 36 ppm. Additionally, a 3-week recovery period did not result in any significant changes in auditory brainstem responses (pre- and post-recovery) in nine of the workers.

Other studies have examined workers for styrene-induced vestibular effects. Significant alteration in tests of central vestibulocular and optocular motor movements (i.e., static posturography, smooth eye pursuit, saccade, and vestibulocular reflex tests) were observed in workers at a plastic boat manufacturing facility exposed to a TWA styrene concentration of 18 ppm (Möller et al. 1990). No indications of labyrinthine or peripheral vestibular lesions were observed. Toppila et al. (2006) also found significant alterations in postural stability in workers at fiberglass-reinforced plastic boat manufacturing facilities exposed to 25 ppm styrene. In contrast, Calabrese et al. (1996) did not find significant alterations in visual suppression tests or postural stability in fiberglass plant workers exposed to 36 ppm styrene. Significant

alterations in vestibulocular reflex were found. A 3-week recovery period did not result in significant changes in the test results.

Workers exposed to styrene in several industries at mean concentrations of 5–125 ppm had mild sensory neuropathy characterized by decreased sensory conduction amplitude and increased duration, but there were too few people to define a NOAEL (Rosen et al. 1978). Peripheral neuropathy and reduced nerve conduction velocity was also reported in an individual following a 2-day exposure to an unknown amount of styrene (and other chemicals) (Fung and Clark 1999). Leg weakness, leg muscle cramps, and paresthesia were also reported in two styrene workers (Gobba et al. 1995). Moderate sensorimotor neuropathy of the demyelinating type was diagnosed in both cases based on the clinical symptoms and the decreased motor nerve conduction velocity in the peroneal nerve and decreased sensory nerve conduction velocity in the sural and median nerves. Alterations in nerve conduction velocity have also been observed in styrene workers. Decreased peripheral conduction velocities in the median and tibial nerves and prolonged latencies of peripheral and cortical somatosensory evoked potentials were observed in female styrene workers exposed to 30–130 ppm (midpoint of the range is 50 ppm) (Štětkářová et al. 1993). Significant decreases in ulnar and peroneal maximum conduction velocities and increased peroneal motor distal latencies were observed in fiber reinforced workers exposed with urinary mandelic acid levels (end of shift) of ≥250 mg/L, as compared to referent workers. Motor distal latencies in the workers with urinary mandelic acid levels ≥250 mg/L were also significantly lower than in workers with urinary mandelic acid levels <250 mg/L (Yuasa et al. 1996). In contrast, no alterations in motor or sensory nerve conduction velocity in the ulnar, median, deep peroneal, or posterior tibial nerve were observed in workers exposed a to TWA styrene concentration of 30 ppm (based on urinary mandelic acid excretion) (Seppäläinen and Härkönen 1976), and no alteration in motor or sensory nerve conduction velocity was observed in workers exposed to approximately 100 ppm for a median of 4 years (Triebig et al. 1985). Although Seppäläinen and Härkönen (1976) did not find alterations in nerve conduction velocity, they found abnormal EEGs in 24% of the styrene workers, as compared to reported values for the normal population. The mean urinary mandelic acid level (975 mg/dm^3) was higher in workers with abnormal EEGs compared to those with normal readings (750 mg/dm^3). Similarly, a significantly higher absolute EEG power in alpha band in the fronto-temporal region of the brain was found in workers with high styrene exposures, as compared to workers with low-level exposure (Matikainen et al. 1993a).

The majority of the available animal neurotoxicity studies have focused on hearing impairment. Hearing loss and a loss of outer hair cells (OHC) in the organ of Corti were observed in rats acutely exposed to 1,000 ppm (Campo et al. 2001; Lataye et al. 2003) or 1,600 ppm (Crofton et al. 1994). In contrast, acute

exposure of guinea pigs to 1,000 ppm did not result in hearing loss or OHC damage (Lataye et al. 2003). Intermediate-duration exposure studies have consistently found hearing loss and loss of OHC in rats exposed to ≥750 ppm styrene (Campo et al. 2001; Lataye et al. 2000, 2001; Loquet et al. 2000; Pouyatos et al. 2002; Pryor et al. 1987; Yano et al. 1992). Exposure to 600–650 ppm resulted in OHC losses but no alterations in hearing threshold (Loquet et al. 1999; Makitie et al. 2002; Pouyatos et al. 2002). A NOAEL of 300 ppm was identified by Makitie et al. (2002).

Other neurological effects that have been observed in animal studies include lethargy and unsteady gait in mice exposed to 250 ppm for 2 weeks (Cruzan et al. 1997), an increase in astroglial alterations at 320 ppm (Rosengren and Haglid 1989), a decrease in nerve conduction velocity in rats exposed to 2,000 ppm, but not 200 ppm, for 32 weeks (Yamamoto et al. 1997), and concentration-related alterations in nystagmus elicited by optokinetic, vestibular, simultaneous optokinetic-vestibular, and saccadic stimulation in rats exposed to 830–4,000 ppm styrene for at least 60 minutes (actual duration of exposure was not reported) (Niklasson et al. 1993).

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

Information on the reproductive effects of styrene in humans is available from epidemiological studies of the reproductive outcomes of females employed in the various industrial operations in which styrene is used. However, exposures to styrene were not adequately quantified in any of the studies cited. In one study, spontaneous abortions among 9,000 Finnish chemical workers from 1973 to 1976 were analyzed (Hemminki et al. 1980). The risk of spontaneous abortion expressed as number of abortions per 100 pregnancies) was significantly higher in women employed in styrene production compared to all women in Finland 15.0 vs. 5.5). However, this increase was not detected in a follow-up study of the same workers (Hemminki et al. 1984). An increase in the occurrence of spontaneous abortions was also observed in a study of 76 women involved in processing polystyrene plastics (McDonald et al. 1988); the ratio of observed to expected abortions was 1.58 (95% CI 1.02–2.35). The possible embryotoxic effects of styrene on 67 female lamination workers compared to 67 age-matched controls were evaluated in a second study (Härkönen and Holmberg 1982). The number of births was significantly lower among the workers exposed to styrene. This result was explained in part by a greater number of induced abortions in the styrene-exposed group. The number of spontaneous abortions was not elevated in the exposed

women. No increased risk of spontaneous abortions among workers processing polymerized plastics or heated plastics made of vinyl chloride or styrene was reported (Lindbohm et al. 1985). The authors reported that the statistical power of the study was low due to the small study population. These studies are not conclusive since the workers were exposed to chemicals other than styrene in the workplace and the concentrations of styrene were not adequately reported. Two studies have examined the potential of styrene to induce menstrual disturbances. A significant increase in the incidence of oligomenorrhea was observed in petrochemical industry workers; the adjusted odds ratio was 1.65 (95% CI 1.05–2.55) (Cho et al. 2001). Although the odds ratio includes an adjustment for exposure to other aromatic chemicals, there was potential for exposure to other chemicals; only three workers were exposed to styrene only and none of these women reported oligomenorrhea. In contrast, no significant alterations were observed in women working at reinforced plastics facilities with a mean styrene exposure level of 52 ppm for women directly exposed to styrene and 13 ppm for those indirectly exposed (Lemasters et al. 1985). Several studies have examined levels of prolactin, follicle stimulating hormone, and luteinizing hormone levels in female styrene workers; the results of these studies are discussed in Section 3.2.1.2, Endocrine Effects.

Several studies have also examined styrene's potential to induce male reproductive effects. A significant decrease in sperm concentration, total sperm count, percentage of normal sperm, and percentage of nonvital sperm and an increase in sperm velocity were observed in 23 workers employed at a styrene manufacturing facility for approximately 6 months, as compared to levels during the first week of employment (Kolstad et al. 1999a; results are also presented in Kolstad et al. 1999b). No significant relationships between urinary mandelic acid level and sperm density, total sperm count, or proportion of sperm with normal morphology were observed. A positive correlation between mandelic acid levels and the percentage of nonvital sperm was found, but the trend test was not statistically significant. In a large multinational study of male styrene workers, no significant alterations in time-to-pregnancy were found; the odds ratio, adjusted for maternal age, use of oral contraceptives, maternal and paternal smoking habits, time-to-pregnancy starting year, length of employment, and country, was 0.79 (95% CI 0.59–1.05) (Kolstad et al. 2000). Additionally, no significant alterations in fertility rates were found when workers were divided into groups based on length of exposure, period of attempting pregnancy, or exposure group (monitoring data from a subset of facilities were used to divide workers into different exposure groups) (preliminary data from this study was reported by Kolstad et al. 1999c). Similarly, no significant alterations in time to pregnancy among styrene workers exposed to high or intermediate/low exposure levels (based on urinary mandelic acid levels) (Sallmén et al. 1998).

Two animal studies have examined the reproductive toxicity of styrene following inhalation exposure. No statistically significant alterations in the frequency of abnormal sperm heads were observed in mice 3–5 weeks after exposure to 300 ppm for 5 days (Salomaa et al. 1985). In a two-generation study in rats (Cruzan et al. 2005b), no significant alterations in reproductive performance, estrous cycle length, spermatogenic parameters, or histological alterations in reproductive tissues were observed at concentrations up to 500 ppm.

3.2.1.6 Developmental Effects

Limited information concerning developmental effects of styrene in humans is available from studies of delivery outcome of women employed in the plastics industry (processing styrene or polyurethane plastics). Case-control studies performed in Sweden and Norway did not detect an increase in the odds ratio for developmental effects (stillbirth, infant death, malformations, low birth weight) in women who worked in the plastic industry (Ahlborg et al. 1987). However, actual levels of styrene exposure were not known for either group of workers. Another study did not find significant increases in the occurrence of congenital malformations in children of men or women working at reinforced plastics facilities (Härkönen et al. 1984). A <4% lower birthweight were observed in the children of women who worked in the reinforced plastics industry in areas with elevated levels of styrene (mean concentration of approximately 82 ppm) during pregnancy (Lemasters et al. 1989). However, this decrease was not statistically significant ($p=0.08$). These studies suggest that developmental effects in exposed workers are not of major concern, but the data are not adequate to exclude this effect. Moreover, interpretation of the results is complicated due to exposure of the workers to other chemicals in the workplace such as toluene, xylene, acetone, methylene chloride, and methyl ketone (Lemasters et al. 1989), as well as thermal degradation products of styrene polymers (Ahlborg et al. 1987). Workers may also be exposed to aerosols containing aldehydes, ketones, alcohols, esters, acids, and anhydrides. An expert panel convened by the National Toxicology Program (NTP 2006) concluded that the human data are not sufficient to evaluate the potential developmental toxicity of styrene in humans.

The developmental toxicity of styrene has been examined in several animal studies. The average fetal crown-rump length was significantly reduced in rats exposed to 300 ppm on gestational days 6–15, but was not affected in rats exposed to 600 ppm (Murray et al. 1978); the investigators concluded that this effect was not treatment-related. A few skeletal variants such as lumbar spurs and delayed ossification of sternebrae occurred in the styrene-exposed litters at a higher incidence than the control litters; however, the occurrence of this effect was similar to historical controls. No developmental effects were observed

in rabbits exposed to 600 ppm styrene on days 6–18 of gestation (Murray et al. 1978). Although there was a significant increase in the incidence of unossified sternebrae in the 600 ppm group, it did not exceed that found in historical control data. No significant alterations in the number of live fetuses, dead/resorbed fetuses, or malformed fetuses were observed in the offspring of mice exposed to 250 ppm styrene on days 6–16 of gestation (Kankaanpää et al. 1980). In the same study, exposure of hamsters to 1,000 ppm of styrene on days 6–18 of gestation resulted in a significant increase in the number of dead or resorbed fetuses; no other alterations were observed (Kankaanpää et al. 1980). No effects were observed at 750 ppm. Similarly, no developmental effects were observed in a two-generation study in which rats were exposed to 500 ppm prior to mating, during mating, and during gestation and lactation (Cruzan et al. 2005b). In contrast, Katakura et al. (1999, 2001) reported a significant increase in neonatal deaths in the offspring of rats exposed to 300 ppm on gestational days 6–20.

Two studies have examined potential neurodevelopmental effects. Some minor alterations in tests of developmental milestones (incisor eruption), functional observational battery tests (forelimb grip strength), and swimming maze test were observed in the F2 offspring of rats exposed to 500 ppm in a two-generation study; these alterations were attributed to a lower body weight rather than a neurodevelopmental effect of styrene (Cruzan et al. 2005a). No alterations in locomotor activity, acoustic startle response, or brain morphology and weights were observed in this study. Another study found delays in righting reflex and incisor eruption in the offspring of rats exposed to 300 ppm on gestational days 6–20 (Katakura et al. 2001). This study (Katakura et al. 1999, 2001) also found alterations in homovanillic acid levels in the cerebrum and 5-hydroxyindoleacetic acid levels in the hippocampus of the offspring in the 300 ppm group.

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

A number of studies have examined the carcinogenic potential of styrene in workers at styrene manufacturing and polymerization facilities, reinforced plastics facilities, and styrene-butadiene manufacturing facilities and among community members exposed to elevated styrene workers. Although there are several epidemiologic studies which suggest there may be an association between styrene exposure and an increased risk of leukemia and lymphoma, the evidence is generally inconclusive due to

multiple chemical exposures and inadequate documentation of the levels and durations of exposure to styrene.

Of the industries examined, workers employed at glass-reinforced plastics manufacturing facilities are likely to be exposed to higher levels of styrene and have lower potential for exposure to other carcinogenic agents. Some studies of glass-reinforced plastic workers have found suggestive evidence of increased cancer risks, particularly in workers with longer exposures to higher levels of styrene. No alterations in the number of deaths from cancer were observed in workers with high styrene exposure (mean levels at two facilities were 42.5 and 71.5 ppm) (Okun et al. 1985). In a follow-up study of these workers (Ruder et al. 2004), a significant increase in the number of deaths from urinary tract cancer (standardized mortality ratio [SMR] 3.44; 95% CI 1.26–7.50) was observed among workers with high styrene exposure; a trend for increasing SMRs for urinary tract cancer with increasing duration of exposure was also observed. The SMRs were not significantly elevated for other cancer types. In a very large epidemiological study of nearly 16,000 workers in the styrene plastic industry, the death rate from leukemia was twice as high in areas of high exposure as in areas of low exposure (Wong 1990); however, there were no statistically significant differences. In a follow-up study conducted 12 years later (Wong et al. 1994), significant increases in deaths from all cancers (SMR 115.5, 95% CI 104.8–127.1), cancer of the esophagus (SMR 191.7; 95% CI 104.8–321.7), bronchus, trachea, or lung (SMR 140.6; 95% CI 119.3–164.0), cervix or uteri (SMR 283.5; 95% CI 135.9–521.3), and female genital organs (SMR 201.6; 95% CI 107.4–344.8) were observed. However, no relationships between styrene exposure (exposure level or duration of exposure) and deaths from these cancer types were found. No significant increases in the incidence of non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma, leukemia, or all lymphohematopoietic malignancies were observed in workers at Danish reinforced plastics facilities in which 50–100% of the workers were involved in reinforced plastics production (Kolstad et al. 1993, 1994). However, when workers were divided by first year of employment, there was a significant increase in leukemia (standard incidence ratio [SIR] 1.69, 95% CI 1.09–2.49) among workers with a latency of ≥10 years and first year of employment of 1964–1970; when the data were analyzed by the length of employment, the incidence of leukemia was only significantly elevated among workers employed for <1 year. Significant increases in incidence were also observed for pancreatic cancer in workers with a high probability of styrene exposure (incidence rate ratio of 2.2; 95% CI 1.1–4.5) and urinary bladder cancer in workers with the highest probability of exposure and employed for >1 year (incidence rate ratio of 2.1; 95% CI 1.1–4.1) (Kolstad et al. 1995). No significant alterations in the incidence of leukemia, lymphoma, or other cancers were observed in styrene workers at eight British reinforced plastic manufacturing facilities (Coggon et al. 1987). In a large international cohort of workers

employed in the reinforced plastics industry (this cohort included the British cohort examined by Coggon et al. 1987 and the Danish cohort examined by Kolstad et al. 1993, 1994, 1995), no significant alterations in no excess in mortality from all cancer or cancer of the lymphatic and hematopoietic tissues were observed (Kogevinas et al. 1993, 1994). However, significant increases in the incidence of lymphatic and hematopoietic neoplasms were observed in workers with a latency of at least 10 years (relative risk [RR] 2.90; 95% CI 1.29–6.48 in workers with a latency of 10–19 years and RR 3.97; 95% CI 1.30–12.13 for workers with a latency of ≥20 years) and in workers exposed to ≥100 ppm styrene (RR 3.11; 95% CI 1.07–9.06 for workers exposed to 100–119 ppm; RR 3.08; 95% CI 1.04–9.08 for workers exposed to 120–199 ppm; RR 3.59; 95% CI 0.98–13.14 for workers exposed to ≥200 ppm), as compared to unexposed workers. An increase in the number deaths from malignant lymphomas was also observed in workers exposed to 120–199 ppm styrene (RR 7.15; 95% CI 1.21–42.11).

An increase in lymphatic leukemia (4 observed deaths versus 0.5 expected) in workers exposed to polymer extrusion fumes, solvents, and colorants, but was not found to be related to duration or level of exposure (Ott et al. 1980). In a follow-up to this study, which followed the workers for another 11 years (Bond et al. 1992), a nonsignificant increase in the number of deaths from lymphatic and heamtopoietic tissue cancers (SMR 144; 95% CI 95–208) was observed. Statistically significant increases in the number of deaths from lymphatic and hematopoietic cancer were observed in workers exposed to 1–4 ppm styrene and a 15-year minimum latency (SMR 160; 95% CI 102–238); however, significant alterations were not found in workers exposed to ≥5 ppm or with longer latency periods. In another study of workers involved in the styrene production, polymerization, or processing, a statistically significant excess of lymphoma deaths (3 deaths observed versus 0.56 expected) was reported; 2 of the 3 deaths occurred in men <40 years of age who had been exposed for at least a year (Hodgson and Jones 1985). However, the lack of association with actual exposure levels or specific durations and the small number of observed deaths requires cautious interpretation. No significant alterations in the number of deaths from cancer were observed in workers (1960 subjects) exposed to styrene in a production and polymerization facility (Frentzel-Beyme et al. 1978). In a study of workers at a styrene-polystyrene manufacturing facility who had at least 5 years of exposure, there were no significant increases in cause-specific mortality (Nicholson et al. 1978). However, when workers employed for <5 years were included in the analysis, there was an apparent increase in the number of deaths from lymphoma or leukemia (statistical analysis not conducted).

A number of older studies provide suggestive evidence of increased risk of lymphatic and hematopoietic cancers in workers at styrene-butadiene rubber manufacturing facilities (Matanoski and Schwartz 1987;

Matanoski et al. 1990; McMichael et al. 1976; Meinhardt et al. 1982); however, these studies provided limited exposure data and did not adjust for contribution of 1,3-butadiene to the overall cancer risk. A case-control study (Matanoski et al. 1993, 1997; Santos-Burgoa et al. 1992) provides suggestive evidence that the increase in leukemia was due to exposure to 1,3-butadiene rather than to styrene exposure. However, increases in the risk of lymphosarcoma and myeloma were associated with styrene exposure (Matanoski et al. 1997). More recent studies of styrene-butadiene workers include adjustments for 1,3-butadiene exposure. A cohort mortality study conducted by Delzell and associates (Delzell et al. 1996; Macaluso et al. 1996) examined workers at many of the same styrene-butadiene rubber manufacturing facilities examined by Matanoski and associates and Meinhardt and associates. In this examination of 15,649 male synthetic rubber workers employed for at least 1 year at one of eight styrene-butadiene rubber manufacturing facilities in the United States or Canada, significant increases in deaths from leukemia were observed among hourly employees (SMR 143; 95% CI 104–191), particularly among workers employed for ≥10 and ≥20 years since hire (SMR 224; 95% CI 149–323) (Delzell et al. 1996). When workers were divided by year of hire and age at death, leukemia deaths were elevated in workers who were hired between 1950 and 1959 (SMR 200; 95% CI 122–310) and who were <55 years of age at the time of death (SMR 179; 95% CI 104–287). Using calculated estimates of exposure levels to 1,3-butadiene, styrene, and benzene, Macaluso et al. (1996) found that 75% of the cohort was exposed to 1,3-butadiene with a median cumulative exposure of 11.2 ppm-years, 83% of the cohort was exposed to styrene with a median cumulative exposure of 7.4 ppm-years, and 25% of the cohort was exposed to benzene with a cumulative exposure of 2.9 ppm-years. Among workers with leukemia, 86% had 1,3-butadiene exposure and 90% had styrene exposure; median cumulative exposure levels of 1,3-butadiene and styrene were about 3 times higher than the rest of the cohort. Workers with a cumulative exposure of 20–79 ppm 1,3-butadiene had a relative risk of leukemia mortality (after adjustment by race, age, and cumulative styrene exposure) that was 50% higher than workers with a cumulative exposure of 0.1–19 ppm and workers with a cumulative exposure of >80 ppm had a 70% higher relative risk than the low exposure group; the progressive of relative risk with increasing cumulative exposure was statistically significant. Although a similar progression was observed for cumulative styrene exposure, the trend was not statistically significant. A follow-up to the Delzell et al. (1996) study, which tracked deaths for an additional 7 years (Sathiakumar et al. 2005), found similar results. A significant increase in deaths from leukemia was observed among hourly workers employed for >10 years and hired 20–29 years earlier (SMR 258; 95% CI 156–403). Increases in deaths for colorectical cancer among workers employed for >10 years and hired 20–29 years earlier (SMR 147; 95% CI 103–205) and deaths from prostate cancer among workers employed for <10 years and hired >30 years earlier (SMR 155; 95% CI 113–206). Significant increases deaths from leukemia were observed in

workers involved in polymerization, coagulation, and finishing processes, maintenance workers, and laboratory workers; these workers had the highest potential exposure to 1,3-butadiene, styrene, and possibly dimethyldithiocarbamate. Subsequent analysis of these data using updated exposure assessments (Cheng et al. 2007; Delzell et al. 2001; Graff et al. 2005) found that the increased risk of leukemia was positively associated with 1,3-butadiene exposure. Positive associations between cumulative 1,3-butadiene exposure (ppm-years) and leukemia and between cumulative styrene exposure and leukemia were observed; the associations were only statistically significant at the highest cumulative exposure levels for 1,3-butadiene (≥362.2 ppm-years) or styrene (≥60.4 ppm) (Delzell et al. 2001). However, when the relative risks were adjusted for 1,3-butadiene and dimethyldithiocarbamate cumulative exposure, cumulative styrene exposure was no longer significantly associated with leukemia (Delzell et al. 2001; Graff et al. 2005). Because styrene, 1,3-butadiene, and dimethyldithiocarbamate exposure were correlated, it is difficult to separate the risks for each individual compound.

Several population-based studies have examined the possible carcinogenicity of styrene. A case-control study found a significant increase in prostate cancer (odds ratio of 5.5; 95% CI 1.4–21.8) and rectal cancer (odds ratio of 5.1; 95% CI 1.4–19.4) among workers with medium to high exposure to styrene (Gerin et al. 1998). Workers in the following professions were considered to have medium to high styrene exposure: motor vehicle painters, motor vehicle repairers, firemen, and plastic mouldmakers. Another study found a significant increase in the incidence of rectal cancer (SIR 3.11; 95% CI 1.14–6.77) among individuals with occupational exposure to styrene (Antilla et al. 1998). A limitation of both of these studies is the lack of exposure information, including levels of styrene and confounding exposure to other chemicals; thus, it is difficult to ascribe the increased cancer risks to styrene exposure. Loughlin et al. (1999) examined former students who attended a high school adjacent to synthetic styrene-butadiene rubber production facilities between 1963 and 1993 and found no significant alterations in deaths from lymphatic and hematopoietic cancer. Two studies have examined the possible association between styrene exposure and breast cancer. A case-control study by Cantor et al. (1995) found significant elevations in the risk of breast cancer among women possibly exposed to styrene in the workplace. Coyle et al. (2005) found a significant higher incidence of age-adjusted breast cancer rate in men and women, women, and women ≥50 years of age and living in counties with EPA toxics release inventory (TRI) facilities with on-site releases of styrene. As with the other population-based studies, these studies did not monitor styrene levels or exposure to other potentially carcinogenic chemicals and thus provided limited information on the carcinogenic potential of styrene.

The carcinogenicity of styrene has been examined in three studies in rats (Conti et al. 1988; Cruzan et al. 1998; Jersey et al. 1978; Maltoni et al. 1982) and one study in mice (Cruzan et al. 2001). No significant increases in the incidence neoplastic lesions were observed in rats exposed to styrene concentrations as high as 1,000 ppm 6 hours/day, 5 days/week for 2 years (Cruzan et al. 1998). Similarly, exposure of female rats to 600 or 1,000 ppm styrene 6 hours/day, 5 days/week for 21 months did not result in styrene-related increases in the incidence neoplastic tumors (Jersey et al. 1978); a high incidence of chronic murine pneumonia in the control and 1,000 ppm male rats precludes the use of the male data for assessing the carcinogenic potential of styrene. There was a significant trend (Cochran-Armitage test conducted by ATSDR) for increased incidence of malignant mammary tumors in female rats exposed to styrene 4 hours/day, 5 days/week for 52 weeks (Conti et al. 1988); the incidences were 6/60, 6/30, 4/30, 9/30, 12/30, and 9/30 in the 0, 25, 50, 100, 200, and 300 ppm groups, respectively. No other significant increases in specific tumors were observed in this study (Conti et al. 1988; Maltoni et al. 1982). The findings of the Conti et al. (1988) study conflict with those of Cruzan et al. (1998), who found a concentration-related decrease in mammary tumors in female rats exposed to similar or higher styrene concentrations for a longer duration (20/60, 13/44, 9/43, 5/38, and 2/59 in female rats exposed to 0, 50, 200, 500, or 1,000 ppm, respectively). The decrease in body weight observed in the female rats exposed to ≥200 ppm may have influenced the occurrence of mammary tumors. In contrast to the results in rat studies, significant increases in the incidence of bronchioalveolar carcinoma were observed in female mice exposed to 160 ppm 6 hours/day, 5 days/week for approximately 2 years (Cruzan et al. 2001). The incidences of bronchioloalveolar carcinoma were 0/50, 0/50, 2/50, 0/50, and 7/50 in the 0, 20, 40, 80, and 160 ppm female mice, respectively). Significant trends for increasing incidences of bronchioalveolar ademona were also observed for the male and female mice; the respective incidence of adenomas was 15/50, 21/50, 35/50, 30/50, and 33/50 in males and 6/50, 16/50, 16/50, 11/50, and 24/50 in females. The incidence of adenoma was significantly higher than controls in males exposed to 40, 80, or 160 ppm and in females exposed to 20, 40, or 160 ppm.

As discussed by IARC (2002) and Cruzan et al. (2002), the lung tumors observed in the mice are likely due to the *in situ* formation of styrene 7,8-oxide resulting in cytotoxicity and increased cell proliferation. The relevance of these tumors to humans has been questioned due to species differences in the metabolism of styrene in the lungs. In rats and mice, Clara cells have the ability to metabolize styrene to styrene 7,8-oxide in the lung, whereas humans have limited ability to metabolize styrene to styrene 7,8-oxide in the lung. A physiologically based pharmacokinetic (PBPK) model predicted that the total amount of styrene oxide in the terminal bronchioles in mice is 10 times higher than in rats and 100-fold higher than in humans. In addition to these quantitative differences in the generation of styrene 7,8-oxide

between rats and mice, there are qualitative differences in styrene metabolism. Mice produce higher levels of the R-enantiomer of styrene oxide, as compared to rats; the R-enantiomer has been shown to be more potent pneumotoxic than the S-enantiomer. The ratio of R- to S-enantiomers ranges from 2.2 to 2.87 in mice exposed to 20–160 ppm styrene and from 0.7 to 0.73 in rats exposed to 50–1,000 ppm. Thus, mice appear to be very sensitive to the induction of lung tumors and the mechanism of inducing lung tumors is not likely to be relevant to humans. Although the mechanism involved in the development of lung tumors in mice may not be applicable to humans, other mechanisms of styrene carcinogenicity may be relevant for humans.

3.2.2 Oral Exposure

No studies were located regarding health effects in humans after oral ingestion of styrene. Based on the animal data that follow, the oral toxicity of styrene in humans would be expected to be low to moderate.

3.2.2.1 Death

No deaths in humans from ingesting styrene have been reported in the evaluations of case studies (EPA 1989c; Gosselin et al. 1984; NIOSH 1983).

The approximate reported oral LD_{50} for male and female rats was 5,000 mg/kg (Wolf et al. 1956). A 100% survival rate and 100% mortality rate were reported in rats exposed to single oral doses of styrene (observation period 2 weeks) at 1,600 and 8,000 mg/kg, respectively (Spencer et al. 1942). Death in this study was mainly due to pronounced irritation of the esophagus and stomach. In another study, female mice were given a single oral dose of 1,350 mg/kg styrene on the 17th day of pregnancy (Ponomarkov and Tomatis 1978). After weaning, the progeny received the same dose once per week. The treatment was suspended after 16 weeks due to high mortality among the progeny (including both males and females). Fifty percent of the males and 20% of the females had died after 20 weeks, despite the suspension of treatment at week 16. The cause of death was liver necrosis and lung congestion. A high mortality rate was reported in 40 female rats exposed to 250 mg/kg/day styrene for 52 weeks (Conti et al. 1988). Mortality was significantly elevated in male and female rats administered styrene by gavage at a dosage level of 2,000 mg/kg/day for 78 weeks (NCI 1979b). In this study, mortality was unaffected at dosage levels of 500 and 1,000 mg/kg/day in male and female rats. Male mice administered styrene at doses of 150 or 300 mg/kg/day for 78 weeks showed increased mortality; however, the female mice did not.

The highest reliable LOAEL values and LD_{50}s values in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding endocrine, metabolic, musculoskeletal, or dermal/ocular effects in humans or animals after oral exposure to styrene.

For the following systemic effects resulting from oral exposure to styrene, the highest NOAEL values and all reliable LOAEL values for each species and duration category are recorded in Table 3-3 and plotted in Figure 3-2.

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to styrene.

Severe lung congestion was observed in mice that were the offspring of dams given a single oral dose of styrene at 1,350 mg/kg on the 17th day of gestation and that continued to receive the same dose once per week after weaning (Ponomarkov and Tomatis 1978). The lung congestion was noted following 16 weeks of styrene administration. No respiratory effects were observed in rats exposed to 35 mg/kg/day styrene in drinking water for 105 weeks (Beliles et al. 1985).

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans following oral exposure to styrene.

No cardiovascular effects were observed in rats chronically exposed to 35 mg/kg/day in drinking water (Beliles et al. 1985).

Gastrointestinal Effects. Abdominal pain was reported by 11% of the residents of two apartment buildings exposed to elevated levels of styrene in drinking water for 3 days (Arnedo-Pena et al. 2003). The concentration of styrene in the water was 900 μg/L and the dose was estimated to be 0.026 mg/kg/day. Based on other symptoms and the higher prevalence of symptoms among residents living near the contaminated water tank, it is likely that some of the observed effects were due to inhalation exposure of styrene vapors from the repair of a firewater tank adjacent to the drinking water tank.

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Table 3-3 Levels of Significant Exposure to Styrene - 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	1 d (GO)				5000 (LD50)	Wolf et al. 1956 Styrene	
Neurological								
2	Rat	once (GO)			200 M (increased dopamine receptor binding)		Agrawal et al. 1982 Styrene	
3	Rat (Wistar)	14 d 1 x/d (GO)			100 M[b] (impaired learning)		Husain et al. 1985 Styrene	
Developmental								
4	Rat (Sprague-Dawley)	Gd 11 (GO)		300			Daston et al. 1991 Styrene	
5	Rat (Sprague-Dawley)	2 x/d Gd 6-15 (GW)		300 F			Murray et al. 1978 Styrene	
INTERMEDIATE EXPOSURE								
Immuno/ Lymphoret								
6	Rat (UF)	5 d/wk 4 wk (GO)		196 M	294 M (impaired immune response)		Dogra et al. 1992 Styrene	
7	Mouse (Swiss)	5 d/wk 4 wk (GO)		23 M	30 M (impaired immune response)		Dogra et al. 1992 Styrene	

Table 3-3 Levels of Significant Exposure to Styrene - 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
Neurological								
8	Rat	90 d 1 x/d (GO)			200 M (increased dopamine receptor binding)		Agrawal et al. 1982 Styrene	
9	Rat (Long- Evans)	5 d/wk 8 wk (GO)			500 M (impaired learning)		Bushnell 1994 Styrene	
10	Rat	15 d 1 x/d (G)			906 M (increased serotonin and noradrenaline and decreased monoamine oxidase levels)		Husain et al. 1980 Styrene	
11	Rat (Wistar)	15 days (GO)		250 F			Khanna et al. 1994 Styrene	
Reproductive								
12	Rat	90 d (continuous) (W)		35			Beliles et al. 1985 Styrene	
13	Rat	60 d 6 d/wk 1 x/d (GO)		200 M		400 M (marked degeneration of seminiferous tubules, decreased spermatozoa)	Srivastava et al. 1989 Styrene	
Developmental								
14	Rat	Ld 1-21 (GO)		200 M	400 M (altered testicular enzyme levels and decreased spermatozoa counts)		Srivastava et al. 1992a Styrene	

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Table 3-3 Levels of Significant Exposure to Styrene - 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
15	Rat (Wistar)	6 d/wk pnd 1-61 (GO)		100 M	200 M (decreased testes weight and spermatozoa counts)		Srivastava et al. 1992b Styrene	
16	Rat (NS)	Gd 1-21, Gd 1- Ld 14-21, or LD 1- Ld 14-21 (GO)			200 (increased dopamine receptor binding)		Zaidi et al. 1985 Styrene	
Cancer								
17	Mouse	16 wk 1 d/wk (GO)				1350 (CEL: lung tumors)	Ponomarkov and Tomatis 1978 Styrene	
CHRONIC EXPOSURE								
Death								
18	Rat	78 wk 5 d/wk 1 x/d (GO)				2000 (decreased survival in males and females)	NCI 1979b Styrene	

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Table 3-3 Levels of Significant Exposure to Styrene - 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
Systemic								
19	Rat	105 wk 7 d/wk (W)	Resp	35			Beliles et al. 1985 Styrene	
			Cardio	35				
			Gastro	35				
			Hemato	35				
			Musc/skel	35				
			Hepatic	35				
			Renal	35				
			Dermal	35				
			Other	35				
20	Rat	120 wk 1 d/wk 1 x/d (GO)	Hepatic	500			Ponomarkov and Tomatis 1978 Styrene	
			Renal	500				
21	Dog	561 d 1 x/d (GO)	Hemato	200	400 (Heinz body formation)		Quast et al. 1979 Styrene	
Cancer								
22	Mouse	78-103 wk 5 d/wk 1 x/d (GO)				300 (CEL: lung tumors)	NCI 1979b Styrene	

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

b The acute-duration oral MRL of 0.1 mg/kg/day; dose divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans and 10 human variability).

Cardio = cardiovascular; CEL = cancer effect level; d = day(s); F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; (GO) = gavage in oil; (GW) = gavage in water; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; pnd = post-natal day; Resp = respiratory; x = time(s); (W) = drinking water; wk = week(s)

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

Acute (≤14 days)

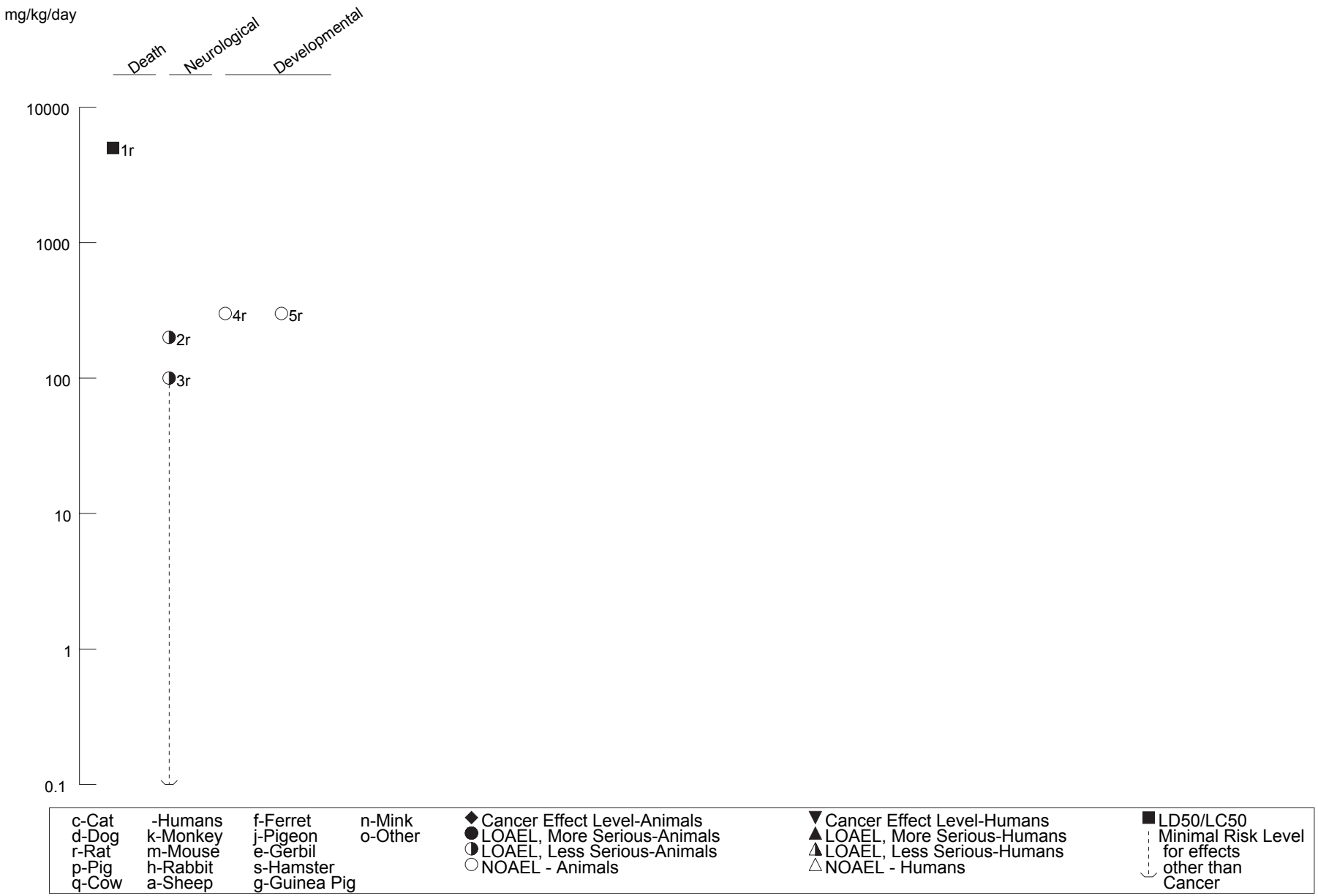

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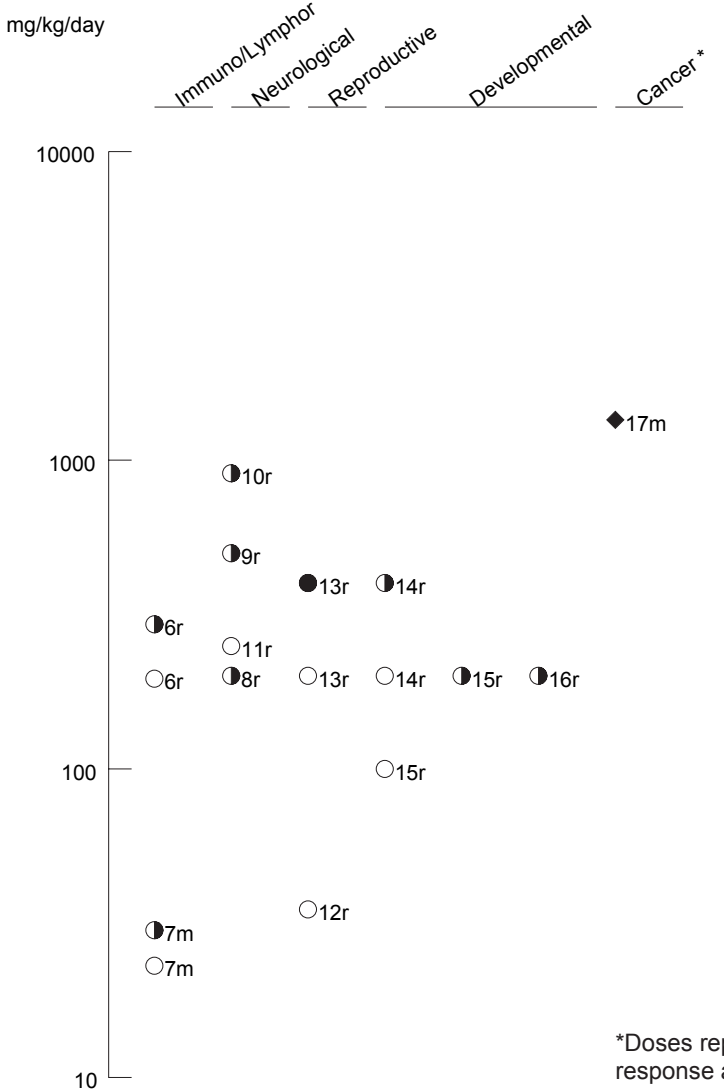
Figure 3-2 Levels of Significant Exposure to Styrene - Oral *(Continued)*

Intermediate (15-364 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink
d-Dog	k-Monkey	j-Pigeon	o-Other
r-Rat	m-Mouse	e-Gerbil	
p-Pig	h-Rabbit	s-Hamster	
q-Cow	a-Sheep	g-Guinea Pig	

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

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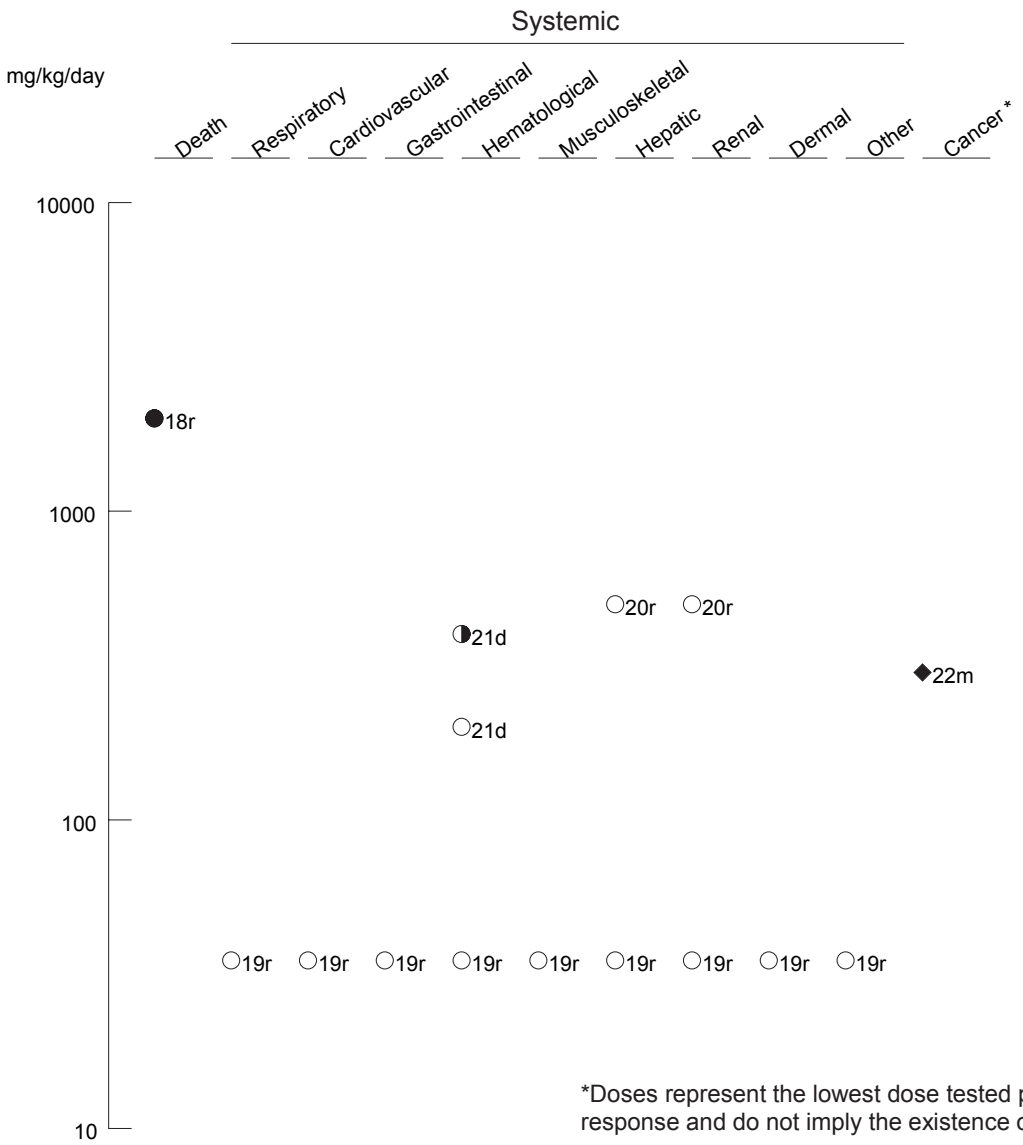
Figure 3-2 Levels of Significant Exposure to Styrene - Oral *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat -Humans f-Ferret n-Mink
d-Dog k-Monkey j-Pigeon o-Other
r-Rat m-Mouse e-Gerbil
p-Pig h-Rabbit s-Hamster
q-Cow a-Sheep g-Guinea Pig

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

No gastrointestinal effects were observed in rats chronically exposed to 35 mg/kg/day styrene in drinking water (Beliles et al. 1985).

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to styrene.

Intra-erythrocytic Heinz bodies were regularly detected in a dose-related manner in male and female dogs chronically exposed to 400 or 600 mg/kg/day groups and sporadically in females in the 200 mg/kg/day group (Quast et al. 1979). There were occasional decreased red blood cell counts, hemoglobin levels, and erythrocyte sedimentation rates in males and females in the 600 mg/kg/day groups. Increased hemosiderin deposits and intranuclear inclusions in liver were noted in animals dosed with 600 mg/kg/day. This was probably secondary to the effects on the red blood cells. The formation of intra-erythrocytic Heinz bodies was readily reversible upon discontinuing the administration of styrene in the 600 mg/kg/day group after 470 days of exposure. No hematological effects were observed in rats chronically exposed to 35 mg/kg/day (Beliles et al. 1985).

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to styrene.

Some animal studies have reported hepatic effects; however, the inconsistency of the findings and poor reporting of the data preclude drawing conclusion on the hepatotoxicity of orally administered styrene. Small areas of focal necrosis was observed in the livers of rats administered 400 mg/kg styrene in groundnut oil 6 days/week for 100 days (Srivastava et al. 1982). Because the incidence or statistical analysis data were not reported, it is not possible to determine whether 400 mg/kg is an adverse effect level. This study also found alteration in mitochondrial and microsomal enzymes at 200 and 400 mg/kg; the significance of these alterations in the absence of histological damage is not known. An increase in liver weights was observed in rats administered 400 or 677 mg/kg via gavage 5 days/week for 6 months (Wolf et al. 1956); no histological alterations were observed. Although the alterations in the liver weight were considered slight at 400 mg/kg and moderate at 677 mg/kg, the magnitude of the change and statistical significance is not known; slight and moderate alterations in body weight were also observed at these dose levels. Hepatic glutathione content was reduced in rats orally administered 900 mg/kg styrene for 7 consecutive days (Das et al. 1981); the toxicological significance of this effect is not known. As noted above, increased numbers of hemosiderin deposits and intranuclear crystalline inclusions were

reported in the hepatocytes of dogs orally administered 600 mg/kg/day of styrene by gavage for 316 days (Quast et al. 1979). This was presumably secondary to Heinz body formation, and no other hepatic histological effects were in this study. No hepatic effects were observed in rats exposed to 35 mg/kg/day styrene in drinking water for 105 weeks (Beliles et al. 1985) or in rats administered 500 mg/kg styrene 1 day/week for 120 weeks (Ponomarkov and Tomatis 1978).

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to styrene.

A decrease in renal glutathione content and decreased glutathione-S-transferase activity was noted in rats orally administered 900 mg/kg styrene for 7 days (Das et al. 1983). Growth depression and slightly increased kidney weight were reported in female rats administered 400 and 667 mg/kg, 5 days/week for 6 months (Wolf et al. 1956); the magnitude and statistical significance of the effect were not reported. Histopathological examination of kidney tissue showed no abnormalities; thus, the changes in organ weight were not considered adverse. In another study, female rats and mice were exposed to 1,350 mg/kg/day of styrene on the 17th day of gestation; the offspring were also administered styrene, by gavage, 1 day/week for 120 weeks. No statistically significant increases in the incidence of kidney lesions were observed in rats exposed to 500 mg/kg (Ponomarkov and Tomatis 1978). No histological alterations were observed in the kidneys of rats chronically exposed to 35 mg/kg/day styrene in drinking water (Beliles et al. 1985).

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to styrene.

The World Health Organization (WHO 1983) reviewed a Russian study conducted by Sinitskij in which styrene was fed to 36 rabbits at doses of 250 mg/kg for 58 days, 5 mg/kg for 216 days, and 0.5 mg/kg for 202 days. Impairment of the immunological defense system was indicated by a nearly total suppression of leukocyte phagocytic activity. Although no statistical analysis was provided, the data showed a dose-response relationship for both the severity of the effect and the time of onset. Similarly, impaired host resistance was observed in mice exposed to 30 mg/kg/day and infected with encephalomyocarditis or a rodent strain of malaria and rats exposed to 294 mg/kg/day and infected with a rodent hookworm parasite (Dogra et al. 1992).

3.2.2.4 Neurological Effects

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

Neurobehavioral effects and alterations in neurochemicals have been observed in animal studies. Significant learning impairment was observed in an operant behavioral test in rats administered 500 mg/kg styrene in corn oil, 5 days/week for 8 weeks (Bushnell 1994). A reversal of the effect was not observed 1 year after exposure termination. Another study found significantly increased mean percent avoidance response, indicative of impaired learning, in rats administered 100 or 200 mg/kg/day styrene for 14 days (Husain et al. 1985). No alterations in foot shock-induced aggressive behavior or amphetamine-induced motor activity were observed in young rats administered via gavage 250 mg/kg/day styrene for 15 days (Khanna et al. 1994). However, significant alterations were observed in similarly exposed rats maintained on a low protein diet (8% casein versus 20% in normal diet).

Significant increases in serotonin levels in the hypothalamus, hippocampus, and midbrain were observed in rats administered 200 mg/kg/day for 14 days (Husain et al. 1985); no alterations in dopamine or noradrenaline levels were observed. Exposure to a higher dose (906 mg/kg/day) for 15 days resulted in increases in serotonin and noradrenalin in brain tissue (Husain et al. 1980). Neither study found significant alterations in brain dopamine levels. Another study found a significant increase in dopamine receptor binding, as assessed using labeled spiroperidol binding, in rats administered 200 or 400 mg/kg/day for 1 day or 90 days (Agrawal et al. 1982).

The highest NOAEL and LOAEL values for neurological effects in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to styrene.

Marked degeneration in the seminiferous tubules and decreased spermatozoa were observed in rats administered 400 mg/kg styrene via gavage 6 days/week for 60 days (Srivastava et al. 1989). No adverse reproductive effects were observed in a three-generation reproduction study in which rats were exposed to 35 mg/kg/day in drinking water (Beliles et al. 1985). In another study, marked degeneration of seminiferous tubules and decreased spermatozoa counts were observed in adult rats administered 400 mg/kg styrene via gavage 6 days/week for 60 days (Srivastava et al. 1989). Significant decreases in

sorbitol dehydrogenase and acid phosphatase levels and increases in lactate dehydrogenase, glucose-6-phosphate dehydrogenase, β-glucoronidase, and γ-glutamyl transpeptidase levels were also observed at this dose level; the investigators considered these enzymes to be markers for testicular function.

The highest NOAEL values and all reliable LOAEL values for reproductive effects in rats in the acute and intermediate duration categories are recorded in Table 3-3 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to styrene.

No developmental effects were observed in the offspring of rats receiving a single gavage dose of 300 mg/kg on gestational day 11 (Daston et al. 1991) or in rats administered 300 mg/kg/day (150 mg/kg administered twice daily) on gestational days 6–15 (Murray et al. 1978).

Other developmental studies have focused on the impaired development of the reproductive system or neurodevelopmental effects. Decreases in spermatozoa counts (measured on postnatal days 61 and 91) were observed in offspring of rats administered to 400 mg/kg styrene on lactation days 0–21; no effects were observed at 200 mg/kg/day (Srivastava et al. 1992a). However, decreases in spermatozoa counts were observed in rats exposed to 200 mg/kg 6 days/week on postnatal days 1–61 (Srivastava et al. 1992b). Both studies found significant alterations in testicular enzyme levels at the same dose level as spermatozoa effects; these enzymes were considered markers of testicular function. A decrease in dopamine receptor binding, as assessed using labeled spiroperidol, was observed in the offspring of rats administered to 200 mg/kg/day styrene throughout gestation and lactation or only during lactation, but was not observed in rat pups only observed during gestation (Zaidi et al. 1985). This decrease in receptor binding was attributed to an increase in the number of dopamine receptors rather than an alteration in binding affinity. Impaired amphetamine-induced locomotor activity and apomorphine-induced stereotypy were also observed in the pups exposed during gestation and lactation.

The highest NOAEL value for developmental effects is recorded in Table 3-3 and plotted in Figure 3-2.

3.2.2.7 Cancer

No studies were located regarding cancer effects in humans after oral exposure to styrene.

Investigations of the carcinogenic potential of styrene in animals after oral exposure have yielded variable results. No significant alterations in the incidence of neoplastic tumors were observed in rats exposed to gavage doses as high as 250 mg/kg 4–5 days/week for 52 weeks (Conti et al. 1988; Maltoni et al. 1982) or 2,000 mg/kg 5 days/week for 78–103 weeks (NCI 1979b) or in rats exposed to 35 mg/kg/day styrene in drinking water for 2 years (Beliles et al. 1985). In contrast, significant increases in the incidence of lung tumors were observed in mice receiving gavage doses of 300 mg/kg 5 days/week for 78–103 weeks (NCI 1979b). The incidences of bronchiolo-alveolar carcinoma in male mice were 0/20, 3/44, and 5/43 in mice exposed to 0, 150, or 300 mg/kg, respectively, and the respective combined incidences of bronchiolo-alveolar carcinoma and adenoma in male mice were 0/20, 6/44, and 9/43. The incidence of bronchiolo-alveolar carcinoma in the 300 mg/kg group was similar to the incidence in untreated historical controls (12%), but lower than the incidence in historical vehicle controls 0/40; however, the National Cancer Institute (NCI 1979b) noted that the incidence in historical vehicle controls is based on too small a number of animals for meaningful use of historical control data. Two studies conducted by Ponomarkov and Tomatis (1978) examined the carcinogenicity of styrene following gestation and postnatal exposure. In the offspring of mice administered 1,350 mg/kg on gestation day 17 with continued exposure of the weanling mice to this dose level (1 day/week) for 16 weeks, a significant increase in lung tumors was observed during the 100-week observation period; this dose was also associated with treatment-related toxicity and mortality. In the second study, the mice were exposed to 300 mg/kg on gestation day 17 followed by exposure to 300 mg/kg for 120 weeks (1 day/week) beginning at weaning. No significant alterations in tumor incidence were observed.

3.2.3 Dermal Exposure

No studies were located regarding health effects in humans after dermal exposure to styrene.

3.2.3.1 Death

No studies were located regarding lethality in humans or animals after dermal exposure to styrene.

3.2.3.2 Systemic Effects

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

Dermal Effects. Marked irritation with denaturation of the skin was noted when styrene was applied in small amounts over a 4-week period to the shaved abdomen of rabbits at 20,000 mg/kg total dose) (Spencer et al. 1942).

Ocular Effects. Moderate conjunctival irritation and transient corneal injury of the eyes were observed when undiluted styrene was tested in rabbit eyes (Wolf et al. 1956). The effects were produced immediately (within 3 minutes) by a single administration of two drops (about 0.1 mL) and persisted throughout the 7-day observation period.

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

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

The genotoxicity of styrene has been examined in numerous *in vivo* studies of workers and laboratory animals; these data are summarized in Table 3-4. Chromosomal damage, DNA strand breaks, and mutagenic effects have frequently been studied in workers exposed to styrene in the production of reinforced plastic products and styrene/polystyrene production. In general, these studies are limited by the fact that workers in these industries are often exposed to chemicals other than styrene, such as methylene chloride and epoxide resins, and many studies did not control for potential confounding factors such as age, sex, and smoking status. Chromosomal aberrations have been reported in numerous studies of workers exposed to styrene for 1–25 years in reinforced plastic operations (Anwar and Shamy 1995; Artuso et al. 1995; Hogstedt et al. 1979; Mäki-Paakkanen et al. 1991; Meretoja et al. 1977, 1978; Somorovská et al. 1999; Tomanin et al. 1992); however, other studies have not found significant increases in chromosomal aberrations (Andersson et al. 1980; Hansteen et al. 1984; Jablonicka et al. 1988; Mäki-Paakkanen 1987; Nordenson and Beckman 1984; Thiess et al. 1980; Vodicka et al. 2004; Watanabe et al. 1981). The results of the Artuso et al. (1995) and Tomanin et al. (1992) studies provide suggestive evidence that the occurrence of chromosomal aberrations is concentrated-related. Significant increases in

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

Species (test system)	End point	Results	Reference
Human Studies			
Human lymphocytes	Chromosomal aberrations	+	Meretoja et al. 1977
Human lymphocytes	Chromosomal aberrations	+	Meretoja et al. 1978
Human lymphocytes	Chromosomal aberrations	+	Hogstedt et al. 1979
Human lymphocytes	Chromosomal aberrations	+	Anwar and Shamy 1995
Human lymphocytes	Chromosomal aberrations	+	Artuso et al. 1995
Human lymphocytes	Chromosomal aberrations	+	Mäki-Paakkanen et al. 1991
Human lymphocytes	Chromosomal aberrations	+	Tomanin et al. 1992
Human mononuclear leukocytes	Chromosomal aberrations	+	Somorovská et al. 1999
Human lymphocytes	Chromosomal aberrations	–	Thiess et al. 1980
Human lymphocytes	Chromosomal aberrations	–	Andersson et al. 1980
Human lymphocytes	Chromosomal aberrations	–	Watanabe et al. 1981
Human lymphocytes	Chromosomal aberrations	–	Nordenson and Beckman 1984
Human lymphocytes	Chromosomal aberrations	–	Hansteen et al. 1984
Human lymphocytes	Chromosomal aberrations	–	Maki-Paakkanen 1987
Human lymphocytes	Chromosomal aberrations	–	Jablonicka et al. 1988
Human lymphocytes	Chromosomal aberrations	–	Vodicka et al. 2004
Human lymphocytes	Sister chromatid exchange	+	Yager et al. 1993
Human lymphocytes	Sister chromatid exchange	+	Laffon et al. 2002
Human lymphocytes	Sister chromatid exchange	+	Karakaya et al. 1997
Human lymphocytes	Sister chromatid exchange	+	Artuso et al. 1995
Human lymphocytes	Sister chromatid exchange	+	Hallier et al. 1994
Human lymphocytes	Sister chromatid exchange	+	Andersson et al. 1980
Human lymphocytes	Sister chromatid exchange	–	Mäki-Paakkanen et al. 1991
Human lymphocytes	Sister chromatid exchange	–	Hansteen et al. 1984
Human lymphocytes	Sister chromatid exchange	–	Watanabe et al. 1981
Human lymphocytes	Sister chromatid exchange	–	Maki-Paakkanen 1987
Human lymphocytes	Micronuclei	+	Nordenson and Beckman 1984
Human lymphocytes	Micronuclei	+	Hogstedt et al. 1983
Human lymphocytes	Micronuclei	+	Laffon et al. 2002
Human lymphocytes	Micronuclei	–	Karakaya et al. 1997

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

Species (test system)	End point	Results	Reference
Human lymphocytes	Micronuclei	–	Anwar and Shamy 1995
Human lymphocytes	Micronuclei	–	Mäki-Paakkanen et al. 1991
Human lymphocytes	Micronuclei	–	Tomanin et al. 1992
Human lymphocytes	Micronuclei	–	Vodicka et al. 2004
Human erythrocytes	Mutations in glycophorin A	+	Bigbee et al. 1996
Human erythrocytes	Mutations in glycophorin A	+	Compton-Quintana et al. 1993
Human lymphocytes	HPRT mutations	±	Vodicka et al. 1995
Human mononuclear leukocytes	DNA Single strand breaks	+	Somorovská et al. 1999
Human lymphocytes	DNA Single strand breaks	+	Shamy et al. 2002
Human lymphocytes	DNA Single strand breaks	+	Mäki-Paakkanen et al. 1991
Human leukocytes	DNA Single strand breaks	+	Walles et al. 1993
Human lymphocytes	DNA Single strand breaks	+	Vodicka et al. 1995
Human lymphocytes	DNA Single strand breaks	–	Vodicka et al. 2004
Human lymphocytes	Unscheduled DNA synthesis	+	Pero et al. 1982
Laboratory animal studies			
Mouse bone marrow	Chromosomal aberrations	–	Sbrana et al. 1983
Rat bone marrow	Chromosomal aberrations	–	Sinha et al. 1983
Mouse bone marrow	Sister chromatid exchange	±	Simula and Priestly 1992
Mouse spleen, lung, erythrocytes	Sister chromatid exchange	+	Kligerman et al. 1992, 1993
Mouse bone marrow, liver cells, and alveolar macrophages	Sister chromatid exchange	+	Conner et al. 1980
Mouse bone marrow	Sister chromatid exchange	±	Simula and Priestly 1992
Rat lymphocyte	Sister chromatid exchange	+	Kligerman et al. 1992, 1993
Mouse bone marrow and polychromatic erythrocytes	Micronuclei	±	Norppa 1981
Mouse bone marrow	Micronuclei	±	Simula and Priestly 1992
Mouse bone marrow	Micronuclei	–	Engelhardt et al. 2003
Mouse spleen, lung, erythrocytes	Micronuclei	–	Kligerman et al. 1992, 1993
Mouse bone marrow	Micronuclei	–	Simula and Priestly 1992
Rat lymphocyte	Micronuclei	–	Kligerman et al. 1992, 1993
Mouse bone marrow and lymphocytes	DNA single strand breaks	–	Vodicka et al. 2001a
Mouse kidney, liver, lung, testes, and brain	DNA	+	Walles and Orsen 1983

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

Species (test system)	End point	Results	Reference
Mouse liver	Unscheduled DNA synthesis	–	Clay 2004

– = negative result; + = positive result; ± = weakly positive result; DNA = deoxyribonucleic acid

chromosomal aberrations alterations were observed in high exposure groups (20–326 or 27–104 ppm), but not in workers exposed to lower exposure levels (0.5–24 ppm). As with the occurrence of chromosomal aberrations, mixed results have been observed in studies of sister chromatid exchange (Andersson et al. 1980; Artuso et al. 1995; Hallier et al. 1994; Hansteen et al. 1984; Karakaya et al. 1997; Laffon et al. 2002; Mäki-Paakkanen 1987; Mäki-Paakkanen et al. 1991; Watanabe et al. 1981; Yager et al. 1993) and micronuclei formation (Anwar and Shamy 1995; Hogstedt et al. 1983; Karakaya et al. 1997; Laffon et al. 2002; Mäki-Paakkanen et al. 1991; Nordenson and Beckman 1984; Tomanin et al. 1993; Vodicka et al. 2004) in styrene workers. Other genotoxicity studies in styrene workers have found significant increases in glycophorin A mutations (Bigbee et al. 1996; Compton-Quintana et al. 1993), DNA single strand breaks (Mäki-Paakkanen et al. 1991; Shamy et al. 2002; Somorovská et al. 1999; Walles et al. 1993; Vodicka et al. 1995), and increases in unscheduled DNA synthesis (Pero et al. 1982).

Studies in laboratory animals have found significant increases in the occurrence of sister chromatid exchange (Conner et al. 1980; Kligerman et al. 1992, 1993; Simula and Priestly 1992). However, the results for chromosomal aberrations (Sbrana et al. 1983; Sinha et al. 1983), micronuclei formation (Engelhardt et al. 2003; Kligerman et al. 1992, 1993; Norppa 1981; Simula and Priestly 1992), DNA single strand breaks (Vodicka et al. 2001a), and unscheduled DNA synthesis (Clay 2004) have been weakly positive or negative.

Styrene has been tested for genotoxic potential in a variety of *in vitro* systems, as summarized in Table 3-5. In the absence of metabolic activation, styrene has not produced gene mutations in *Salmonella typhimurium* (DeMeester et al. 1981; Dunkel et al. 1985; Vainio et al. 1976) or *Escherichia coli* (Dunkel et al. 1985); mixed results have been found in the presence of metabolic activation. Increases in chromosomal aberrations (Jantunen et al. 1986) and sister chromatid exchange (Norppa et al. 1983) have been found in human lymphocytes in the absence of metabolic activation.

3.4 TOXICOKINETICS

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

The uptake of styrene following inhalation exposure in humans and animals is rapid (Ramsey and Andersen 1984; Ramsey and Young 1978; Ramsey et al. 1980; Withey and Collins 1979; Withey and Karpinski 1985). Pulmonary retention of inhaled styrene in humans is approximately 2/3 of the

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

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium (two strains, plate incorporation method)	Gene mutation	+	–	Vainio et al. 1976
S. typhimurium (three strains, plate incorporation method)	Gene mutation	–	–	Vainio et al. 1976
S. typhimurium (three strains, vapor exposure – disiccator test)	Gene mutation	+	–	DeMeester et al. 1981
S. typhimurium (four strains, vapor exposure – disiccator test)	Gene mutation	–	–	DeMeester et al. 1981
S. typhimurium (five strains, preincubation method)	Gene mutation	–	–	Dunkel et al. 1985
Escherichia coli (one strain, preincubation method)	Gene mutation	–	–	Dunkel et al. 1985
Mammalian cells:				
Human lymphocytes	Sister chromatid exchange	No data	+	Norppa et al. 1983
Human lymphocytes	Chromosomal aberrations	No data	+	Jantunen et al. 1986

— = negative result; + = positive result

administered concentrations (Engstrom et al. 1978a, 1978b). For example, male human subjects were exposed to styrene in inspired air during 30-minute rest and three 30-minute work periods on a bicycle ergometer. The mean uptake was approximately 63% (range was 59–70%) of the amount of inspired styrene. In exercising volunteers exposed to 50 ppm styrene for 2 hours, an average of 66.5% of the inhaled styrene was absorbed (Johanson et al. 2000). Another study in volunteers exposed to 50 ppm styrene for 2 hours during exercise calculated that 64% of the styrene was absorbed (Norstöm et al. 1992). Exposures of rats to styrene concentrations of 50–2,000 ppm for 5 hours yielded blood uptakes that showed a continued and increasing rapid absorption, proportional to the styrene air level (Withey and Collins 1979). Plateau levels of styrene in rats' blood were reached within 6–8 hours during exposures ranging from 80 to 1,200 ppm styrene for up to 24 hours (Ramsey and Young 1978).

3.4.1.2 Oral Exposure

No studies were located regarding absorption in humans after oral exposure to styrene.

The absorption of styrene from the gastrointestinal tract was rapid and complete in rats deprived of food overnight and administered, via gavage, 9.3 mg/kg styrene in aqueous solution. A peak blood level of 6 μg/mL was reached in a few minutes. There was a much slower uptake of the styrene administered in vegetable oil (Withey 1976). Styrene administered in vegetable oil at a total dose of 32.61 mg produced a peak level of 12 μg/mL. This was reached at about 100 minutes (Withey 1976).

3.4.1.3 Dermal Exposure

Limited data indicate that absorption of styrene via the dermal route is probably low compared to absorption via other routes. When liquid styrene was applied to the forearms of male subjects, the absorption rate was estimated to be 9–15 mg/cm^2/hour (Dutkiewicz and Tyras 1968). By contrast, the rate of absorption through human skin was very low (1 ± 0.5 μg/cm^2/minute) in subjects who dipped one hand into liquid styrene (Berode et al. 1985). The higher absorption rate reported by Dutkiewicz and Tyras (1968) likely included the disappearance rate of the solvent from the surface of the skin (Guillemin and Berode 1988). Riihimaki and Pfaffli (1978) demonstrated that in humans, dermal exposure to moderate concentrations of styrene vapor (300 and 600 ppm) resulted in percutaneous penetration corresponding to approximately 0.1–2% of the amount estimated to be absorbed from the respiratory tract. Similarly, Limasset et al. (1999) did not find significant differences in the levels of urinary metabolites in workers wearing total protective equipment (insulating suit and respiratory mask) and those wearing a respiratory mask only.

Although absorption of styrene applied to the abdomen of rabbits was reported, there was no information on absorption rates (Spencer et al. 1942). Dermal exposure of rats to a neat solution of styrene resulted in peak blood levels of 5.3 µg/mL within 1 hour of exposure (Morgan et al. 1991).

3.4.2 Distribution

A blood:air partition coefficient of 40.2 and fat:air partition coefficient of 3,476 were calculated for rats (Gargas et al. 1989). Fisher et al. (1997) calculated a human blood:air partition coefficient of 69.74 and a breast milk:blood partition coefficient of 2.17.

In a study of 81 adults without occupational exposure to styrene, average blood styrene levels were 0.221 µg/L; in comparison, blood styrene levels in reinforced plastics industry workers were 1,068–1,590 µg/L at the end of workshift and 60–119 µg/L in the morning after exposure (Brugnone et al. 1993).

3.4.2.1 Inhalation Exposure

Inhalation studies in both humans and animals resulted in the widespread distribution of styrene with the highest concentration in adipose tissue.

Three humans were exposed to 8–20 ppm styrene which resulted in a mean daily uptake of 193–558 mg styrene (Engstrom et al. 1978b). The concentration of styrene in adipose tissue was 2.8-8.1 mg/kg at the beginning of the week and 4.7–11.6 mg/kg at the end of the week. The authors estimated the half-life of styrene in the subcutaneous fat of humans to be about 72 hours. Subsequent studies by this author confirmed this estimate and reported the half-life of styrene in adipose tissue to be 2–5 days (Engstrom et al. 1978a).

Fiberglass factory workers exposed to >50 ppm of styrene for 8-hour work shifts had blood styrene levels which ranged from 120 to 684 µg/L at the end of the shift (Apostoli et al. 1983). The concentrations of urinary MA and phenylglyoxilic acid (PGA) were 133–2,100 and 107–685 mg/L, respectively. These levels were also determined at the end of the work-shift. Distribution of styrene was also studied in adult men exposed to about 70 ppm of styrene for 2 hours during light physical exercise (Wigaeus et al. 1983). Blood styrene reached a level of approximately 2,000 µgl/L after 75 minutes. The concentration of styrene in adipose tissue was about 5,000 µg/kg after 30–90 minutes of exposure.

Rats were exposed for 5 hours to styrene at concentrations ranging from 50 to 2,000 ppm (Withey and Collins 1979). Tissue concentrations of styrene in the heart, liver, lung, kidney, spleen, brain, and perirenal fat demonstrated different patterns of distribution as the dose increased. The styrene concentration in perirenal fat was 10 times greater than in other organs. The largest amounts of styrene were found in the subcutaneous fat of male rats exposed to about 45 ppm of radioactively labeled styrene in the inspired air for 1–8 hours (Carlsson 1981). The concentration increased steadily during the first 4 hours of exposure. Styrene concentrations in brain tissue and muscles were about 70% of the arterial blood value. Other investigators (Ramsey and Andersen 1984; Ramsey and Young 1978; Savolainen and Pfaffli 1978; Withey 1976) demonstrated that higher levels of styrene in adipose tissue increase with higher exposures to styrene. Styrene was found to distribute to the fetuses of pregnant rats after inhalation exposure, but at concentrations much lower than those measured in maternal organs and tissues (Withey and Karpinski 1985).

3.4.2.2 Oral Exposure

No studies were located regarding distribution in humans after oral exposure to styrene.

An oral dose of 20 mg/kg of ^{14}C styrene was administered to male and female rats (Plotnick and Weigel 1979). Tissue levels peaked at 4 hours or earlier after dosing. Less than 10% of the administered dose was found in the stomach, small intestine, and large intestine 8 hours after dosing. The kidney had the highest concentration of radioactivity at all time intervals, with decreasing amounts in the liver and pancreas. Fat tissue showed increased levels after 2 hours. All tissue levels were below 1 μg/g at 24 hours and at 48 and 72 hours were below the limit of detection. Excretion data from the Plotnick and Weigel (1979) study are presented in Section 3.4.4.2.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans after dermal exposure to styrene.

Immersion of rats' tails in pure liquid styrene for 1 hour resulted in styrene levels in the liver and brain that were estimated to be between 50 and 70% of the concentrations found in the same organs after 4-hour inhalation exposure to a vapor concentration of 11.8 g/m^3 (Shugaev 1969). A skin:air partition coefficient of 91.9 was calculated for rat skin (Mattie et al. 1994).

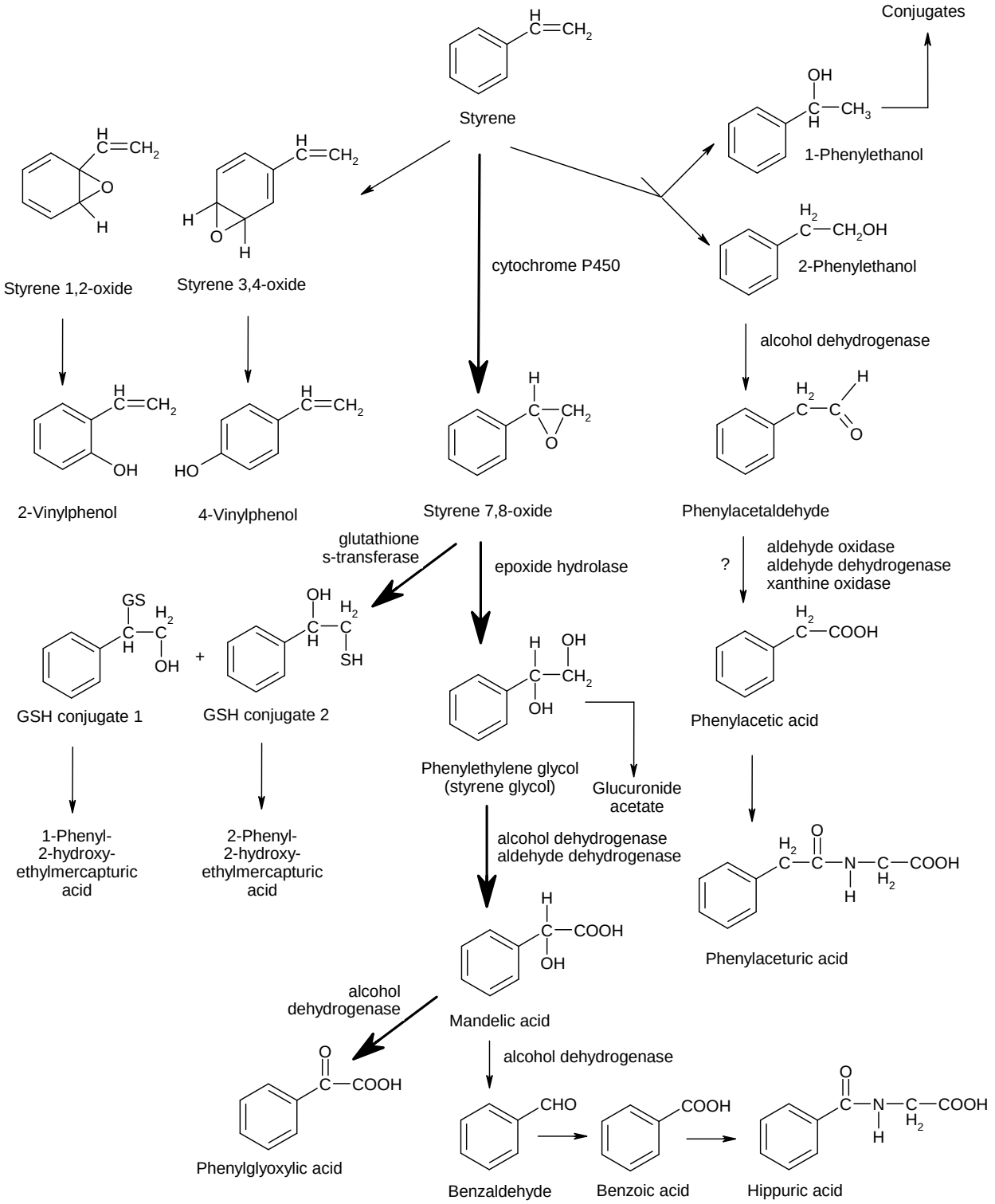
3.4.3 Metabolism

There have been numerous *in vivo* studies, conducted primarily via inhalation, and *in vitro* studies that address the metabolism of styrene in humans and animals. There are several metabolic pathways for styrene (Cruzan et al. 2002; IARC 2002; Sumner and Fennel 1994), as illustrated in Figure 3-3. The primary pathway is oxidation of the side chain by cytochrome P450 to form styrene 7,8-oxide. Styrene oxide is predominantly metabolized by epoxide hydrolase to form styrene glycol; the styrene glycol is subsequently converted to mandelic acid, phenylglyoxylic acid, and hippuric acid. Styrene 7,8-oxide can also be conjugated with glutathione to ultimately form phenylhydroxylethylmercapturic acids. A minor pathway of styrene metabolism involves the formation of phenylacetaldehyde from styrene 7,8-oxide or cytochrome P450 conversion of styrene to pheylethanol and subsequent metabolism to phenylacetic acid. An alternative minor pathway involves ring oxidation resulting in the production of styrene 3,4-oxide, which is further metabolized to 4-vinylphenol.

As summarized by Cruzan et al. (2002), over 95% of the styrene urinary metabolites excreted by humans are derived from styrene glycol (mandelic acid, phenylglyoxylic acid, hippuric acid) compared to 49–59% in mice and 68–72% in rats. In mice and rats, 25–35% of the metabolites are derived from glutathione conjugation (mercapturic acids). The remaining metabolites derive from phenylacetic acid production (12–22% in mice and 3–5% in rats) and ring oxidation (4–8% in mice and <1% in rats). Trace amounts of mercapturic acids (DePalma et al. 2001) and 4-vinylphenol (Manini et al. 2002, 2003) have also been detected in humans; both metabolites each account for <1% of the total styrene metabolites.

The liver is the primary site of styrene metabolism and the source of styrene oxide in the blood (Cruzan et al. 2005). However, styrene is metabolized in other tissues, particularly the lung and nasal cavity following inhalation exposure, and it is this localized metabolism that results in the observed toxicity and/or carcinogenicity in these tissues. Studies in humans, mice, and rats indicate that styrene metabolism is concentration-dependent. At air concentrations of <200–300 ppm, most of the inhaled styrene is metabolized, only small amounts are exhaled unchanged, and there is little accumulation (Filser et al. 1993; Ramsey and Andersen 1984). At concentrations >300 ppm, metabolism was pregressively limited by metabolic capacity and was saturated (V_{max}) at 700 ppm in rats and at 800 ppm in mice (Filser et al. 1993). Löf and Johanson (1993) estimated that metabolic saturation occurs at 100–200 ppm in humans; however, a subsequent analysis by this group (Jonsson and Johanson 2002) found that the V_{max} was 40% higher.

Figure 3-3. Scheme for Styrene Metabolism in Humans and Animals



GSH = glutathione

Source: Adapted from IARC 2002; Manini et al. 2002; Sumner and Fennel 1994

Comparisons of cytochrome P450, epoxide hydrolase, and glutathione S-transferase activity in the liver and lungs of rats and mice demonstrated 2–15-fold higher activities in the liver (Mendrala et al. 1993). In both tissues, previous exposure to styrene did not result in a dose-related increase in enzyme activity. In contrast, cytochrome P450 from human lung has a very limited ability to metabolize styrene to styrene oxide (Carlson et al. 2000; Nakajima et al. 1994); the capacity was 100-fold lower than in rat lung microsomes (Cruzan et al. 2002).

A number of cytochrome P450 isozymes have the capacity to catalyze styrene to styrene oxide. In human livers, CYP2B6 was the most active isoform; the activities of CYP1A2 and CYP2E1 were about half that of CYP2B6 (Nakajima et al. 1993). Kim et al. (1997) found that CYP2E1 was the main isoform at low styrene concentrations and CYP2B6 at high styrene concentrations. In mice and rats, the CYP1A1 and CYP2B1, respectively, were the most active isoforms in the liver (Nakajima et al. 1993). In the lungs, CYP2F1 was the most active isoform in human (Nakajima et al. 1994) lung microsomes. In mice and rat lung and nasal cavity microsomes, CYP2F2 and CYP2E1 are the predominant isoforms (Hynes et al. 1999).

Studies by Mendrala et al. (1993) compared the kinetic constants of cytochrome P450 from the livers of humans, mice, and rats. The affinity of cytochrome P450 for styrene (based on K_m valves) was similar for the three species. However, the mouse had the greatest capacity to form styrene 7,8-oxide from styrene based on the V_{max} values and relative liver and body size, and humans had the lowest capacity. In contrast, marked differences in the K_m values for epoxide hydrolase were found between species; the K_m values were 0.01, 0.74, and 0.13-0.23 mmol for humans, mice, and rats, respectively. These results suggest that humans have a greater affinity to metabolize styrene 7,8-oxide and is more efficient at low levels of styrene oxide, as compared to rodent species.

Styrene oxide exists as two enantiomeric forms (R) and (S). As with other aspects of styrene metabolism, species differences in the ratio of R enantiomer and S enantiomer have been detected. In human liver samples, the R:S ratio was 0.15 at low styrene concentrations (0.016 mM) and 1.4 at high styrene concentrations (1.1 mM) (Wenker et al. 2001). In mouse and rat liver microsomes (incubated with 2 mM styrene), the R:S ratio was 0.57 and 1.18, respectively (Hynes et al. 1999). In the lungs, the R:S ratio was 2.4 and 0.52 for mice and rats, respectively.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Several studies have demonstrated that styrene is almost totally excreted as urinary metabolites in humans, and at higher doses, the elimination profile indicates saturation of metabolic excretion or processes (Ramsey and Young 1978; Ramsey et al. 1980). Most of the inhaled styrene is excreted in urine as MA and PGA. In a study of the excretion of styrene and its metabolites resulting from a 100-ppm/8-hour inhalation exposure, 2.6% of the total uptake was excreted as unchanged styrene in exhaled air (Guillemin and Berode 1988). The metabolites MA, PGA, and hippuric acid were excreted in the urine at 56.9, 33, and 7.5% of the absorbed dose, respectively. In exercising volunteers exposed to 50 ppm styrene for 2 hours, 0.7–2.2% of the retained dose was exhaled as unchanged styrene (Johanson et al. 2000). Peak levels of styrene in the urine were measured immediately after exposure termination, whereas urinary excretion of MA and PGA peaked at 2 hours after exposure termination. MA excretion accounted for 6–29% of the estimated retained dose and PGA excretion accounted for 4–6%; the halftime excretion rates of MA and PGA were 2.2–4.2 and 3.5–13.9 hours, respectively. Phenylaceturic acid and hippuric acid was also detected in the urine samples collected 2 hours after exposure termination. At this time point, MA account for 73% of the total excreted metabolites, PGA 18%, phenylaceturic acid 4.5%, and hippuric acid 5.7%. In styrene workers exposed to 29–42 ppm styrene, both R-mandelic acid and S-mandelic acid were detected in the urine (Hallier et al. 1995). The ratio of R- to S-mandelic acid ranged from 0.7 to 1.2 in 19 of the 20 workers; in the last worker, the ratio was 2.2.

An alternative pathway for the metabolism of styrene 7,8-oxide is conjugation with glutathione, resulting in the excretion of mercapturic acids. Low levels of mercapturic acids have been detected in workers exposed to an unspecified amount of styrene (Maestri et al. 1997a). The mean concentrations of styrene metabolites were 580 mg/g creatinine mandelic acid, 174 mg/g creatinine phenylglyocylic acid, 1.517 mg/g N-acetyl-S-(1-phenyl-2-hydroxyethyl)-cysteine S-enantiomer, 0.0637 mg/g N-acetyl-S-(1-phenyl-2-hydroxyethyl)-cysteine R- enantiomer, and 1.519 mg/g N-acetyl-S-(2-phenyl-2-hydroxyethyl)-cysteine. Another study of styrene workers (exposure level of 29–41 ppm) only detected styrene-specific mercapturic acid in 1 of 20 workers (Hallier et al. 1995). Similarly, in volunteers exposed to 50 ppm for 2 hours during exercise, N-acetyl-S-(2-phenyl-2-hydroxyethyl)-cysteine was not detected in urine samples collected up to 5 hours after exposure termination (Norström et al. 1992).

In volunteers exposed to 80 ppm styrene, styrene is cleared from the blood in a biphasic manner, indicating a two-compartment pharmacokinetic model. The half-lives for the rapid and slow clearance

phases are 0.58 and 13.0 hours, respectively. The half-life of styrene in subcutaneous adipose tissue of humans is 2–5 days (Engstrom et al. 1978a). The quantities of the major metabolites of styrene in urine compared with the quantity of styrene eliminated unchanged in expired air indicated that approximately 97% is cleared by the metabolic route (Ramsey et al. 1980).

Another human inhalation study determined that between 59 and 66% of inhaled styrene (50–200 ppm) was retained after a 4–8-hour exposure (Guillemin and Bauer 1979). Urinary elimination of MA was biphasic with a half-life for the first phase of 4 hours and for the second phase, 25 hours. These findings were comparable to those reported by Engstrom et al. (1976). The half-life of urinary elimination of PGA was determined to be 11 hours. This was regarded by the authors as being the first phase of elimination since MA is a precursor of PGA.

A lactational transfer pharmacokinetic model developed by Fisher et al. (1997) predicted that exposure to 50 ppm styrene would result in 0.650 mg styrene being ingested by a nursing infant over a 24-hour period.

Styrene is almost totally excreted as urinary metabolites in animals. The blood elimination curve for rats is biphasic exponential at 80 and 200 ppm styrene over 6 hours. For exposures >600 ppm exposure levels for 6 hours duration), a nonlinear blood elimination curve following Michaelis-Menten kinetics was observed. In going from 80 to 1,200 ppm (a 15-fold increase) the area under the blood concentration curves increases by 112-fold (Ramsey and Young 1978; Young et al. 1979). Rats exposed to 50–2,000 ppm styrene by inhalation for 5 hours exhibited a dose dependent biphasic pattern of elimination (Withey and Collins 1979).

3.4.4.2 Oral Exposure

No studies were located regarding excretion in humans after oral exposure to styrene.

Styrene was rapidly excreted in the urine of male and female rats administered 20 mg/kg of ^{14}C styrene with 90% of the dose detected in the urine within 24 hours of administration (Plotnick and Weigel 1979). Less than 2% of the dose was found in the feces. Detectable tissue levels were not found 48 and 72 hours after administration. In mice administered 200 mg/kg for 70 days, 26.4, 13.3, and 19.0% of urinary metabolites excreted on day 70 were mandelic acid, phenylglyoxylic acid, and hippuric acid (Sbrana et al. 1983). Approximately 80% of the dose was excreted in the first 24 hours.

3.4.4.3 Dermal Exposure

In a study of the absorption of liquid styrene applied to the forearms of male volunteers, about 13% of the absorbed dose was excreted as MA (Dutkiewicz and Tyras 1968).

No studies were located regarding excretion in animals after dermal exposure to styrene.

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. 1987a). 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-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.

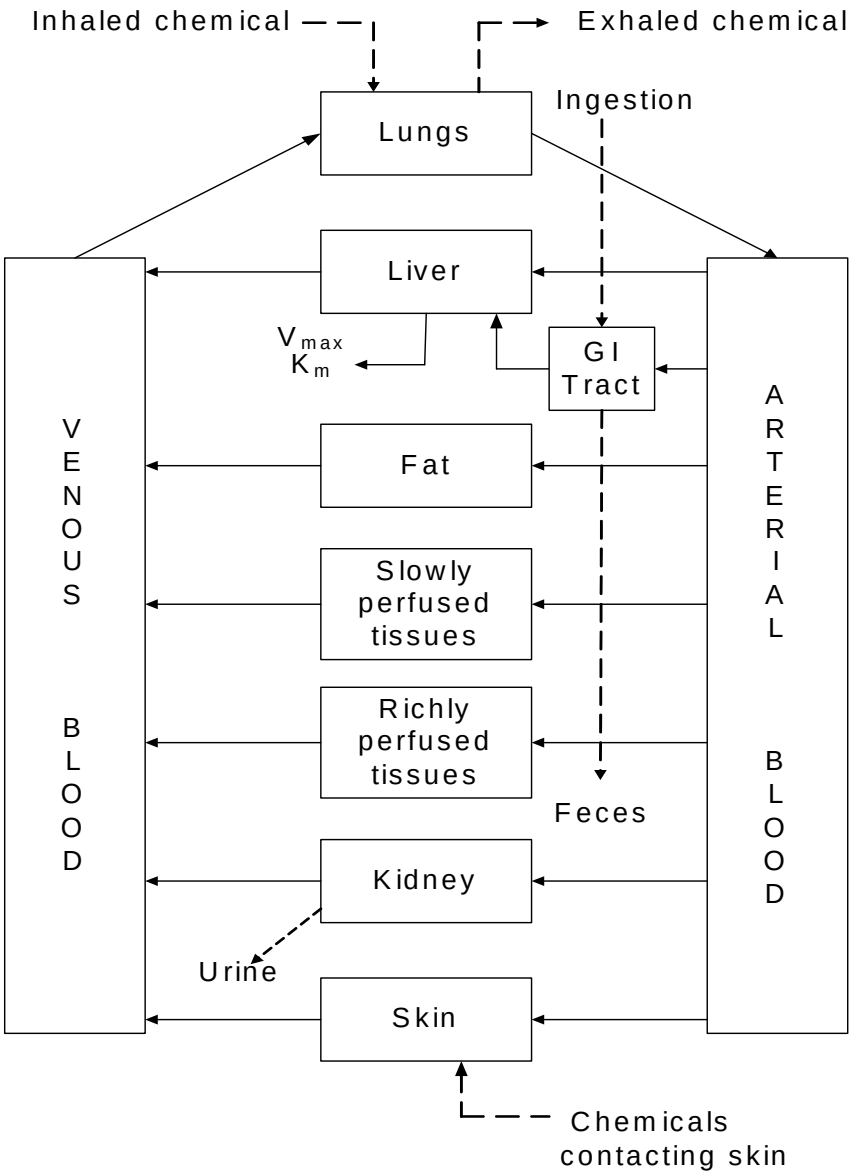
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many 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 styrene 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 investigators have developed toxicokinetic models for styrene (Csanády et al. 1994, 2003; Jonsson and Johanson 2002; Leavens and Bond 1996). The Csanády et al. (1994, 2003) model is useful for evaluating the carcinogenic risk associated with inhalation exposure to styrene. As discussed in Section 3.2.1.7, species differences exist in the metabolism of styrene in the lungs of rats, mice, and humans; these differences result in increased sensitivity of mice. Jonsson and Johanson (2002) developed a population-based PBPK model for styrene, which decreased the intraindividual variability for estimating the metabolic capacity for styrene in humans. Leavens and Bond (1996) described initial work on developing a model for co-exposure to 1,3-butadiene and styrene in mice. Some of these models provide strong support for the observed differences in styrene toxicity between rats, mice, and humans. As discussed further in Section 3.5.3, some have primarily focused on the species differences in the metabolism of styrene and metabolic differences between rats, mice, and humans.

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

3.4.5.1 Summary of PBPK Models

A number of investigators have developed toxicokinetic models for styrene. The earliest model was developed by Ramsey and Anderson (1984) to relate styrene exposure concentrations quantitatively to blood concentrations. A model developed by Csanády et al. (1994, 2003) is useful in describing the blood/tissue time course of styrene and styrene oxide in rats, mice, and humans following multiple routes of administration. A model developed by Sarangapani et al. (2002) expands these models and adds a description of styrene and styrene oxide levels in multiple compartments of the respiratory tract; this model is described below.

Additionally, Jonsson and Johanson (2002) developed a population-based PBPK model for styrene, which decreased the intraindividual variability for estimating the metabolic capacity for styrene in humans. Leavens and Bond (1996) described initial work on developing a model for co-exposure to 1,3-butadiene and styrene in mice.

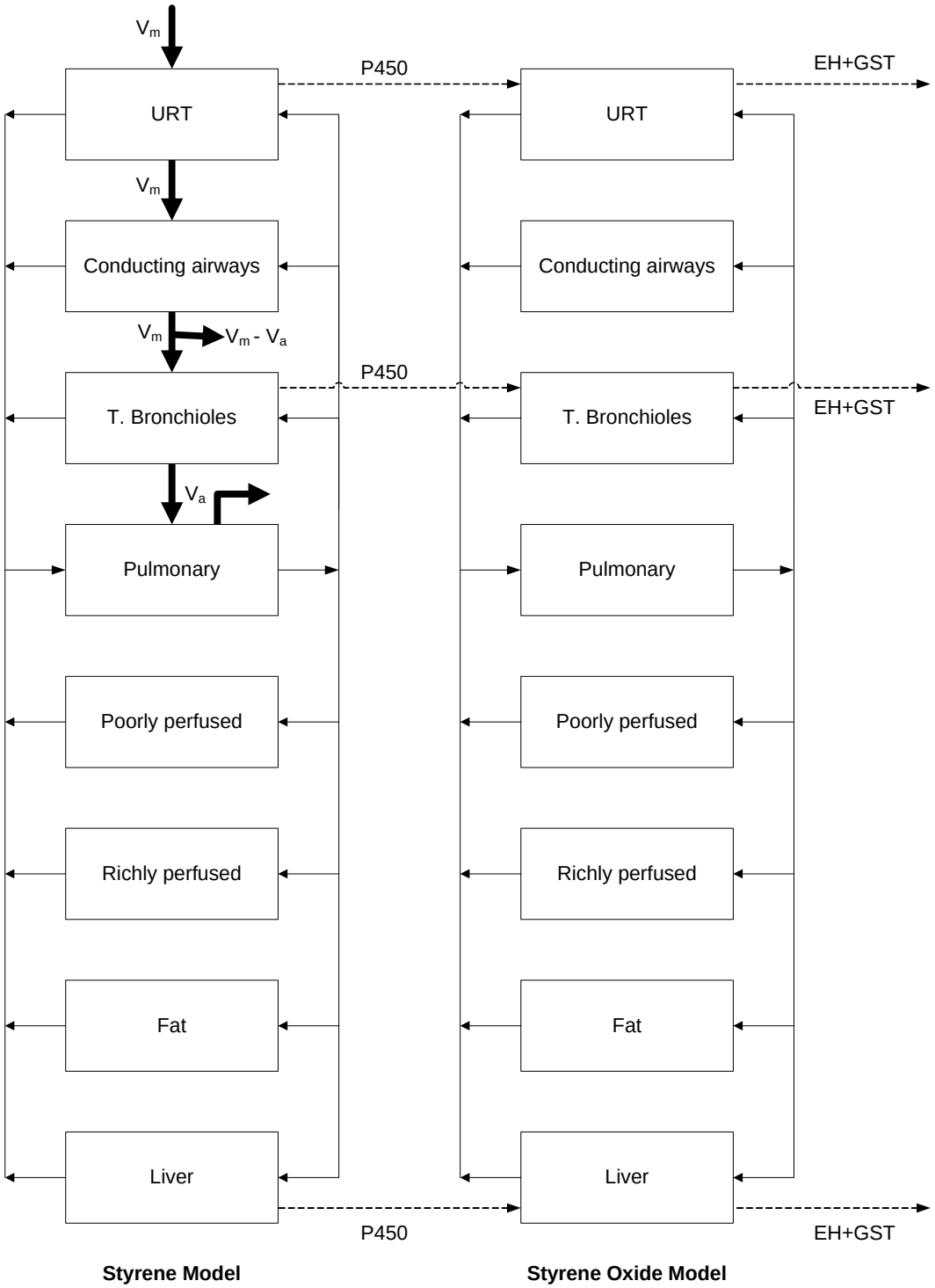
3.4.5.2 Discussion of Model

Risk assessment. The Sarangapani model quantifies the observed differences in the occurrence of lung tumors between rats, mice, and humans.

Description of the model. The Sarangapani model is a mode of action based PBPK model developed to predict blood, liver, and respiratory tract tissue (particularly the terminal bronchioles) levels of styrene and styrene oxide and allow for interspecies extrapolations. The model has a nested architecture with a model for styrene and a linked submodel for styrene oxide. Both models consist of four respiratory tract tissue compartments (upper respiratory tract, conducting airways, terminal bronchioles, and pulmonary) and systemic tissue compartments for liver, fat, richly perfused tissue, and poorly perfused tissue. The metabolism of styrene to styrene 7,8-oxide and the detoxification of styrene 7,8-oxide by epoxide hydrolase and glutathione S-transferase was modeled to occur in the liver and selected regions of the lung. The styrene oxide formed in the parent model in any tissue compartment was passed to the corresponding tissue compartment in the metabolite submodel; the schematic of the PBPK model is presented in Figure 3-5. Inhalation was the only exposure route considered in this model.

Mass balance equations were used to account for the transport of styrene across the lumen and tissue subcompartments in the upper, conducting, and transitional airways and in the liver. Additionally mass balance equations were used to describe the production of styrene 7,8-oxide, elimination of styrene oxide,

Figure 3-5. Styrene and Styrene Oxide Models used in the Sarangapani PBPK Model



EH = epoxide hydrase; GST = glutathione S-transferase; PBPK = physiologically based pharmacokinetic; T. Bronchioles = transitional bronchioles; URT = upper respiratory tract; Va = alveolar ventilation; Vm = minute ventilation or pulmonary ventilation;

Source: Sarangapani et al. 2002

and kinetics of cytosolic glutathione in the liver and lung. Most of the physiological and flow parameters (Table 3-6) and respiratory tract-specific physiological parameters (Table 3-7) used in the model were obtained from the literature. Tissue volumes for the respiratory tract compartments were estimated by multiplying the appropriate surface areas with the tissue thickness. The kinetic constants used for hepatic and lung cytochrome P450, epoxide hydrolase, and glutathione S-transferase are presented in Table 3-8. Stereospecific kinetic parameters (Table 3-9) and steady-state concentrations of R-styrene oxide and S-styrene oxide were used to account for species differences in the stereospecific metabolism of styrene to styrene oxide.

Validation of the model. Ten independent data sets ranging from closed chamber data to concentration measurements of styrene and styrene oxide in multiple tissues following multiple routes of exposure were used to validate various dose metrics in the mouse, rat, and human. The model provided good fit across species and at multiple target sites, including the whole lung for both styrene and styrene oxide. The model was not validated for styrene and styrene oxide levels in the transitional bronchioles due to the lack of experimental data.

Target tissues. The model was used to predict steady-state styrene oxide levels in arterial blood and the terminal bronchioles.

Species extrapolation. The model predicted that the levels of styrene oxide in the transitional bronchioles are approximately 10- and 100-fold lower in rats and humans, as compared to mice, thus suggesting that humans would be 100-fold less sensitive than mice to styrene-induced lung tumors.

Interroute extrapolation. Interroute extrapolation was not attempted in this model.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Styrene is rapidly absorbed through the respiratory tract (Ramsey and Andersen 1984; Ramsey and Young 1978; Ramsey et al. 1980; Withey and Collins 1979; Withey and Karpinski 1985) with a mean uptake of approximately 60–70% in humans (Johanson et al. 2000; Norström et al. 1992). A concentration-dependent uptake efficiency was found in the upper respiratory tract of rats and mice (Morris 2000). In rats, the uptake efficiency was 23.7% at 5 ppm and 10.1% at 200 ppm; in mice, uptake efficiency decreased from 41.7% at 5 ppm to 9.6% at 200 ppm. Based on the decreased uptake efficiency

Table 3-6. Physiological and Flow Parameters Used in the Sarangapani PBPK Model

Parameter	Mouse	Rat	Human
Body weight (g)	25	250	70,000
Tissue volume as fraction of body weight			
Liver	5.5	3.66	2.6
Richly perfused tissues	10	12.34	8.5
Poorly perfused tissues	70	70	60
Fat	7	6.5	21.4
Blood	7.5	7.5	7.5
Minute ventilation (mL/minute)	24	150	15,000
Pulmonary ventilation (mL/minute)	12–1	75–110	10,500
Cardiac output (mL/minute)	14	110	5,200
Tissue blood flow as fraction of cardiac output			
Liver	15–30	15–30	22.7
Richly perfused tissues	48	28.7	43
Fat	5.9	7	5.2
Upper airways	1.0	1.0	0.25
Conducting airways	0.5	2.1	0.75
Transitional airways	0.1	0.15	0.67
Partition coefficients for styrene			
Blood:air	40	40	48
Liver:blood	2	2	2
Fat:blood	87	87	50
Tissue:blood	1.3	1.3	1.3
Partition coefficients for styrene 7,8-oxide			
Blood:air	2,000	2,000	2,000
Liver:blood	1	1	1
Fat:blood	14	14	14
Tissue:blood	0.6	0.6	0.6
Styrene:styrene oxide tissue-phase diffusivity (cm^2/minute)	0.0002	0.0002	0.0002
Styrene:styrene oxide air-phase diffusivity (cm^2/minute)	6	6	6

Source: Sarangapani et al. 2002

Table 3-7. Respiratory Tract-Specific Physiological Parameters Used in the Sarangapani PBPK Model

Parameter	Mouse	Rat	Human
Tissue thickness (cm)			
Upper airway epithelium	0.005	0.005	0.005
Conducting airway epithelium	0.0025	0.0025	0.0025
Transitional airway epithelium	0.001	0.001	0.001
Pulmonary airway epithelium	0.0003	0.00025	0.0005
Mucus	0.0005	0.001	0.001
Upper airway submucosa	0.01	0.01	0.01
Conducting airway submucosa	0.005	0.005	0.005
Transitional airway submucosa	0.002	0.002	0.002
Surface area (cm^2)			
Upper airway compartment	2.7	13.2	138
Conducting airway compartment	8.87	48.3	2,000
Transitional airway compartment	0.48	5.5	,6220
Pulmonary airway compartment	500	3,400	540,000
Mass transfer coefficient (cm/min)			
Upper airway air-phase	7,200	7,200	1,980
Conducting airway air-phase	312	228	181
Transitional airway air-phase	1,136	481	158
Tissue liquid phase	32	16	19.2
Intracompartment clearance (cm^3/minute)	10	40	400
Lung microsomal protein (mg/mL)	3.8	3.8	3.8
Liver microsomal protein (mg/mL)	13	11	23
Lung cytosolic protein (mg/mL)	68	60	43
Liver cytosolic protein (mg/mL)	94	90	45

Source: Sarangapani et al. 2002

Table 3-8. Kinetic Parameters Used in the Sarangapani PBPK Model

Parameter	Mouse	Rat	Human
V_{max} Cytochrome P450 (nmol/minute/mL)			
Liver	200	50–150	50
Upper airway	183	98	50
Transitional airway	362	46.4	1.7
K_m Cytochrome P450 (nmol/mL)	10	10	10
V_{max} Epoxide hydrolase (nmol/minute/mL)			
Liver	200	250	900
Upper airway	250	250	500
Transitional airway	250	250	500
K_m Epoxide hydrolase (nmol/mL)	100	100	100
V_{max} Glutatione S-transferase (nmol/minute/mL)			
Liver	11,000	6,300	1,400
Upper airway	1,000	1,000	300
Transitional airway	1,000	1,000	300
K_m Glutathione S-transferase for styrene (nmol/mL)	2,500	2,500	2,500
K_m Glutathione S-transferase for styrene oxide (nmol/mL)	700	700	500
Liver glutathione basal concentration (nmol/mL)	8,300	6,300	6,000
Upper airway glutathione basal concentration (nmol/mL)	1,000	2,500	1,000
Transitional airway glutathione basal concentration (nmol/mL)	1,000	1,000	1,000
Glutathione production rate (per minute)	0.012	0.012	0.012

Source: Sarangapani et al. 2002

Table 3-9. Stereospecific Kinetic Parameters for Styrene and Styrene Oxide Metabolism in Rodents Used in the Sarangapani PBPK Model

	Mouse		Rat	
Parameter	R	S	R	S
V_{max} Cytochrome P450-liver (nmol/minute/mL)	108.3	91.7	33.8	59.2
V_{max} Cytochrome P450-terminal bronchioles (nmol/minute/mL)	211.8	88.2	130	250
K_m Cytochrome P450 (nmol/mL)	10	10	10	10
K_m Epoxide hydrolase, liver (nmol/mL)	66.7	133.3	570	151
K_m Epoxide hydrolase, terminal bronchioles (nmol/mL)	125	125	200	50
K_m Epoxide hydrolase (nmol/mL)	29	155	29	155
V_{max} Glutatione S-transferase-liver (nmol/minute/mL)	4,400	6,600	2,400	3,600
V_{max} Glutatione S-transferase-terminal bronchioles (nmol/minute/mL)	400	600	400	600
K_m Glutathione S-transferase for styrene oxide (nmol/mL)	700	2,000	700	2,000
K_m Glutathione S-transferase for glutathione (nmol/mL)	2,500	2,500	2,500	2,500

Source: Sarangapani et al. 2002

observed in rats and mice following exposure to the cytochrome P450 inhibitor, metyrapone, Morris (2000) suggested that styrene was metabolized *in situ* and this metabolism enhanced styrene uptake. In humans, blood styrene levels reached steady state after 75 minutes of exposure to 70 ppm (Wigaeus et al. 1983). The elimination of styrene from blood was biphasic, with a half-time of 1 minute for the rapid distribution phase and 40.8 minutes for the elimination phase. Styrene is rapidly distributed throughout the body with the highest concentrations found in adipose tissue. In rats, the styrene concentration in the adipose tissue was approximately 50-fold higher than in muscle; the biological half-time was 6.3 hours in adipose tissue and 2.4–2.0 hours in the blood, liver, kidney, spleen, muscle, and brain (Teramoto and Horiguchi 1979). In humans, styrene is primarily excreted in the urine as mandelic acid and phenyoxyglylic acid. The half-times of mandelic acid and phenylglyoxylic acid in the urine were 3.6 and 8.8 hours, respectively, in humans exposed to 70 ppm for 2 hours (Wigaeus et al. 1983); another study reported elimination half-times of 2.2–4.2 hours for mandelic acid and 3.5–13.9 hours for phenylglyoxylic acid following a 2-hour exposure to 50 ppm styrene (Johanson et al. 2000).

3.5.2 Mechanisms of Toxicity

A large number of studies have investigated the mechanism of styrene carcinogenic activity, particularly the increased susceptibility of mice. Increases in malignant lung tumors have been observed in mice exposed to 160 ppm 6 hours/day, 5 days/week for approximately 2 years (Cruzan et al. 2001) and following gavage exposure to 300 mg/kg/day administered 5 days/week (NCI 1979b); however, neoplastic tumors have not been observed in rats exposed to concentrations as high as 1,000 ppm 6 hours/day, 5 days/week for 2 years (Cruzan et al. 1998) or 2,000 mg/kg/day 5 days/week for 2 years (NCI 1979b), suggesting that mice are particularly sensitive. As reviewed by IARC (2002), Cohen et al. (2002), and Cruzan et al. (2002), genotoxic and nongenotoxic modes of action have been proposed. Although styrene itself does not appear to be DNA reactive, styrene 7,8-oxide is DNA reactive and has been shown to form stable N_2 and O_6 adducts of deoxyguanosine. Styrene oxide, DNA adducts, and genotoxic effects have been detected in humans, rats, and mice. Styrene (styrene 7,8-oxide is the likely causative agent) has been shown to be mutagenic in bacteria, and exposure can result in increased frequency of sister chromatid exchange, chromosomal aberrations, micronucleated cells, and DNA strand breaks. However, elevated levels of blood styrene oxide do not explain the species differences in tumor formation. In humans, styrene 7,8-oxide is rapidly hydrolyzed by epoxide hydrolase as evidenced by the high levels of mandelic acid, phenylglyoxylic acid, and hippuric acid detected in the urine. Styrene 7,8-oxide is relatively stable in rats and mice, and elevated levels have been detected in blood. The blood

levels of styrene oxide in rats exposed to 1,000 ppm is 100-fold higher than the levels in mouse exposed to 20–40 ppm; however, tumors have not been detected in rats.

Although the nongenotoxic potential mode of action also implicates styrene 7,8-oxide as the causative agent, it involves cytotoxic damage at the target tissue, the lung. In the mouse lung, styrene is primarily metabolized by cytochrome P450, particularly the CYP2F2 isoform, in the Clara cell. The continued exposure to styrene 7,8-oxide results in Clara cell cytotoxicity, increased cell proliferation, bronchiolar epithelial hyperplasia, and eventually lung tumors. Several species differences account for the increased sensitivity of mice, compared to rats and likely humans. Humans appear to have a lower capacity to metabolize styrene in the lung compared to rats and a much lower capacity compared to mice and humans and rats have fewer Clara cells than mice. Mouse Clara cells metabolize higher levels of styrene than rat Clara cells and produce a higher proportion of R-styrene oxide than S-styrene oxide, as compared to rats. It has been estimated that mice produce 15 times more R-styrene oxide than rats; in humans, the S enantiomer also predominates. This is particularly important since R-styrene oxide is a more potent pneumotoxicant than S-styrene oxide. In mice and rats, a portion of the styrene oxide generated is metabolized via glutathione conjugation. Mice appear to be more susceptible to glutathione depletion than rats, and glutathione depletion has been observed in mouse lung tissue at exposure concentrations of 80–300 ppm.

IARC (2002) concluded that the proposed mechanism involving the metabolism of styrene to styrene 7,8-oxide is the mouse Clara cell is the likely mode of action resulting in lung tumors in mice. This mode of action is not likely to be relevant to humans to a biologically significant extent. However, this mechanism and a genotoxic mode of action have not been excluded for humans, and styrene is considered a possible human carcinogen.

3.5.3 Animal-to-Human Extrapolations

Species differences exist in the metabolism of styrene in humans, rats, and mice; these differences are discussed in greater detail in Section 3.4.3. Although all three species predominantly metabolize styrene to styrene 7,8-oxide, there are species differences in the subsequent metabolism of styrene 7,8-oxide. As discussed in the metabolism section, styrene 7,8-oxide is primarily hydrolyzed to mandelic acid via epoxide hydrolase in humans. In rats and mice, styrene 7,8-oxide is also conjugated to form mercapturic acids and styrene is metabolized to phenylacetic acid and/or 4-vinylphenol. In rats, 68–72% of the styrene metabolites in urine are generated from the epoxide hydrolase pathway and 26–35% are from the

glutathione transferase pathway; in mice, 48–59 and 20–35% arise from the epoxide hydrolase and glutathione transferase pathways, respectively (Cruzan et al. 2002). In contrast, 95–100% of the styrene 7,8-oxide is metabolized via the epoxide hydrolase pathway; only trace amounts of mercapturic acids have been detected in human urine. The difference in metabolism could result in significant increases in styrene 7,8-oxide levels in the body following exposure to high levels of styrene which may result in depletion of glutathione. Additionally, a small percentage of styrene can under ring oxidation resulting in the formation of 4-vinylphenol. Ring-opened compounds account for 4–8% of the urinary metabolites in mice, less than 1% in rats, and were not detected in humans. The production of 4-vinylphenol is potentially significant mode of action because it is considered to be more toxic to the liver and lung than styrene or styrene oxide (Cruzan et al. 2005b).

However, these differences in the hepatic metabolism of styrene do not account for all of the observed species differences in styrene toxicity. As discussed in Section 3.2, mice appear to be especially sensitive to styrene toxicity in the liver, nasal olfactory, and lung. In the respiratory tract, the species differences between rats and mice are due to local metabolism of styrene to R-styrene oxide and/or other oxidized metabolites. The higher rate of metabolism in mice and higher production of the more reactive enantiomer likely result in increased susceptibility. The fact that humans have a more limited ability to metabolize styrene in the respiratory tract and possibly a higher potential to detoxify styrene oxide suggests that mice are not a good model for end points in which styrene oxide is the causative agent.

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 1997). 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 *in vitro* studies were located regarding endocrine disruption of styrene.

There is some evidence in styrene workers and in female rats that inhalation exposure to styrene may disrupt the tuberoinfundibular dopaminergic system. Significant alterations in serum prolactin levels have been observed in male and female workers exposed to air concentrations as low as 50 ppm (Bergamaschi et al. 1996, 1997; Luderer et al. 2004; Mutti et al. 1984b); a regression model predicts that exposure to ≥20 ppm would result in significant elevations in serum prolactin levels (Luderer et al. 2004). Elevated serum prolactin levels have also been observed in female rats acutely exposed to 150 ppm (Umemura et al. 2005); alterations have not been observed in male rats exposed to concentrations as high as 1,500 ppm (Jarry et al. 2002; Umemura et al. 2005). As noted by NTP (2006), the clinical significance of the increased serum prolactin levels, in the absence of other reproductive effects, is not known. Styrene exposure does not appear to adversely affect thyroid stimulating hormone levels in humans (Arfini et al. 1987) or rats (Umemura et al. 2005) or follicle stimulating hormone or luteinizing hormone in humans (Arfini et al. 1987).

No significant alterations were observed in a gonadal sex differentiation assay using genetic male frogs exposed to styrene (Ohtani et al. 2001).

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).

No studies were identified that examined the toxicity of styrene in children. Several occupational exposure studies have examined the developmental toxicity of styrene; these studies did not find statistically significant alterations in the occurrence of stillbirths, infant deaths, malformations, or birth weight (Ahlborg et al. 1987; Härkönen et al. 1984; Lemasters et al. 1989). However, an expert panel (NTP 2006) evaluating these data concluded that the human studies were not sufficient to evaluate developmental toxicity due to the low statistical power of the studies and the lack of adequate information on exposure.

In general, animal studies have not found styrene-related developmental effects following inhalation exposure in rats (Murray et al. 1978), mice (Kankaanpää et al. 1980), rabbits (Murray et al. 1978), or hamsters (Kankaanpää et al. 1980) or oral exposure in rats (Daston et al. 1991; Murray et al. 1978); additionally, no developmental effects were observed in a rat two-generation study (Cruzan et al. 2005b). An expert panel determined that there was sufficient animal data to conclude that styrene does not cause developmental toxicity in rats following inhalation or oral exposure or in rabbits following inhalation exposure. No studies examined styrene toxicity following exposure of young laboratory animals.

Studies in adults, particularly reinforced plastics industry workers, have identified the nervous system as the most sensitive target of styrene toxicity. Inconsistent results have been found in animal neurodevelopmental toxicity studies. Minor alterations in forelimb grip strength and swimming ability were observed in F2 offspring of rats exposed to 500 ppm styrene; however, the investigators (Cruzan et al. 2005a) attributed these alterations to a lower body weight rather than a neurodevelopmental effect of styrene. Another inhalation study found impaired righting reflex in the offspring of rats exposed to 300 ppm during gestation (Katakura et al. 2001). Similarly, impaired amphetamine-induced locomotor

activity and apomorphine-induced stereotypy were observed in the offspring of rats orally administered 200 mg/kg/day styrene during gestation and lactation (Zaidi et al. 1985).

No human or animal data were located on the toxicokinetic properties of styrene in children or immature animals or possible age-related differences in the toxicokinetics of styrene. A lactational toxicokinetic model predicted that styrene can by transferred via maternal milk (Fisher et al. 1997). Subsequent sections of this chapter (Sections 3.8, 3.10, and 3.11) discuss the available information on biomarkers, interactions, and methods for reducing toxic effects. The available information is from adults and mature animals; no child-specific information was identified. It is likely that this information will also be applicable to children.

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).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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. Identification of 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) may be difficult. Biomarkers of exposure to styrene 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 styrene 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 Styrene

The elimination of styrene via expired air may be used to identify exposure to styrene (Guillemin and Berode 1988; Stewart et al. 1968). Only a small percentage of unchanged styrene is expired after cessation of exposure. There are no adequate studies correlating post-exposure exhaled styrene with previous exposure levels. Assessment of occupational exposure involving measurement of unchanged styrene in urine has been reported (Dolara et al. 1984). In this study of workers, the styrene air concentrations were 3.8–14 ppm and the urinary concentrations of styrene were 0.7–4.1 µg/L. Urinary mutagenic activity was also evaluated in this study and was not a good indication of exposure to styrene. Only a small fraction of unchanged styrene is recovered in the urine. However, measurement of styrene in urine is a reliable indicator of styrene exposure if the exposure is recent (Dolara et al. 1984; Gobba et al. 1993; Guillemin and Berode 1988; Pezzagno et al. 1985).

Analysis of unchanged styrene in blood may be used as a qualitative indicator of styrene exposure (Antoine et al. 1986). In one study, styrene was detected in the blood of humans exposed to 80 ppm (Ramsey et al. 1980). The maximum blood concentration at the end of exposure was 0.92 ± 0.26 µg/mL. The half-life values for rapid and slow clearance curves were 0.58 and 13 hours, respectively. In another study, the concentration of styrene in blood (0.2–3.7 mg/L) increased with the level and duration of styrene exposure (Baselt 1988a).

The presence of styrene in adipose tissue is also an indicator of exposure. The concentration of styrene in the adipose tissue of two workers exposed to 7.5–20 ppm of styrene during a work week suggested a half-life of 5.2 days for one worker and 2.8 days for the other worker. The elimination time was estimated to be 5 weeks (Engstrom et al. 1978b).

Levels of occupational exposure to styrene may also be estimated by measurement of styrene metabolites such as MA and PGA in urine (Bartolucci et al. 1986; Elia et al. 1980; Engstrom et al. 1976; Sedivec et al. 1984; Sollenberg et al. 1988). However, large intra-individual differences in MA and PGA urinary concentrations have been reported. A study of the inter- and intra-individual differences found that PGA levels were less variable than MA levels (Symanski et al. 2001) and variability was higher in post-shift urine samples compared to pre-shift urine samples. Expressing MA and PGA levels in units of mg per gram creatinine decreased the source intra-individual variability. Some studies found a good correlation between the time-weighted styrene exposure and urinary MA concentrations (Chua et al. 1993; Engstrom et al. 1976; Härkönen et al. 1974), while other studies found a better correlation with the sum of urinary MA and PGA at the end of the work period (Elia et al. 1980; Ong et al. 1994; Sollenberg et al. 1988). A good correlation between environmental styrene levels and urinary PGA levels has also been found (Chua et al. 1993). Total MA and PGA measured the morning after exposure may be a more reliable biological indicator of styrene exposure in factories where there is high variability in the environmental styrene concentration (Bartolucci et al. 1986).

Reference levels of styrene urinary metabolites likely to be observed in workers exposed to the time-weighted average concentrations by inhalation have been reported. The American Conference of Governmental Industrial Hygienists (ACGIH 2006) recommends a biological exposure index of 400 mg/g creatinine for the sum of MA and PGA in urine.

3.8.2 Biomarkers Used to Characterize Effects Caused by Styrene

The nervous system is the most sensitive target of styrene toxicity in humans. Styrene affects both sensory (color vision, hearing, vestibular) and motor (nerve conduction velocity) function. Impaired performance on neurobehavioral tests and diminished color vision may be indicative of styrene exposure; however, these effects are not specific to styrene and have been observed following exposure to other solvents such as toluene. Several studies have found significant associations between performance on tests of color discrimination (Eguchi et al. 1995; Kishi et al. 2001) or reaction time (Mutti et al. 1984a) and levels of styrene urinary metabolites (mandelic acid and/or phenylglyoxylic acid).

Other investigators have proposed the use of genotoxicity biomarkers. Cytogenetic monitoring of peripheral lymphocytes as a biomarker of effect has been proposed (DeJong et al. 1988; Pero et al. 1982). Future biomarkers may include hemoglobin adducts. Using unscheduled DNA synthesis (UDS) as an indicator of DNA damage, the lymphocytes of 38 individuals occupationally exposed to styrene were evaluated. The induced UDS was significantly increased for the group exposed to 1–40 ppm styrene (Pero et al. 1982). Measurement of chromosome aberration in peripheral blood lymphocyte has been used for many years to monitor the biologic effects of genotoxic chemicals. However, due to high background levels of chromosomal aberration and exposures to other genotoxic workplace chemicals, the sensitivity of this biomarker for the effects of styrene is probably not adequate (DeJong et al. 1988). The role of hepatic glutathione in the toxicity of styrene has been proposed as inhibiting the covalent binding of styrene. This has been confirmed in animal studies by decreased glutathione in styrene-exposed animals (Parkki 1978). However, its use as a biomarker of effect in humans remains to be demonstrated since data on the adverse effects of styrene on the human liver are insufficient.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Styrene metabolism is known to be inhibited by the presence of other chemicals such as toluene, trichloromethylene, and ethyl benzene. The biotransformation of styrene in rats to PGA, MA, and hippuric acid was suppressed by co-administration of toluene (Ikeda et al. 1972). This may be due to competitive inhibition of oxidative mechanisms. Similar results were reported by Ikeda and Hirayama (1978) in rats when styrene metabolism was inhibited by the administration of trichloroethylene. Urinary metabolites of styrene may be markedly reduced when humans or animals are concurrently exposed to organic solvents that inhibit styrene metabolism.

In numerous polymer industries, workers are exposed to styrene and 1,3-butadiene, and several animal studies have found that styrene affects the metabolism and toxicity of 1,3-butadiene (Laib et al. 1992; Leavens and Bond 1996; Leavens et al. 1997) and 1,3-butadiene affects the metabolism of styrene (Leavens et al. 1996); however, the affect of 1,3-butadiene on styrene toxicity has not been well examined. In other industries, workers are co-exposed to styrene and acrylonitrile; in rats receiving an intraperitoneal dose of styrene and gavage dose of acrylonitrile, increases in serum creatinine and asparate aminotransferse levels were observed, as compared to styrene-only exposure (Normandeau et al. 1984).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to styrene than will most persons exposed to the same level of styrene 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 styrene, or compromised function of organs affected by styrene. Populations who are at greater risk due to their unusually high exposure to styrene are discussed in Section 6.7, Populations with Potentially High Exposures.

Styrene is a hazardous substance found in the workplace with much lower levels found in the environment. Therefore, the populations at risk are workers in industries making polystyrene plastics, coating, polyester resins, and other products. Although no populations of unusually susceptible individuals have been identified for styrene, based on the targets of styrene toxicity, an assumption can be made that persons with pre-existing respiratory or neurological problems would be at risk for the irritant action and central nervous system effects of styrene, respectively.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to styrene. 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 styrene. 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 styrene:

Bronstein AC, Currance PL. 1988. Emergency care for hazardous materials exposure. St. Louis, MO: The C.V. Mosby Company, 221-222.

Ellenhorn MJ, Barceloux DG. 1988. Medical toxicology: Diagnosis and treatment of human poisoning. New York, NY: Elsevier, 956-959.

Haddad LM, Winchester JF. 1990. Clinical management of poisoning and drug overdose. 2nd ed. Philadelphia, PA: W.B. Saunders Company, 1226-1228.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to styrene may occur by inhalation, ingestion, or dermal contact. General recommendations for reducing absorption of styrene following exposure include removing the exposed

individual from the contaminated area and removing the contaminated clothing. If the eyes and skin were exposed, they should be flushed with water. Since aspiration of styrene into the lung can cause pulmonary edema and hemorrhage, some authors advise against the use of emetics, but recommend administration of water for dilution of gastric lavage (Bronstein and Currance 1988; Haddad and Winchester 1990). Following acute inhalation exposure, administration of oxygen and use of mechanical ventilation to support respiration have been suggested (Bronstein and Currance 1988; Ellenhorn and Barceloux 1988; Haddad and Winchester 1990). Administration of aminophylline and inhaled bronchodilators may be required to treat bronchospasm (Ellenhorn and Barceloux 1988). Furthermore, cardiac monitoring has been suggested. Supportive treatment may be needed for neurological effects of styrene exposure (Haddad and Winchester 1990).

3.11.2 Reducing Body Burden

Styrene is metabolized by the body, and most styrene that is absorbed is excreted in the urine as metabolites of the parent compound. Styrene is cleared rapidly from the human body. Its half-life is several hours in the blood and about 2–4 days in subcutaneous adipose tissue (see Section 3.4). No method is commonly used to enhance the elimination of the absorbed dose of styrene.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

In humans, central nervous system depression and upper respiratory tract irritation were reported following acute exposure to higher styrene concentrations (see Section 3.2). Studies in animals indicate that chronic styrene exposure causes liver and kidney effects and may induce cancer. Styrene oxide was found to be the active mutagenic metabolite of styrene in several studies (de Raat 1978; Donner et al. 1979; Norppa et al. 1979, 1980a, 1980b, 1981, 1984, 1988; Pohlova et al. 1985; Vainio et al. 1976). Based on these studies, it can be concluded that styrene is a typical indirect mutagen that needs metabolic activation to be able to bind covalently to macromolecules (e.g., nucleic acids). In one of the possible metabolic pathways, styrene oxide is further metabolized to hydroxyphenylethyl mercapturic acid. The reaction utilizes glutathione (Bond 1989). The mutagenic activity of styrene oxide was decreased in the presence of glutathione in *S. typhimurium* TA100 (Yoshikawa et al. 1980). This experiment, therefore, suggests that glutathione may reduce the mutagenic effects of styrene oxide.

The formation of styrene oxide may also contribute to other effects following styrene exposure. Glutathione decreases the cytotoxicity of many reactive chemicals by acting as a scavenger of toxic metabolites. Exposure of rodents to high levels of styrene caused depletion of glutathione content in the

liver cells of these animals (Das et al. 1983; Vainio et al. 1979). Glutathione has been suggested to decrease the hepatoxicity by preventing styrene oxide reaction with other endogenous macromolecules. Similarly, depletion of glutathione was found in all regions of rat brain following exposure to styrene oxide (Dixit et al. 1982; Trenga et al. 1991). The authors speculated that the depletion of brain glutathione may lead to an increased concentration of free styrene oxide with increased binding to cellular nucleophiles. This process would contribute to oxidative injury to neuronal and glial cells and may be a part of styrene-induced neurotoxicity. However, that styrene itself, being a lipophilic compound, may disrupt the nerve membrane function in a manner similar to anesthetic agents.

Although results from *in vitro* studies in bacteria and *in vivo* animal studies demonstrate that exogenous glutathione precursors may decrease the effects of styrene toxicity, the benefit of this treatment is not known in humans. For low-level exposure cases, the endogenous glutathione levels are not likely to be decreased to a significant extent. Therefore, exogenous glutathione precursors such as N-acetylcysteine are not likely to be effective in mitigating the toxic effects of styrene. Exogenous doses of reducing agents may be useful following acute high dose exposure to styrene. In this case, a significant depletion of glutathione may occur as a result of the presence of high levels of styrene oxide. However, there are no clinical data available to date that support the use of this treatment.

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 styrene 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 styrene.

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 Styrene

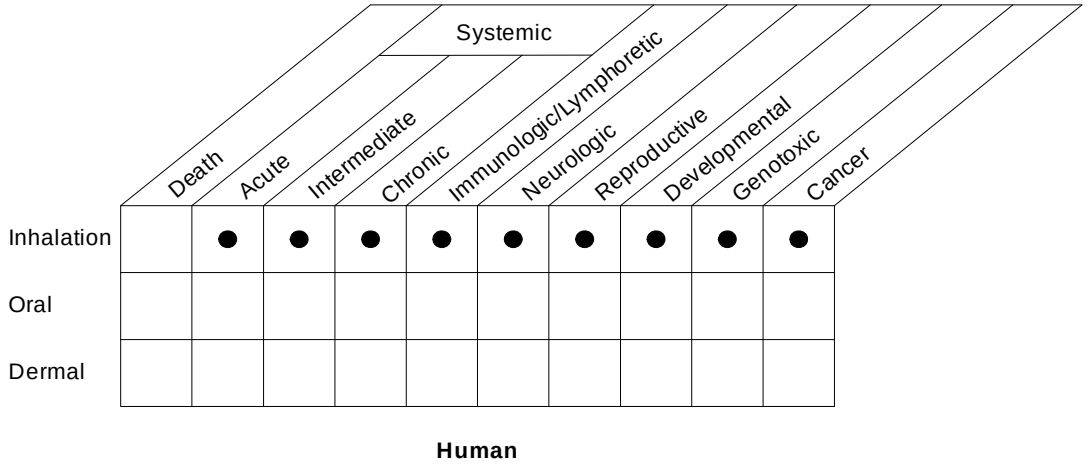
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to styrene are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of styrene. Each dot in the figure indicates that one or more 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.

There is information on most categories of human toxicity via the inhalation route from occupational studies. However, there are limited data on humans exposed to styrene by the oral or dermal routes. Data from animal studies are more extensive, with studies available for most areas of toxicity resulting from exposure via the oral and inhalation routes. Little is known about the effects of dermal exposure to styrene in animals.

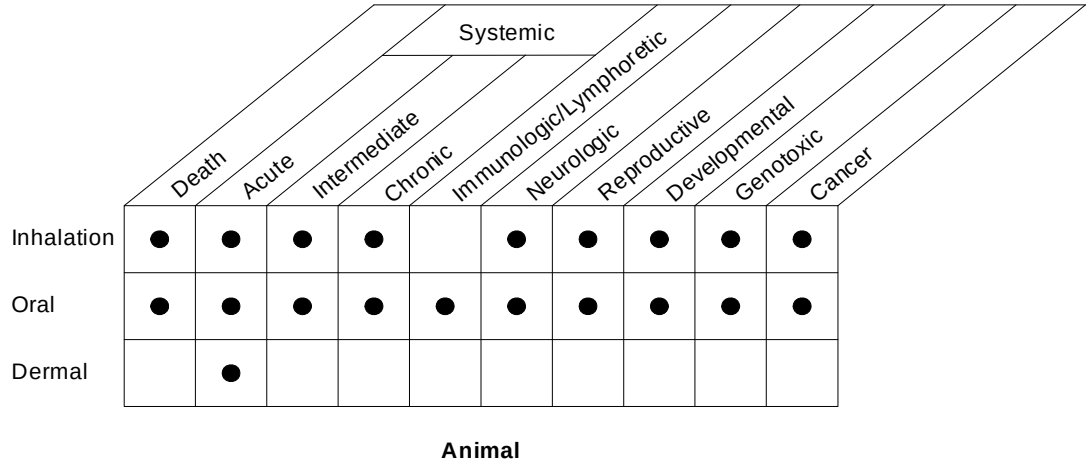
3.12.2 Identification of Data Needs

Acute-Duration Exposure. The possibility for brief human exposure to high concentrations of styrene exists in occupational settings, and might also exist near major spills. Exposure of the general public to episodic high concentrations of styrene at hazardous waste sites, in the home, or in the general environment is unlikely. The respiratory tract and central nervous system are the likely target organ systems for inhaled styrene (Alarie 1973; Carpenter et al. 1944; DeCeaurriz et al. 1983; Kankaanpää et al. 1980; Murray et al. 1978; Seeber et al. 2004; Spencer et al. 1942; Stewart et al. 1968). Animal studies have reported hepatic (Cruzan et al. 1997, 2001; Morgan et al. 1993a, 1993b, 1993c; Vainio et al. 1979) and nasal (Cruzan et al. 2001) effects and hearing impairments (Campo et al. 2001; Crofton et al. 1994; Lataye et al. 2003). Available toxicokinetic data suggest that the mouse may be more sensitive to the hepatic and nasal toxicity of styrene than humans; thus, these data are not suitable for derivation of an acute-duration inhalation MRL. Studies have also examined potential reproductive (Salomaa et al. 1985) and developmental (Kankaanpää et al. 1980; Murray et al. 1978) effects; the highest doses tested in these studies were NOAELs. An acute-duration inhalation MRL based on a NOAEL for neurological effects in

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



Human



Animal

● Existing Studies

humans (Seeber et al. 2004) was derived. Episodic high-level exposures to styrene from contaminated food or water are unlikely. Few studies have examined the toxicity of styrene following exposure to an acute oral dose. Abdominal discomfort was observed in residents exposed to elevated levels of styrene in drinking water (Arnedo-Pena et al. 2003); concomitant inhalation exposure to styrene limits the utilization of this study for MRL derivation. A study in rats identified a LOAEL for neurotoxicity (Husain et al. 1985) and another rat study examined potential developmental effects, but found no adverse effects (Daston et al. 1991). An acute-duration oral MRL was derived using the neurotoxicity study conducted by Husain et al. (1985). Although data on the toxicity of styrene following acute inhalation or oral exposure were considered adequate for the derivation of MRLs, the databases are limited to a few studies; additional studies confirming the dose-response relationships would increase the confidence in these MRLs. Dermal exposure to styrene at significant levels is unlikely except in the case of workplace spills and dermal absorption is probably low based on limited human studies. However, the almost complete lack of dermal toxicity data in animals and humans creates a degree of uncertainty on this issue. Therefore, single-dose dermal studies would be useful in determining target organs and thresholds for dermal exposure. In designing these types of studies, precautions should be taken to avoid concomitant inhalation exposure.

Intermediate-Duration Exposure. Information on the toxicity of styrene in humans following intermediate-duration inhalation exposure is limited to a study examining potential reproductive effects in workers (Lindbohm et al. 1985); however, exposure information was not provided. Inhalation studies in animals have reported damage to the nasal olfactory epithelium in rats (Cruzan et al. 1997, 2005a, 2005b; Ohashi et al. 1986) and mice (Cruzan et al. 1997, 2001), liver damage in mice (Cruzan et al. 1997), eye irritation in rats (Cruzan et al. 1997) and guinea pigs (Spencer et al. 1942), ototoxicity in rats (Campo et al. 2001; Lataye et al. 2000; Loquet et al. 1999, 2000; Makitie et al. 2002; Pouyatos et al. 2002; Pryor et al. 1987; Yano et al. 1992), and impaired nerve conduction velocity (Yamamoto et al. 1997). A two-generation study in rats did not find reproductive, developmental, or neurodevelopmental effects (Cruzan et al. 2005a, 2005b), but another study did find neurodevelopmental effects (Katakura et al. 1999, 2001). However, additional studies are needed, as the data are not considered sufficient to derive an intermediate-duration inhalation MRL. Chronic exposure studies provide strong evidence that the nervous system is the most sensitive target of styrene toxicity; studies examining neurological function of workers exposed to styrene for <1 year would provide valuable data for deriving an intermediate-duration inhalation MRL. Oral exposure studies of intermediate-duration are limited to a small number of animal studies and no human data; observed effects include impaired learning in rats (Bushnell 1994) and decreases in spermatozoa in rats (Srivastava et al. 1989, 1992a, 1992b). The results of the Srivastava

studies have been questioned by the NTP Expert Panel (NTP 2006) because the findings are inconsistent with the lack of reproductive effects found in an inhalation two-generation study conducted by Cruzan et al. (2005b). The LOAELs identified in these studies were higher than the lowest LOAEL identified in an acute-duration neurotoxicity study (Husain et al. 1985). The intermediate-duration database was considered inadequate for derivation of an MRL because an intermediate-duration oral MRL based on the LOAELs identified in the intermediate-duration oral studies would be higher than the acute-duration oral MRL. Studies in animals are needed to establish dose-response relationships for neurotoxicity, the presumed sensitive end point. Additionally, more studies examining the potential reproductive toxicity of ingested styrene are needed to confirm the results of the Srivastava studies. One study examined the dermal toxicity of styrene in rabbits (Spencer et al. 1942); basic information on the adverse effects of intermediate-duration dermal exposure to styrene in animals is also needed due to the sparsity of available data.

Chronic-Duration Exposure and Cancer. A large number of occupational exposure studies have examined the chronic toxicity of styrene. Systemic toxicity studies have examined endocrine (Bergamaschi et al. 1997; Mutti et al. 1984b), hematological (Checkoway and Williams 1982; Thiess and Friedheim 1978), hepatic (Hotz et al. 1980; Lorimer et al. 1978), or renal (Verplanke and Herber 1998; Viau et al. 1987; Vyskocil et al. 1989) end points; most studies relied on biomarkers of toxicity. The most widely examined end point is neurotoxicity and the available data suggest that this is the most sensitive end point. Examined neurological end points included color vision (Campagna et al. 1995, 1996; Chia et al. 1994; Eguchi et al. 1995; Fallas et al. 1992; Gobba et al. 1991; Gong et al. 2002; Kishi et al. 2001; Mutti et al. 1984a), vestibular effects (Calabrese et al. 1996; Möller et al. 1990), hearing impairment (Morata et al. 2002; Morioka et al. 1999; Muijser et al. 1988; Śliwińska-Kowalska et al. 2003), symptoms of neurotoxicity (Checkoway et al. 1992; Cherry et al. 1980; Edling et al. 1993; Viaene et al. 1998, 2001), performance on neurobehavioral tests (Cherry et al. 1980; Edling et al. 1993; Gamberale et al. 1976; Jegaden et al. 1993; Lindstrom et al. 1976; Mutti et al. 1984a; Tsai and Chen 1996; Viaene et al. 1998, 2001), nerve conduction velocity (Behari et al. 1986; Murata et al. 1991; Rosen et al. 1978), olfactory alterations (Dalton et al. 2003), and EEG alterations (Härkönen et al. 1984; Seppäläinen and Härkönen 1976). Other human studies have examined reproductive (Härkönen and Holmberg 1982; Hemminki et al. 1980) and developmental (Ahlborg et al. 1987; Lemasters et al. 1989) end points. The chronic toxicity of styrene has also been examined in rat (Cruzan et al. 1998; Jersey et al. 1978) and mouse (Cruzan et al. 2001) studies. The occupational exposure studies were considered adequate for derivation of a chronic-duration inhalation MRL for styrene. Further research to define the dose-response curve more fully and to identify a chronic inhalation NOAEL for neurological effects

would be valuable and would help to reduce uncertainty in the MRL. Data on chronic oral exposure to styrene is only available through animal studies (Beliles et al. 1985; Conti et al. 1988; NCI 1979b; Quast et al. 1979). In these studies, the most sensitive indicator of toxicity appears to be Heinz body formation in red blood cells in dogs (Quast et al. 1979) and the EPA has calculated a chronic oral RfD based on this study (IRIS 2007). However, there is some doubt regarding the chronic oral NOAEL, and whether hematological effects are really more sensitive than neurological effects. Moreover, decreased survival has been noted in rats at exposure levels only slightly higher than the no-effect level for hematological effects (Conti et al. 1988). Therefore, no chronic oral MRL has been derived. Further studies on the effects of oral exposure, with special emphasis on neurological or neurobehavioral effects, would be valuable. Although chronic dermal exposure by the general public is not likely, there may be some potential for dermal contact with soil at hazardous waste sites. Therefore, data on long-term effects of dermal contact with styrene would be useful.

Taken together, the animal and human data indicate that styrene may possibly be a weak human carcinogen. Although data from epidemiological studies are limited due to concurrent chemical exposures and small cohorts, the data are suggestive of some carcinogenic potential in humans (Antilla et al. 1998; Bond et al. 1992; Cheng et al. 2007; Coggon et al. 1987; Delzell et al. 1996, 2001; Frentzel-Beyme et al. 1978; Gerin et al. 1998; Graff et al. 2005; Hodgson and Jones 1985; Kogevinas et al. 1993, 1994; Kolstad et al. 1993, 1994, 1995; Macaluso et al. 1996; Matanoski and Schwartz 1987; Matanoski et al. 1990; McMichael et al. 1976; Meinhardt et al. 1982; Nicholson et al. 1978; Okun et al. 1985; Ott et al. 1980; Sathiakumar et al. 2005; Wong 1990; Wong et al. 1994). Inhalation and oral exposure studies in rats have not found significant increases in the incidence of neoplastic tumors (Beliles et al. 1985; Conti et al. 1988; Cruzan et al. 1998; Jersey et al. 1978; Maltoni et al. 1979; NCI 1979b). However, inhalation and oral studies in mice have found significant increases in the incidence of neoplastic lung tumors (Cruzan et al. 2001; NCI 1979b). The available data suggest that toxicokinetic differences between rats, mice, and humans result in an increased sensitivity of mice. Clarification of the data is needed in several areas. Almost all of the available epidemiological studies involve concurrent exposures to other chemicals. The role of the metabolism of styrene in humans and animals needs to be clarified and the carcinogenic mechanisms needed to be further elucidated. Additional studies that account for these issues would be valuable.

Genotoxicity. The results of genotoxicity tests for styrene both *in vivo* and *in vitro* are frequently conflicting, and the genotoxic potential of styrene is not clear (Andersson et al. 1980; Beliles et al. 1985; Hogstedt et al. 1979; Meretoja et al. 1977, 1978; Watanabe et al. 1981). The reasons for the mixed or

conflicting genotoxicity results may be differences in the metabolism or detoxification of styrene in the various test systems employed. The role of the metabolite styrene oxide in genotoxicity assays on styrene should be fully evaluated, preferably in mammalian *in vivo* systems. Toxicokinetic studies evaluating the presence, level, and activity of styrene oxide in humans will influence the interpretation of genotoxicity studies on styrene and their relevance to public health.

Reproductive Toxicity. Occupational exposure studies have examined male and female styrene workers to evaluate potential reproductive effects; however, most of these studies did not quantify styrene exposure or exposure to other compounds, thus, interpretation of results is difficult. Inconsistent results have been reported for spontaneous abortions with some studies reporting significant increases (Härkönen and Holmberg 1982; Hemminki et al. 1980; McDonald et al. 1988) and others reporting no effect (Härkönen and Holmberg 1982; Hemminki et al. 1980, 1984; Lindbohm et al. 1985). Oligomenorhhea was observed in one study of workers (Cho et al. 2001), but not in another study (Lemasters et al. 1985). Studies in male workers have found alterations in sperm parameters (Kolstad et al. 1999a), but no alterations in time-to-pregnancy (Kolstad et al. 2000; Sallmén et al. 1998) or fertility rates (Kolstad et al. 1999c). A two-generation inhalation study (Cruzan et al. 2005b) and three-generation oral study (Beliles et al. 1985) in rats showed no styrene-related reproductive effects. However, testicular effects have been observed in an oral exposure study (Srivastava et al. 1989), but not in two inhalation studies (Cruzan et al. 2005b; Salomaa et al. 1985). Additional reproductive data on occupationally-exposed males would be useful in evaluating the existing animal data that indicates altered testicular function and studies in females would be useful in evaluating the inconsistent findings in the existing studies.

Developmental Toxicity. Data on the developmental effects of inhalation exposure to styrene are available in humans and animals. Occupational exposure studies (Ahlborg et al. 1987; Härkönen et al. 1984; Lemasters et al. 1989) have not found increases in the occurrence of birth defects or decreases in birth weight. However, interpretation of the results are complicated by exposure to other chemicals and lack of information on exposure levels. Additional occupational studies are needed to adequately assess this end point. Developmental studies in animals via inhalation (Cruzan et al. 2005b; Kankaanpää et al. 1980; Murray et al. 1978) or oral (Beliles et al. 1985) exposure have not found effects on fetal outcome, birth weight, or incidence of abnormalities. However, several studies have reported neurodevelopmental (Katakura et al. 1999, 2001; Zaidi et al. 1985) or reproductive (Srivastava et al. 1992a, 1992b) effects. Additional studies are needed to examine the potential effects on the nervous and reproductive systems of developing organisms. No studies examined the developmental toxicity of styrene following dermal exposure.

Immunotoxicity. Occupational exposure studies have found alterations in lymphocyte subsets (Bergamaschi et al. 1995b; Biró et al. 2002), which may be indicative of reduced cell-mediated immunity and an impaired immune response to concanavalin (Tulinska et al. 2000). Limited data in animals indicate that inhalation (Ban et al. 2006) and oral (Sinitskij discussed in WHO 1983) exposure can also result in impaired immune response. No dermal exposure studies examining immunotoxicity were identified. Human and animal studies provide suggestive evidence that the immune system is a target; additional studies would be useful to further investigate the effect of styrene on immune function.

Neurotoxicity. The neurotoxicity of styrene in workers in the reinforced plastic industry has been extensively examined (Behari et al. 1986; Calabrese et al. 1996; Campagna et al. 1995, 1996; Castillo et al. 2001; Checkoway et al. 1992; Cherry et al. 1980; Chia et al. 1994; Dalton et al. 2003; Edling et al. 1993; Eguchi et al. 1995; Fallas et al. 1992; Fung and Clark 1999; Gamberale et al. 1976; Gobba et al. 1991, 1995; Gong et al. 2002; Härkönen et al. 1984; Iregren et al. 2005; Jegaden et al. 1993; Kishi et al. 2001; Lindstrom et al. 1976; Matikainen et al. 1993a, 1993b; Möller et al. 1990; Morata et al. 2002; Morioka et al. 1999; Muijser et al. 1988; Murata et al. 1991; Mutti et al. 1984a; Niklasson et al. 1993; Rosen et al. 1978; Seppäläinen and Härkönen 1976; Śliwińska-Kowalska et al. 2003; Štětkářová et al. 1993; Triebig et al. 1985, 2001; Tsai and Chen 1996; Viaene et al. 1998, 2001; Yuasa et al. 1996). A variety of neurological effects have been observed in these studies including decreased color discrimination, slowed reaction time, altered performance on neurobehavioral tests of memory and learning, altered vestibular function, altered hearing, reduced nerve conduction velocity, and increased clinical symptoms such as dizziness, tiredness, memory loss, and feeling drunk. Additionally, several experimental studies have examined the effects of acute exposure on vestibular function (Ödkvist et al. 1982; Stewart et al. 1968), clinical symptoms (Seeber et al. 2004; Stewart et al. 1968), and performance on neurobehavioral tests (Seeber et al. 2004). Animal studies have primarily focused on the damage to the organ of Corti and hearing loss (Campo et al. 2001; Crofton et al. 1994; Lataye et al. 2003; Loquet et al. 1999, 2000; Makitie et al. 2002; Pouyatos et al. 2002; Pryor et al. 1987; Yano et al. 1992), although nerve conduction velocity has also been examined (Kulig 1988; Yamamoto et al. 1997). The potential for neurotoxicity has not been examined in humans orally exposed to styrene and a limited number of end points have been examined in animals (Agrawal et al. 1982; Bushnell 1994; Husain et al. 1980, 1985; Khanna et al. 1994). The neurological effects observed in styrene workers were used as the basis of a chronic-duration inhalation MRL. Since this is based on a LOAEL, further studies which define the chronic NOAEL, as well as acute- and intermediate-duration NOAELs, would be valuable especially at levels of styrene causing problems with coordination and psychological function. These and other

neurological effects may play a role in the rate of workplace accidents and the level of performance. Additional studies in mammalian animal models are needed to determine if styrene causes chronic damage to the central and/or peripheral nervous systems and to determine the associated mechanism of toxicity. Also, information is needed to determine if neurotoxicity is a sensitive end point from exposure to styrene via the oral route.

Epidemiological and Human Dosimetry Studies. Numerous studies have examined the toxicity of styrene in workers, as discussed in other sections, most of these studies have focused on neurotoxicity and potential carcinogenicity of styrene. A common limitation of these studies is the poor characterization of exposure levels and possible exposure to other chemicals. Some studies provided no data on styrene exposure levels and other studies provide current exposure levels with limited or no data on past exposure levels. Occupational exposure and experimental studies also provide suggestive evidence of acute upper respiratory tract irritation and eye irritation (Carpenter et al. 1944; NIOSH 1983) Stewart et al. 1968) and possible endocrine effects (elevated levels of serum prolactin) (Arfini et al. 1987; Bergamaschi et al. 1996, 1997; Luderer et al. 2004; Mutti et al. 1984b); additional studies are needed to confirm the results of these studies and to establish dose-response relationships. Additionally, there are suggestive findings that styrene has the potential to induce reproductive effects (Cho et al. 2001; Härkönen and Holmberg 1982; Hemminki et al. 1980; Kolstad et al. 1999c; McDonald et al. 1988); however, poor characterization of styrene exposure, possible exposure to other compounds (particularly for the Cho et al. (2001) study), the low statistical power of the studies, and the lack of positive associations in the follow-up study (Hemminki et al. 1984) to the Hemminki et al. (1980) study limit the usefulness of the studies. Studies of males and female styrene workers examining a variety of reproductive end points and adequately characterized exposure would be useful.

Biomarkers of Exposure and Effect.

Exposure. Available studies indicate that there are good quantitative relationships between styrene metabolites (MA and PGA) in the urine and styrene exposure levels in humans (Bartolucci et al. 1986; Chua et al. 1993; Elia et al. 1980; Engstrom et al. 1976; Härkönen et al. 1978; Ong et al. 1994; Sedivec et al. 1984; Sollenberg et al. 1988; Symanski et al. 2001). Levels of styrene in blood have also been used as a biomarker of exposure (Antoine et al. 1986; Baselt et al. 1988a; Ramsey et al. 1980).

Effect. There are currently no biomarkers specific for the effects of styrene that are not also typical of other central nervous system depressants. Further research is needed to evaluate potential biomarkers of

effect in the areas of chromosome aberrations, psychomotor decrement, hepatic glutathione depletion, and adipose tissue retention of styrene. These potential biomarkers should be evaluated in terms of long-term or chronic exposure periods, and their specificity for exposure to styrene.

Absorption, Distribution, Metabolism, and Excretion. Styrene oxide (styrene epoxide) has been identified as an intermediate metabolite of styrene (Drummond et al. 1989; Engstrom et al. 1976; Korn et al. 1984, 1987; Leibman 1975; Lof et al. 1983; Withey and Collins 1979; Young et al. 1979). However, styrene oxide has only been found in minute amounts in human studies (Lof et al. 1986a). The presence of styrene oxide, a mutagen and carcinogen, may account for some conflicting results and/or interspecies variation in mutagenicity tests and cancer bioassays. The role, if any, of styrene oxide in the overall toxicity of styrene needs to be evaluated by additional metabolism studies to confirm its presence, level, and duration in human tissues. The toxicokinetics of styrene exposure via inhalation are reasonably well defined. However, oral and dermal exposure data are needed to better characterize absorption rates and the elimination ratios of the metabolites (MA and PGA).

Comparative Toxicokinetics. Interspecies variations in styrene metabolism have been established. Differences in the relative proportion of urinary metabolites, which is indicative of different metabolic pathways have been found in humans, mice, and rats. Additionally, there are differences in the kinetic constants for cytochrome P450 and epoxide hydrolase, which result in higher levels of reactive metabolites in the liver, lungs, and nasal epithelium. Also, mice appear to generate a higher proportion of R-styrene oxide than S-styrene oxide; the R-enantiomer is believed to be more cytotoxic. These metabolic differences are believed to result in mice being more sensitive than rats or humans to liver, lung, and nasal toxicity. Potential species differences in the neurotoxicity, the most sensitive end point in humans, have not been examined; if the neurological effects are due to styrene rather than one of its metabolites, the observed species differences may not be relevant. Efforts should continue to identify which animal model best approximates human metabolism of styrene.

Methods for Reducing Toxic Effects. Recommended methods for the mitigation of acute effects of styrene intoxication include mechanical ventilatory support, administration of oxygen, and drug therapy for bronchospasm, if exposure is by inhalation (Bronstein and Currance 1988; Ellenhorn and Barceloux 1988). Thorough washing or flushing with water is recommended for dermal/ocular exposure. Supportive treatment is indicated for neurological effects of styrene exposure (Haddad and Winchester 1990). No information was located concerning mitigation of effects of lower-level or longer-term exposure to styrene. Further information on techniques to mitigate such effects would be useful in

determining the safety and effectiveness of possible methods for treating styrene-exposed populations in the vicinity of hazardous waste sites. This includes further studies on the mechanism(s) of styrene toxicity, so that methods may be developed to interfere with or block styrene's toxic actions in the body.

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.

No studies were identified that examined the toxicity of styrene in children or young laboratory animals. No consistently observed developmental effects have been reported in occupational exposure studies (Ahlborg et al. 1987; Härkönen et al. 1984; Lemasters et al. 1989) or in animal studies (Cruzan et al. 2005b; Daston et al. 1991; Kankaanpää et al. 1980; Murray et al. 1978). The nervous system is the most sensitive target of styrene toxicity in adults. No adverse styrene-related effects were observed in neurobehavioral function tests in rats exposed to styrene during gestation and lactation (Cruzan et al. 2005a); however, neurological effects have been observed in another inhalation study of rats exposed during gestation (Katakura et al. 2001) and in an oral gestation and lactation study (Zaidi et al. 1985). Possible neurological effects have not been assessed following post-weaning exposure; these data would be useful in evaluating whether growing children are more susceptible than adults to styrene-induced neurotoxicity.

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

No ongoing studies on styrene were identified in Federal Research in Progress database (FEDRIP 2007).