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 1,3-butadiene. 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.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of 1,3-butadiene are indicated in Table 3-1 and Figure 3-1. Because cancer effects could occur at lower exposure levels, Figure 3-1 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 1,000,000 (10^{-4} to 10^{-6}), as developed by EPA.

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

3.2.1 Inhalation Exposure

3.2.1.1 Death

Information on the lethality of 1,3-butadiene in humans is limited. A number of occupational exposure studies have examined mortality ratios in 1,3-butadiene workers, the results of these studies are discussed in subsequent sections on the primary effects.

No deaths were seen in B6C3F1 mice exposed to ≤8,000 ppm 1,3-butadiene 6 hours/day, 5 days/week, for 2 weeks (NTP 1984). The majority of rabbits died when exposed to 250,000 ppm 1,3-butadiene for an average of 23 minutes (Carpenter et al. 1944). The LC_{50} values calculated for mice and rats exposed for 2 and 4 hours, respectively, was 122,000 and 129,000 ppm, respectively (Shugaev 1969).

Intermediate-duration exposures produced no deaths in rats exposed for 6 hours/day, 5/days/week, for 13 weeks to 8,000 ppm (Crouch et al. 1979), or in rats, guinea pigs, rabbits, and dogs during 8 months of exposure to 6,700 ppm (Carpenter et al. 1944). Increased mortality was seen in mice exposed to 5,000 ppm, but not 2,500 ppm, for 6 hours/day, 5 days/week, for 14 weeks (NTP 1984). The lowest

intermediate-duration exposure resulting in death was observed in mice receiving 200 ppm for 6-hours/day, 5 days/week, for 40 weeks (NTP 1993), ostensibly from the early development of neoplasms.

During chronic exposure to 625 and 1,250 ppm of 1,3-butadiene for 61 weeks, significantly increased mortality, primarily due to cancer, was found in B6C3F1 mice (NTP 1984). Similar results were obtained in another study using a much lower concentration (20 ppm) (NTP 1993). Exposure of rats to 8,000 ppm 1,3-butadiene resulted in statistically significant increased mortality from cancer when compared with controls (Owen et al. 1987). The LC_{50} values and all reliable LOAEL values for death in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

Respiratory Effects. Workers exposed to 1,3-butadiene gas during the manufacture of rubber complained of irritation of the eyes, nasal passages, throat, and lungs (Wilson 1944). In some, coughing, fatigue, and drowsiness developed. All symptoms disappeared on removal from the gas. The associated exposure levels were not reported.

No effects in respiratory tissues were observed in rats, guinea pigs, rabbits, or dogs inhaling up to 6,700 ppm 1,3-butadiene for 7.5 hours/day, 6 days/week, for 8 months (Carpenter et al. 1944) or in rats or mice exposed to 8,000 ppm 1,3-butadiene for 6 hours/day, 5 days/week, for 13–14 weeks (Crouch et al. 1979; NTP 1984). No effects were observed in lungs of mice exposed to concentrations as high as 625 ppm for 6 hours/day, 5 days/week, for 9 months (NTP 1993).

An increase in chronic inflammation of the nasal cavity, fibrosis, cartilaginous metaplasia, osseous metaplasia, atrophy of the sensory epithelium, and hyperplasia of the respiratory epithelium were observed in mice exposed to 1,250 ppm for 2 years (NTP 1984). Lungs of rats exposed chronically to 8,000 ppm 1,3-butadiene exhibited metaplasia (Owen and Glaister 1990; Owen et al. 1987). Atrophy of the nasal olfactory epithelium was observed in mice exposed to concentrations as high as 1,250 ppm 1,3-butadiene for 6 hours/day, 5 days/week, for 61 weeks (NTP 1993), while alveolar epithelial hyperplasia (a possible precancerous lesion) occurred in mice exposed to 6.25 ppm 6 hours/day, 5 days/week for 2 years (NTP 1993).

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - 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	1 d 4 h/d				129000 (LC50)	Shugaev 1969	
2	Mouse	1 d 2h/d				122000 (LC50)	Shugaev 1969	
3	Rabbit	1 d 23 min/d				250000	Carpenter et al. 1944	
Systemic								
4	Rat	10 d 6 hr/d Gd 6-15	Bd Wt		200 F (decreased maternal body weight gain)	8000 F (45% decreased maternal body weight gain)	Irvine 1981	
Neurological								
5	Human	1 d 6-8 hr/d		8000			Carpenter et al. 1944	
6	Rabbit	1 d 23 min/d				250000 (anesthesia)	Carpenter et al. 1944	
Reproductive								
7	Mouse (B6C3F1)	5 d 6 h/d			1000 M (73% increase in number of abnormal sperm heads)		DOE/NTP 1988a	
8	Mouse CD-1	6 hr/day 5 days			200 M (increased intrauterine death)		DOE/NTP 1988b	

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - 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								
9	Rat (Sprague-Dawley)	10 d 6 hr/d Gd 6-15		1000			DOE/NTP 1987a	
10	Rat	10 d 6 hr/d Gd 6-15		200	8000 (decreased fetal growth) 1000 (major skeletal malformations)		Irvine 1981	
11	Mouse (CD-1)	10 d 6 hr/d GD 6-15			40 M (decreased fetal BW in males)		DOE/NTP 1987b	
INTERMEDIATE EXPOSURE								
Death								
12	Mouse	14 wk 5 d/wk 6 hr/d				5000 (increased mortality)	NTP 1984	
13	Mouse (B6C3F1)	13-52 wk 6 hr/d 5 d/wk				200 (increased mortality from 40 weeks of exposure)	NTP 1993	

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - 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
Systemic								
14	Rat	13 wk 5 d/wk 6 hr/d	Resp	8000			Crouch et al. 1979	
			Cardio	8000				
			Hemato	8000				
			Musc/skel	8000				
			Hepatic	8000				
			Renal	8000				
			Dermal	8000				
			Ocular	8000				
15	Mouse	3-24 wk 6 d/wk 6 hr/d	Hemato			1250 M (macrocytic megaloblastic anemia starting at 6 weeks)	Irons et al. 1986a, b	

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - 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
16	Mouse	14 wk 5 d/wk 6 hr/d	Resp	8000			NTP 1984	
			Cardio	8000				
			Gastro	8000				
			Musc/skel	8000				
			Hepatic	8000				
			Renal	8000				
			Dermal	8000				
			Bd Wt	1250	2500 M (13% decreased body weight)			
17	Mouse (B6C3F1)	13-52 wk 6 hr/d 5 d/wk	Resp		200 M (alveolar epithelial hyperplasia after 40 weeks)		NTP 1993	
			Cardio		200 M (endothelial hyperplasia after 40 weeks)			
			Ocular	200 M	625 M (Harderian gland hyperplasia after 26 weeks)			

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - 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	Mouse (B6C3F1)	40 wk 6 hr/d 5 d/wk	Resp	200 M	625 M (alveolar epithelial hyperlasia)		NTP 1993	
			Cardio	625				
			Gastro	200	625 (forestomach epithelial hyperplasia)			
			Hemato		62.5 M (decreased erythrocyte counts, hemoglobin concentration, and red cell volume)	200 F (macrocytic megaloblastic anemia)		
			Musc/skel	625				
			Hepatic	625 F				
			Bd Wt	625				
Immuno/ Lymphoret								
19	Mouse (B6C3F1)	40 wk 6 hr/d 5 d/wk			625 F (19% reduction in relative thymus weight)		NTP 1993	
20	Mouse	6-24 wk 5 d/wk 6 hr/d			1250 (lymphoid organ histopathology)		Thurmond et al. 1986	
Neurological								
21	Rat	13 wk 5 d/wk 6 hr/d		8000			Crouch et al. 1979	

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - 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
22	Mouse	14 wk 5 d/wk 6 hr/d		8000			NTP 1984	
Reproductive								
23	Mouse (CD)	10 wk 6 h/d 5 d/wk				12.5 M (increase in late fetal deaths, exencephalies, and skull abnormalities)	Anderson et al. 1996	
24	Mouse (CD)	4 wk 6 h/d 5 d/wk		12.5 M	65 M (increases in early fetal deaths)		Anderson et al. 1998	
25	Mouse (B6C3F1)	40 wk 6 hr/d 5 d/wk		62.5 F		200 F (ovarian atrophy)	NTP 1993	
Cancer								
26	Mouse (B6C3F1)	13-52 wk 6 hr/d 5 d/wk				200 M (CEL:lymphocytic lymphoma, histiocytic sarcoma, cardiac hemangiosarcoma, alveolar/bronchiolar adenoma/carcinoma, forestomach squamous cell papilloma/carcinoma, hepatocellular adenoma, Harderian gland adenoma/adenocarcinoma preputial gland carcinoma)	NTP 1993	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form	Comments
CHRONIC EXPOSURE										
Death										
27	Rat	105-111 wk 5 d/wk 6 hr/d		1000			8000	(increased mortality)	Owen et al. 1987, Owen and Glaister 1990	
28	Mouse	61 wk 5 d/wk 6 hr/d					625	(increased mortality)	NTP 1984	
29	Mouse (B6C3F1)	2 yr 6 hr/d 5 d/wk					20	(increased mortality)	NTP 1993	
Systemic										
30	Rat	105-111 wk 5 d/wk 6 hr/d	Resp	1000	8000	(increased organ weight, metaplasia)			Owen et al. 1987, Owen and Glaister 1990	
			Cardio	8000						
			Gastro	8000						
			Hemato	8000						
			Hepatic	8000						
			Renal	1000			8000	(nephrosis)		
			Dermal	8000						
			Ocular	8000						

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form	Comments
31	Mouse	61 wk 5 d/wk 6 hr/d	Resp	625			1250	(atrophy of nasal olfactory epithelium)	NTP 1984	
			Cardio		625	(endothelial hyperplasia)				
			Gastro		625	(epithelial hyperplasia)				
			Musc/skel	1250						
			Hepatic		625	(hepatic necrosis)				
			Renal	1250						
			Dermal	1250						
32	Mouse (B6C3F1)	2 yr 6 hr/d 5 d/wk	Resp		6.25 M	(alveolar epithelial hyperplasia)			NTP 1993	
			Cardio	200	625	(endothelial hyperplasia)				
			Gastro		625 F	(forestomach epithelial hyperplasia)				
					200 F	(forestomach epithelial hyperplasia)				
			Musc/skel	625						
			Hepatic		62.5	(liver necrosis)				
			Bd Wt	625						

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - 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
33	Mouse (B6C3F1)	65 wk 6 hr/d 5 d/wk	Hemato	200		625	(macrocytic megaloblastic anemia)	NTP 1993	
			Bd Wt	625					
Neurological									
34	Rat	105-111 wk 5 d/wk 6 hr/d		8000				Owen et al. 1987, Owen and Glaister 1990	
35	Mouse	61 wk 5 d/wk 6 hr/d		1250				NTP 1984	
Reproductive									
36	Rat	105-111 wk 5 d/wk 6 hr/d		8000				Owen et al. 1987, Owen and Glaister 1990	Only examined histopathology
37	Mouse	61 wk 5 d/wk 6 hr/d				625	(gonadal atrophy)	NTP 1984	
38	Mouse (B6C3F1)	2 yr 6 hr/d 5 d/wk				6.25 F	(ovarian atrophy)	NTP 1993	
39	Mouse (B6C3F1)	65 wk 6 hr/d 5 d/wk		20 F		62.5 F	(ovarian atrophy)	NTP 1993	

Table 3-1 Levels of Significant Exposure to 1,3-butadiene - 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								
40	Rat	105-111 wk 5 d/wk 6 hr/d				1000 (CEL: mammary gland adenoma and sarcoma)	Owen et al. 1987, Owen and Glaister 1990	
41	Mouse	61 wk 5 d/wk 6 hr/d				625 (CEL:alveolar/bronchiolar adenoma or carcinoma, malignant lymphoma, hemangiosarcoma, forestomach squamous cell papilloma or carcinoma, mammary gland carcinoma, ovarian granulosa cell tumor)	NTP 1984	
42	Mouse (B6C3F1)	2 yr 6 hr/d 5 d/wk				6.25 F (CEL: alveolar/bronchiolar adenoma/carcinoma)	NTP 1993	

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

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); F = Female; Gastro = gastrointestinal; Gd = gestational day; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological; LC50 = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to 1,3-Butadiene - Inhalation

Acute (≤14 days)

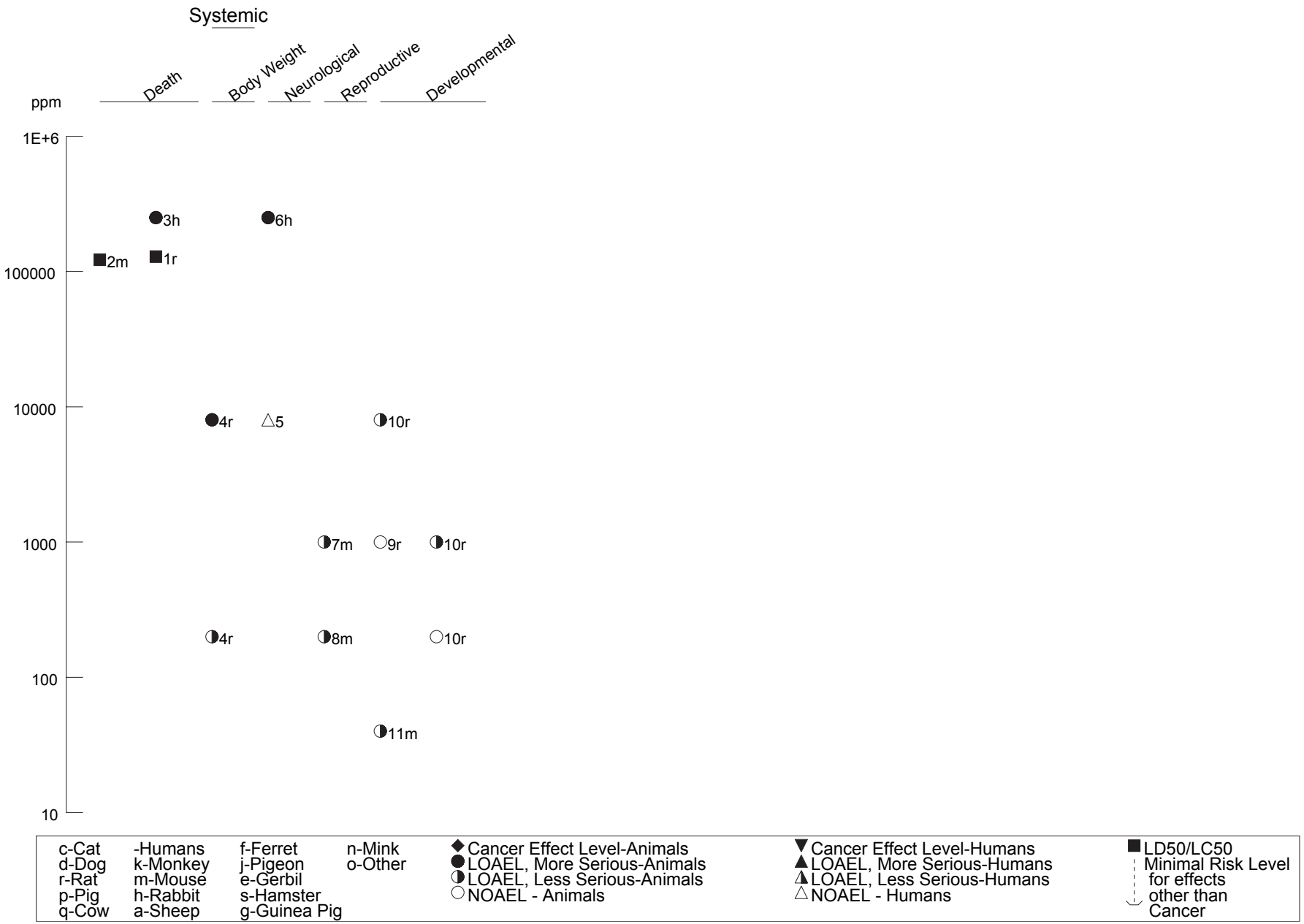


Figure 3-1 Levels of Significant Exposure to 1,3-Butadiene - Inhalation *(Continued)*

Intermediate (15-364 days)

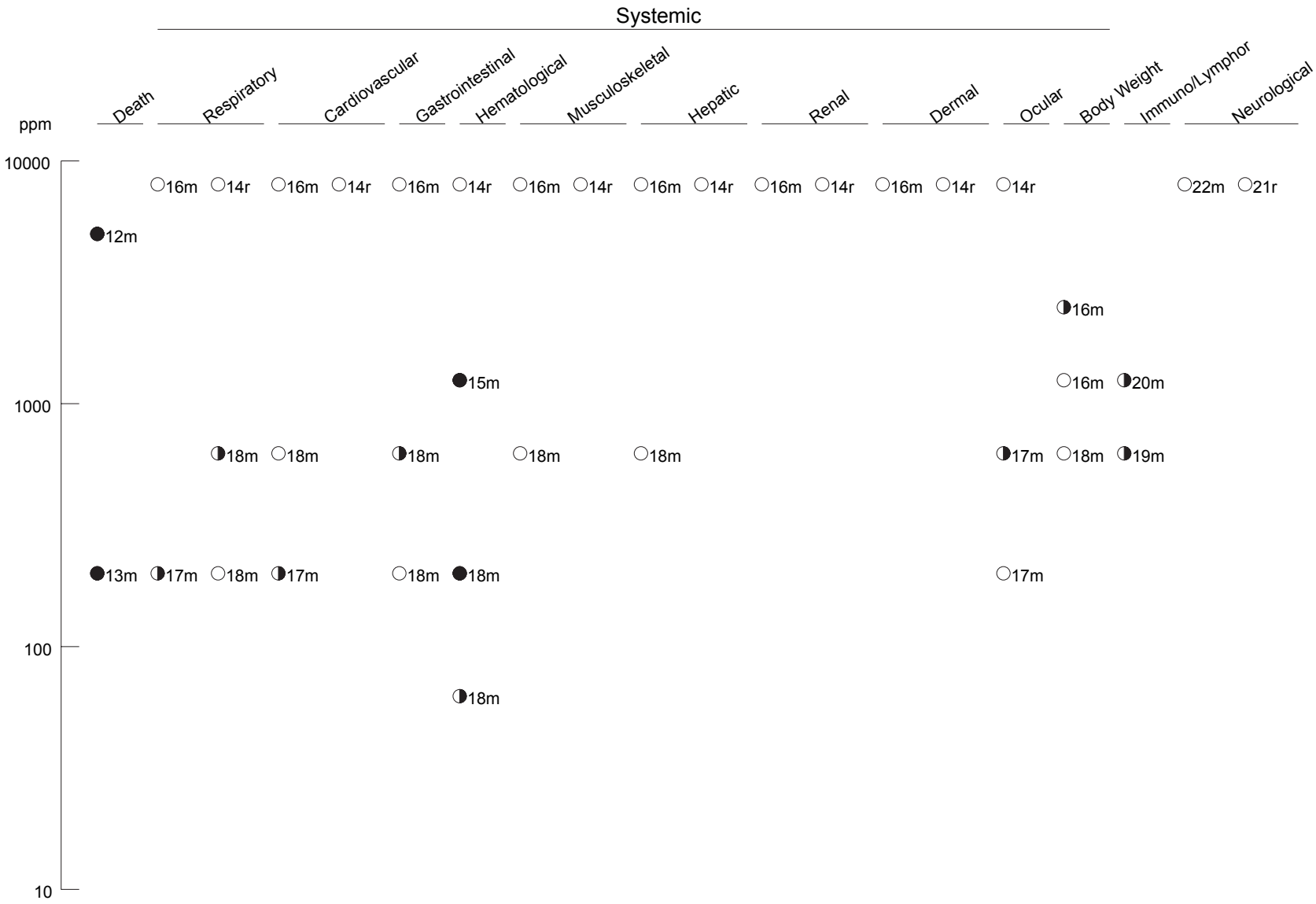
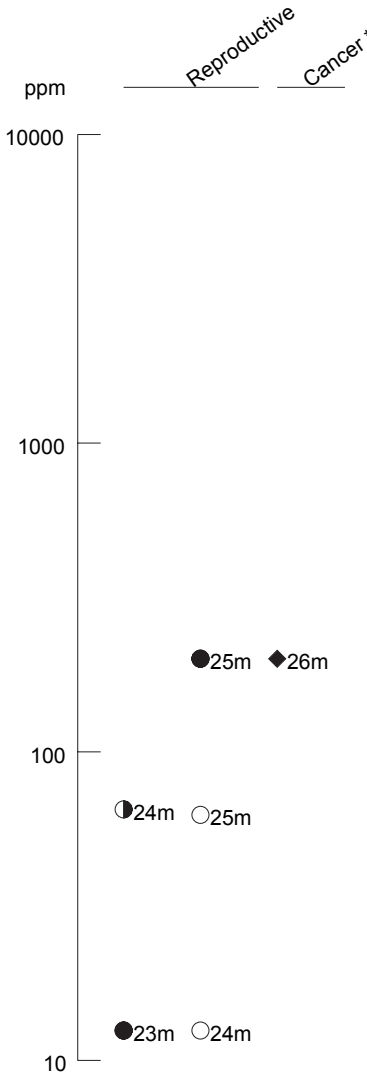


Figure 3-1 Levels of Significant Exposure to 1,3-Butadiene - Inhalation *(Continued)*

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	

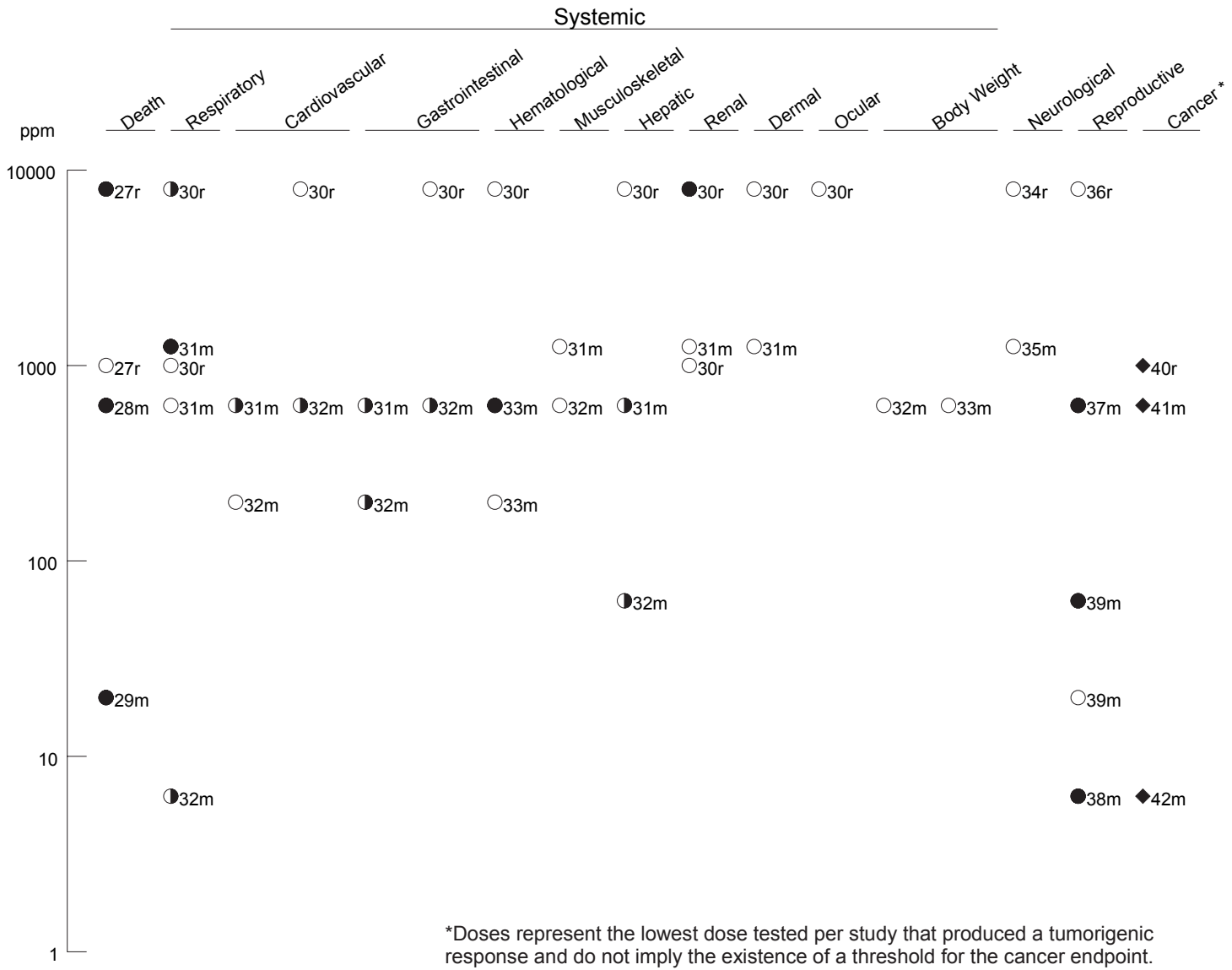
◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

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

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

Figure 3-1 Levels of Significant Exposure to 1,3-Butadiene - 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
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

Cardiovascular Effects. In a retrospective epidemiological study of middle-aged workers in the rubber industry, excessive mortality was noted for certain types of cardiovascular diseases, mainly chronic rheumatic and arteriosclerotic heart diseases (McMichael et al. 1974). Furthermore, increased mortality for arteriosclerotic heart disease was reported among black males in the rubber industry (Matanoski and Schwartz 1987). This result was also reported in an update of the original study (Matanoski et al. 1988, 1990). However, increased mortality from cardiovascular disease was not observed in three other cohorts of SBR and 1,3-butadiene monomer workers (Cowles et al. 1994; Divine and Hartman 1996, 2001; Ward et al. 1995). Thus, it is unclear if cardiovascular disease is likely to be caused by 1,3-butadiene exposure.

No cardiovascular lesions were found in mice or rats exposed to 8,000 ppm 1,3-butadiene 6 hours/day, 5 days/week, for 13–14 weeks (Crouch et al. 1979; NTP 1984). Endothelial hyperplasia in the heart (an early preneoplastic lesion) was observed in mice after exposure to 200 ppm for 6 hours/day, 5 days/week for 40 weeks (NTP 1993) or 625 ppm 6 hours/day, 5 days/week for 61 weeks or 2 years (NTP 1984, 1993). No exposure-related histopathological cardiac lesions were found in rats exposed chronically to up to 8,000 ppm for 2 years (Owen et al. 1987).

Gastrointestinal Effects. No studies were located regarding noncancer gastrointestinal effects in humans after inhalation exposure to 1,3-butadiene.

No significant incidences of gastrointestinal tract lesions were observed in mice following exposure to 8,000 ppm 1,3-butadiene for 6 hours/day, 5 days/week, for 14 weeks (NTP 1984) or 200 ppm for 6 hours/day, 5 days/week for 40 weeks (NTP 1993). In a chronic-duration study, high incidences of forestomach epithelial hyperplasia (a possible preneoplastic lesion) were observed in mice exposed to 625 ppm for 6 hours/day, 5 days/week for 61 weeks (Melnick et al. 1990a; NTP 1984) and for 2 years (NTP 1993). No exposure-related nonneoplastic gastrointestinal lesions were found in rats exposed chronically to up to 8,000 ppm for 6 hours/day, 5 days/week for 2 years (Owen et al. 1987).

Hematological Effects. A hematological survey of workers at a styrene-butadiene rubber plant revealed little indication of bone marrow toxicity among the workers (Checkoway and Williams 1982). Styrene and 1,3-butadiene were the most significant chemicals in the atmosphere; benzene and toluene were present in much lower concentrations. A group of eight tank farm workers (workers who load and unload chemicals from storage tanks; mean level exposure of 20 ppm) demonstrated slightly lower levels

of red blood cells, hemoglobin, platelets, and neutrophils compared with other workers, but these findings were within the normal range. Tsai et al. (2005) examined a number of hematological end points in a petrochemical production facility in which the current time-weighted average (TWA) 1,3-butadiene exposure level was 0.25 ppm; prior to 1997, the TWA concentration was 4.55 ppm. As compared to unexposed controls, no significant alterations in total or differential leukocyte levels, erythrocyte levels, hemoglobin levels, mean corpuscular volume, or platelet levels were found. An older study of workers from one of the facilities examined by Tsai et al. (2005) also found no significant alterations in total and differential leukocyte levels, erythrocyte levels, hemoglobin levels, platelet levels, or mean corpuscular volume (Cowles et al. 1994); the mean 1,3-butadiene concentration was 3.5 ppm.

No signs of blood dyscrasias were found among 164 animals (rats, rabbits, guinea pigs, dogs) exposed to concentrations up to 6,700 ppm of 1,3-butadiene for 8 months (Carpenter et al. 1944). The results were supported by a 3-month study in which no effects on hematological indices were found in rats after exposure to 8,000 ppm of 1,3-butadiene (Crouch et al. 1979).

A treatment-related macrocytic megaloblastic anemia was observed in B6C3F1 and NIH mice exposed to 1,250 ppm 1,3-butadiene for 6 hours/day, 6 days/week for 6–24 weeks, but not after a 3-week exposure to the same concentration (Irons et al. 1986a, 1986b). The bone marrow damage was expressed as reduced numbers of red blood cells, decreased hemoglobin concentration and hematocrit, and increased mean corpuscular volume of circulating erythrocytes. The changes were observed in both strains, independently of the occurrence of murine leukemia viruses in the animals. Male mice exposed to ≥62.5 ppm for 6 hours/day, 5 days/week for 40 weeks exhibited decreased red blood cell counts, hemoglobin concentration, and hematocrit (NTP 1993). Leukopenia and lymphopenia occurred at ≥200 ppm. Females exhibited macrocytic megaloblastic anemia and bone marrow atrophy after exposure to 200 and 625 ppm, respectively, 6 hours/day, 5 days/week for 40 weeks (NTP 1993).

Macrocytic megaloblastic anemia and bone marrow hyperplasia was also observed in mice exposed chronically to 625 ppm 1,3-butadiene 6 hours/day, 5 days/week for 65 weeks (NTP 1993). Surviving females, but not males, in this study exhibited increased bone marrow cellularity.

In contrast to the findings in mice, no effects on hematology or blood chemistry of Sprague-Dawley rats were observed after exposure to 1,000 and 8,000 ppm of 1,3-butadiene for 105–111 weeks (Owen et al. 1987).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects of 1,3-butadiene in humans after inhalation exposure.

No musculoskeletal effects were observed in mice and rats exposed to 8,000 ppm 1,3-butadiene 6 hours/day, 5 days/week for 13–14 weeks (Crouch et al. 1979; NTP 1984) or in mice exposed to 625 ppm 6 hours/day, 5 days/week from 40 or 65 weeks or 2 years (NTP 1993).

Hepatic Effects. No studies were located regarding hepatic effects of 1,3-butadiene in humans after inhalation exposure.

No histopathological changes in livers of rats (Crouch et al. 1979) or mice (NTP 1984) were found after intermediate-duration exposure to 1,3-butadiene. The relative liver weights of both sexes of Sprague-Dawley rats were elevated after the chronic exposure to 1,3-butadiene (1,000 and 8,000 ppm); however, this finding was not associated with any pathological changes (Owen et al. 1987). Mice exposed to ≥625 ppm 6 hours/day, 5 days/week for 61 weeks had a significant increase in liver necrosis (NTP 1984).

Renal Effects. No studies were located regarding renal effects in humans after inhalation exposure to 1,3-butadiene.

The results of urinalysis in 164 animals, including rats, guinea pigs, rabbits, and dogs were all normal after an 8-month exposure to concentrations up to 6,700 ppm of 1,3-butadiene (Carpenter et al. 1944), but the methods were poorly described. These results were supported, however, in rats after 13 weeks of exposure to concentrations up to 8,000 ppm of 1,3-butadiene (Crouch et al. 1979). Nephrosis was found among male rats after 111 weeks of exposure to 8,000 ppm, but not 1,000 ppm, of 1,3-butadiene (Owen et al. 1987). No non-neoplastic renal lesions were observed in mice exposed to 8,000 ppm 6 hours/day, 5 days/week for 14 weeks (NTP 1984) or 625 ppm 6 hrs/day, 5 days/week, for 40 weeks to 2 years.

Dermal Effects. No studies were located regarding dermal effects in humans after inhalation exposure to 1,3-butadiene.

No histopathological dermal changes were found in rats or mice after 13–14 weeks exposure to 8,000 ppm 1,3-butadiene (Crouch et al. 1979; NTP 1984), in rats after 111 weeks exposure to 8,000 ppm (Owen et al. 1987), or in mice after 40 weeks to 2 years exposure to 625 ppm (NTP 1993).

Ocular Effects. Two men reported slight irritation of the eyes and difficulty in focusing on instrument scales during 6–7 hours exposure to 2,000 and 4,000 ppm 1,3-butadiene (Carpenter et al. 1944).

Ophthalmologic examination of the eyes of dogs and rabbits disclosed no signs of injury during the course of exposure to up to 6,700 ppm 1,3-butadiene for 8 months (Carpenter et al. 1944). After the termination of the experiment, histological examination revealed that the sclera, cornea, and ciliary body were normal. Sections of the optic nerve with adjacent retina showed no myelin sheath degeneration. Although the ophthalmological examination was described in detail, the study was limited by the small number of animals used.

No histopathological ocular changes were found in rats or mice after 13–14 weeks exposure to 8,000 ppm (Crouch et al. 1979; NTP 1984) or in rats after 111 weeks exposure to 8,000 ppm 1,3-butadiene (Owen et al. 1987). Increased Harderian gland hyperplasia (a precancerous lesion) was observed in male mice exposed to ≥62.5 ppm 6 hours/day, 5 days/week for 65 weeks and 2 years (NTP 1993); this effect is not relevant to humans because they do not have Harderian glands.

Body Weight Effects. Body weights were reduced by 13% in male mice exposed to 2,500 ppm 1,3-butadiene 6 hours/day, 5 days/week for 14 weeks, but were not significantly different from controls when exposed to 1,250 ppm 6 hours/day, 5 days/week for 61 weeks or when exposed to 625 ppm 6 hours/day, 5 days/week for 2 years (NTP 1993)

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects of 1,3-butadiene in humans after inhalation exposure.

After 3–21 weeks of exposure to 1,250 ppm 1,3-butadiene, an increased expression of murine leukemia virus (MuLV) was observed in hematopoietic tissues of B6C3F1 mice, but not in NIH mice (Irons et al. 1987a). Furthermore, altered regulation of the stem cell development in B6C3F1 mice was reported after a similar exposure (Leiderman et al. 1986).

Intermediate-duration exposure of female mice to 62.5 ppm 1,3-butadiene 6 hours/day, 5 days/week for 40 weeks resulted in a 17% reduction in relative spleen weight, while exposure to 625 ppm for the same

duration resulted in a 19% reduction in thymus weight (NTP 1993). By 65 weeks, relative spleen weights in 625 ppm females had increased to 57% higher than controls.

Immunological changes were detected after evaluation of specific humoral and cell-mediated immunity in B6C3F1 mice exposed to 1,250 ppm 1,3-butadiene for 6, 12, or 24 weeks (Thurmond et al. 1986). Suppression of cytotoxic T-lymphocyte generation to mastocytoma cells was observed after 6 weeks, but recovered after 12 weeks of exposure. The histological examination of lymphoid organs showed depressed spleen cellularity after 24 weeks of exposure; this value is recorded as a LOAEL for immunological effects in Table 3-1 and plotted in Figure 3-1, although it is not known how these changes affect immunocompetency.

3.2.1.4 Neurological Effects

Psychomotor responses of two men inhaling 2,000, 4,000, or 8,000 ppm 1,3-butadiene for 6–8 hours/day on different days were evaluated by Carpenter et al. (1944). At the two higher concentrations, the subjects performed a steadiness test; at the highest concentration, a tapping rate test was also performed. Results after 1,3-butadiene exposure were identical to those obtained before exposure.

Rabbits exposed to 250,000 ppm of 1,3-butadiene went through all stages of anesthesia to death in the average time of 23 minutes (Carpenter et al. 1944). Less than 2 minutes of exposure was required for loss of motor and labyrinth reflexes.

No effects on erythrocyte or brain cholinesterase or on neuromuscular function tests were found in rats exposed to up to 8,000 ppm for 13 weeks (Crouch et al. 1979). In intermediate and chronic exposure studies in mice and rats, no treatment-related histopathological lesions were found in organs and tissues of the nervous system (brain, spinal cord, sciatic nerves) (Crouch et al. 1979; NTP 1984; Owen and Glaister 1990; Owen et al. 1987). Tests results for neurological function (i.e., loss of balance on a rotating cone) were possibly confounded by the mammary tumors interfering with the mobility of rats (NTP, 1984). The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to 1,3-butadiene. A concentration-related increase in the incidence of sperm-head abnormalities occurred in

B6C3F1 mice after exposure to 1,000 (73% increase) and 5,000 ppm (129% increase) of 1,3-butadiene 6 hours/day for 5 days (DOE/NTP 1988a). No impairment of fertility was noted when groups of male and female rats, rabbits, or guinea pigs were exposed to ≤6,700 ppm 1,3-butadiene (Carpenter et al. 1944).

No treatment-related histopathological effects were seen in reproductive organs of rats or mice exposed to 8,000 ppm 1,3-butadiene 6 hours/day, 5 days/week for 13–14 weeks (Crouch et al. 1979; NTP 1984). Reduction in the number of round and elongated sperm heads was seen in mice exposed to 130 ppm 6 hours/day, 5 days/week for 4 weeks, but this was not associated with changes in fertility (Anderson et al. 1998). Ovarian and uterine atrophy occurred in female mice exposed to 200 ppm 6 hours/day, 5 days/week for 40 weeks (NTP 1993), while testicular atrophy was seen in male mice exposed to 625 ppm for the same duration. Exposure of female mice to 62.5 or ≥6.25 ppm 1,3-butadiene 6 hours/day, 5 days/week for 65 weeks or 2 years, respectively, resulted in an increased incidence of ovarian atrophy (NTP 1993). Affected females had no evidence of oocytes, follicles, or corpora lutea. An increase in testicular atrophy and preputial gland hyperplasia was observed in males only after exposure to 625 ppm 6 hours/day, 5 days/week for 2 years (NTP 1993). In contrast, no histological alterations were observed in the gonads of rats exposed to up to 8,000 ppm 6 hours/day, 5 days/week for 2 years (Owen et al. 1987).

In untreated female mice mated with males exposed to ≥200 ppm 6 hours/day for 5 days, a significant increase in the number of females with two or more intrauterine deaths were observed (DOE/NTP 1988b). This effect was only observed in animals mated during the first week post-exposure. Additional effects included increases in early implantation loss at 1,000 ppm during the first post-week of exposure and at 200 and 1,000 ppm during the second week post-exposure; implantation losses were not significantly increased in the mice exposed to 5,000 ppm. Early fetal death was also observed in untreated female mice mated to males exposed to 65 ppm for 6 hours/day, 5 days/week for 4 weeks (DOE/NTP 1988a). Fetal toxicity was observed following the mating of untreated female mice with males exposed to 12.5 ppm 1,3-butadiene 6 hours/day, 5 days/week for 10 weeks (Anderson et al. 1996). Observed effects included an increase in late fetal death, exencephaly, and skull abnormalities.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation exposure to 1,3-butadiene.

When exposed to concentrations up to 8,000 ppm 1,3-butadiene for 6 hours/day, 5 days/week during GDs 6–15, Sprague-Dawley rats showed signs of dose-related maternal and fetal toxicity (Irvine 1981). Depressed body weight gain among dams was observed at ≥200 ppm, and fetal growth (body weight and crown-rump length) was significantly decreased in the 8,000 ppm group. A significant increase in the number of litters with fetuses showing minor skeletal defects was observed at 200 ppm, but not at 1,000 or 8,000 ppm; however, an increase in the number of fetuses with irregular ossification was observed at 8,000 ppm. Significant increases in the number of litters with fetuses showing major skeletal defects were observed at 1,000 and 8,000 ppm. The majority of the major skeletal defects were wavy ribs; abnormalities of the skull, spine, sternum, long bones, and ribs were also observed at 8,000 ppm. A significant increase in the number of litters with fetuses showing minor external/visceral defects was observed at 1,000 ppm, but not at 8,000 ppm. In a study in which female Sprague-Dawley rats were exposed to 40–1,000 ppm during GDs 6–15 (DOE/NTP 1987a), some skeletal abnormalities and ossification reductions were found in the fetuses, but were not statistically significant and were not considered to be treatment-related. In mice, a 5–23% decrease in fetal body weight gain, primarily among male mice, was observed after exposure of dams during GDs 6–15 to 40–1,000 ppm 1,3-butadiene. The magnitude of the decreased fetal body weight in males was 5, 18, and 23% in the 40, 200, and 1,000 ppm groups, respectively. The investigators reported that the decreased fetal body weight was statistically significant in males at all dose levels; however, the statistical method used (ANOVA) did not account for differences in litter size. Increased incidences of extra ribs and reduced ossification of sternebrae were found in fetuses from groups exposed to 200 ppm and 1,000 ppm, respectively (DOE/NTP 1987b).

The highest NOAEL value and all reliable LOAEL values for developmental effects in rats for the acute duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.7 Cancer

Retrospective epidemiological studies of mortality among workers in the rubber industry were conducted in SBR (polymer) production workers and 1,3-butadiene monomer workers. For SBR workers, the primary cohort is comprised of largely overlapping cohorts from multiple SBR facilities in the United

States and Canada examined by investigators at John Hopkins (Matanoski and Schwartz 1987; Matanoski et al. 1989b, 1990) and the University of Alabama at Birmingham (Cheng et al. 2007; Delzell et al. 1996; Graff et al. 2005; Macaluso et al. 1996; Sathiakumar et al. 2005, 2007). Mortality among three independent cohorts of male 1,3-butadiene monomer production workers has been studied and updated on several occasions, including cohorts from Union Carbide (Ward et al. 1995), Texaco (Divine 1990; Divine et al. 1993), and Shell (Cowles et al. 1994).

Matanoski et al. 1990 (in an update of the Matanoski and Schwartz 1987 cohort) found increased standardized mortality ratios (SMRs) of 532 (1 case), 656 (95% confidence interval [CI]=135–1,906), and 482 (95% CI=59–1,762) for lymphosarcoma, leukemia, and other lymphatic neoplasms, respectively, in black SBR production workers; white workers exhibited an SMR of 230 (95% CI=92–473) for other lymphatic neoplasms. In white maintenance workers, SMRs of 144 (95% CI=53–314) and 166 (95% CI=93–275) were observed for esophageal and stomach cancer, respectively. An odds ratio of 9.4 for leukemia was observed in SBR workers in a nested case-control study of the Matanoski and Schwartz (1987) cohort of SBR workers (Matanoski et al. 1989b). No such association was found for exposure to styrene. Several investigators at the University of Alabama at Birmingham examined cancer mortality in a cohort of over 15,000 North American SBR workers (Cheng et al. 2007; Delzell et al. 1996; Graff et al. 2005; HEI 2006; Macaluso et al. 1996; Sathiakumar et al. 2005, 2007). Delzell et al. (1996) found increased SMRs for leukemia of 265 (95% CI=141–453) for maintenance and 431 (95% CI=207–793) for laboratory workers. Macaluso et al. (1996) derived a relative rate value of 4.5 for leukemia development (no confidence interval reported) associated with a cumulative exposure of 80 ppm-years. In an update of these studies (Sathiakumar et al. 2005), an increase in deaths from all types of leukemia (SMR of 258; 95% CI=156–403) was found among hourly employees with 20–29 years since hire and >10 years of employment. Increases in leukemia deaths were also found among workers in the polymerization (SMR of 204; 95% CI=121–322), maintenance labor (SMR of 326; 95% CI=178–456), and laboratory (SMR of 326; 95% CI=178–546) operations. However, no increases in a specific type of leukemia were found. Similarly, Graff et al. (2005) reported increased relative risks for leukemia of 2.9 (95% CI=1.4–6.4) and 3.7 (95% CI=1.7–8.0) among workers with cumulative 1,3-butadiene exposures of 184.7–<435.0 and 425.0 ppm-years. When the relative risks were adjusted for exposure to styrene and dimethyldithio-carbamate, age, and years since hire, only the highest cumulative exposure group had a confidence interval that included 1 (relative risk of 3.0, 95% CI=1.0–9.2). When workers were divided into two categories based on exposure to >100 and ≤100 ppm, associations between 1,3-butadiene cumulative exposure and leukemia were found for both groups, although the association was weaker at the lower concentration (relative risk of 2.0; 95% CI=0.6–6.0 for cumulative exposure to >124.7 ppm-years) than at

the higher concentration (relative risk of 3.7; 95% CI=1.3–11.1 for cumulative exposure of >247.6 ppm-years) and the trend was only statistically significant in the >100 ppm workers (HEI 2006). A more recent paper by Graff and associates (Graff et al. 2009) used uncertainty analysis to evaluate the impact of potential exposure estimate inaccuracies on the leukemia relative risks. The investigators concluded that analysis of the complete probability distribution of 1,3-butadiene exposure estimate supported the association between 1,3-butadiene cumulative exposure and increased leukemia risk. Using personal monitoring device data collected from 1977 to 1991, Sathiakumar et al. (2007) compared measured 1,3-butadiene levels with estimated levels. The mean measured 1,3-butadiene level (across all years and job categories) was 5.2 ppm and the mean estimated concentration was 4.7 ppm; the estimated 1,3-butadiene levels tended to underestimate concentrations that were greater than 7 ppm. Cheng et al. (2007) found a significant trend for the association of leukemia in SBR workers and increasing cumulative exposure or number of "peak" exposures (>100 ppm). They also reported a minimal association (relative rate of 1.03) of leukemia in SBR workers receiving an estimated 5 ppm 1,3-butadiene exposure for 20 years (100 ppm-years). Examination of possible associations between 1,3-butadiene exposure and increased deaths from other cancer types, including non-Hodgkin's lymphoma, Hodgkin's lymphoma, and multiple myeloma were not found in this cohort (HEI 2006). Increases in the risk of colorectal and prostate cancer were observed in some subgroups of SBR workers; however, no consistent exposure-response trends were found (HEI 2006).

These results in SBR workers with 1,3-butadiene exposure are supported by studies in 1,3-butadiene monomer production workers. Downs et al. (1987) calculated an SMR of 235 (no CI reported) for lymphosarcoma and reticular cell sarcoma. No increase in mortality from cancer of gastrointestinal, respiratory, urinary, and skeletal systems was associated with monomer exposure. Divine (1990) and Divine et al. (1993) reported similar results in a follow-up study of this cohort (SMR of 452 [95% CI=165–984]) for lymphosarcoma. Divine and Hartman (2001) followed this cohort for an additional 5 years and found a statistically significant increase in SMR (141; 95% CI=105–186) for all lymphohematopoietic cancer. Subcohort analyses showed that increases in lymphohematopoietic cancers appear to be restricted to monomer workers employed before 1950 and for the shortest duration (Divine and Hartman 1996, 2001). Elevations were also seen for leukemia (SMR of 129; 95% CI=77–204) and non-Hodgkin's lymphoma (SMR of 148; 95% CI=89–231), but the elevation was not statistically significant. Additionally, higher SMRs for leukemia and non-Hodgkin's lymphoma were found in workers with shorter employment durations. No significant associations were found between cumulative 1,3-butadiene exposure (defined as a combination of job exposure class, calendar time, and length of time in job) and the relative risk for lymphohematopoietic cancers, leukemia, or non-Hodgkin's lymphoma

(Divine and Hartman 2001). Analysis of another cohort of 364 monomer workers, 277 of which worked in a U.S. Rubber Reserve plant during World War II, found an SMR of 577 (95% CI=157–1,480) for lymphosarcoma and reticulosarcoma (Ward et al. 1995). No significant association was found between 1,3-butadiene monomer exposure and all, lung, or lympho-hematopoietic cancers in a cohort of 614 monomer workers employed between 1948 and 1989 who were exposed to a relatively low mean concentration of 3.5 ppm, with many measurements below 1 ppm (Cowles et al. 1994).

The major limitations of the epidemiological studies described so far include the lack of precise historic exposure data to 1,3-butadiene, lack of adjustment for smoking, and possible exposure to other chemicals. Irons and Pyatt (1998) suggest that dithiocarbamates, such as dimethyldithiocarbamate (DMDTC), used in the SBR vulcanization process from 1950 to 1965, may have played a significant role in leukemia development that was concentrated in workers employed during this period. However, Santos-Burgoa et al. (1992) and Delzell et al. (2001) and HEI (2006) used multivariate analysis to suggest that the estimates of 1,3-butadiene exposure provided the best correlation with the rates of leukemia, even in the presence of styrene and DMDTC.

Several investigators have evaluated associations between childhood leukemias and ambient 1,3-butadiene emissions. Knox et al. (2005, 2006) associated the occurrence of childhood cancer with proximity of birthplace to industrial 1,3-butadiene and benzene emissions, and roads, railways, waterways, and bus, ferry, or train stations in Great Britain; however, no actual exposure data or estimates were available. Reynolds et al. (2003) calculated leukemia rate ratios (RR), adjusted for age, ethnicity, and sex, of 1.21 (95% CI=1.03–1.42) and 1.32 (95% CI=1.11–1.57) for children in California census tracts ranked highest for combined exposure to 25 hazardous air pollutants (HAPs, including 1,3-butadiene) and highest for point-source HAP exposures, respectively. Likewise, Whitworth et al. (2008) reported a significant association of childhood leukemia incidence with residence in census tracts close to the ship channel (which is in close proximity to petrochemical and chemical manufacturing facilities) in Houston, Texas. These studies do not indicate strong causality between 1,3-butadiene exposure and childhood leukemia, as many chemicals may have contributed to exposure and the actual exposure to 1,3-butadiene, if any, is unknown.

The rodent bioassay database corroborates the association of lympho-hematopoietic neoplasms and occupational exposure to 1,3-butadiene reported in the epidemiology literature. Mice were clearly more sensitive to 1,3-butadiene-mediated tumor development than rats. In rats, increased tumors were not observed following intermediate-duration exposures of up to 8,000 ppm 1,3-butadiene 6 hours/day,

5 days/week for 13 weeks (Crouch et al. 1979). Two-year exposure of rats to 1,000 or 8,000 ppm 6 hours/day, 5 days/week resulted in increased incidences of Leydig cell adenoma, pancreatic exocrine adenoma, uterine sarcoma, mammary gland adenoma and carcinoma, Zymbal gland carcinoma, and thyroid follicular cell tumors (Owen and Glaister 1990; Owen et al. 1987). In mice, exposure to 200 ppm for 40 weeks resulted in increased incidences of lymphocytic lymphoma, histiocytic sarcoma, cardiac hemagiosarcoma, alveolar/bronchiolar adenoma/carcinoma, forestomach squamous cell papilloma/ carcinoma, hepatocellular adenoma, hardarian gland adenoma/adenocarcinoma, and preputial gland carcinoma (NTP 1993). These same tumors developed in mice in as little as 13 weeks after exposure to 625 ppm 6 hours/day, 5 days/week (NTP 1993). Chronic exposure of mice to lower concentrations of 1,3-butadiene also resulted in multi-target organ neoplasm development. Two-year 6-hour/day, 5-day/week exposures of 20 ppm for male mice and 6.25 ppm (the lowest exposure level tested) for female mice resulted in increased incidences of lymphocytic lymphoma, histiocytic sarcoma, cardiac hemagiosarcoma, alveolar/bronchiolar adenoma/carcinoma, forestomach squamous cell papilloma/ carcinoma, hepatocellular adenoma, hardarian gland adenoma/adenocarcinoma, mammary gland carcinoma, adenocanthoma, malignant mixed tumor, and malignant ovarian granulosa cell tumor.

The cancer effect levels (CELs) are recorded in Table 3-1 and plotted in Figure 3-1.

Using the Poisson regression analysis by Health Canada (2000) of the leukemia risk data from a cohort of 15,000 SBR production workers (Delzell et al. 1996), EPA derived a unit risk for inhalation exposure of 0.08 ppm^{-1} (IRIS 2012). This unit risk corresponds to upper bound individual lifetime cancer risks at 10^{-4}–10^{-6} for exposure levels of $1x10^{-3}$–$1x10^{-6}$ ppm, which are plotted in Figure 3-1. It should be noted that EPA derived the cancer risk levels in 2001, and thus, these values may not reflect the results of more recent studies of SBR workers.

The International Agency for Research on Cancer (IARC) has classified 1,3-butadiene as a Group 1 carcinogen (carcinogenic to humans) (IARC 2009). EPA has classified 1,3-butadiene as carcinogenic to humans (EPA 2002; IRIS 2012). The Department of Health and Human Services (NTP 2005) also identified 1,3-butadiene as a “known human carcinogen”.

3.2.2 Oral Exposure

No studies were located regarding health effects in humans or animals after oral exposure to 1,3-butadiene.

3.2.3 Dermal Exposure

Dermal contact with liquid 1,3-butadiene causes a sensation of cold followed by a sensation of burning, which is the result of rapid expansion of pressurized 1,3-butadiene from liquid to gas states (NIOSH, 2005). Although this may cause frostbite, it is specific to an unusual exposure scenario and is not a toxic endpoint. However, the possible toxic effects from dermal absorption of such a concentrated amount of 1,3-butadine are unknown. High gas concentrations may cause mild skin irritation as well (NIOSH, 2005). No other studies were located regarding health effects in humans or animals following dermal exposure to 1,3-butadiene.

3.3 GENOTOXICITY

1,3-Butadiene has been tested for genotoxicity in a number of *in vitro* and *in vivo* studies (Tables 3-2 and 3-3). Positive results have been found in the reverse mutation assay in *Salmonella typhimurium* TA1530 and TA1535 in the absence or in the presence of metabolic activation system (de Meester et al. 1978; Madhusree et al. 2002). However, the interpretation of these results was confounded by the fact that the Petri dishes not containing S-9 mix were contaminated by volatile active metabolites. It was concluded that S-9 mix was necessary to activate 1,3-butadiene into mutagen(s) (De Meester 1988). TA1530 was the most sensitive strain, but 1,3-butadiene mutagenicity was detectable only with metabolic activation in the subsequent study (de Meester et al. 1980). No significant mutagenic effect on *S. typhimurium* strain TA100 with metabolic activation was observed (Victorin and Stahlberg 1988). A weak genotoxic activity was detected in strain TA1535 with rat S-9 (Arce et al. 1989). A weak increase in sister chromatid exchanges was observed in Chinese hamster ovary cells, but only with metabolic activation (Sasiadek et al. 1991). Increased mutations occurred in the hypoxanthione-guanine phosphoribosyl transferase (*hprt)* and *tk* gene loci of human TK6 lymphoblastoid cells (Cochrane and Skopek 1993). On the basis of these data, 1,3-butadiene appears to require metabolic activation to produce genotoxicity.

The genotoxicity of 1,3-butadiene has been examined in several occupational exposure cohorts. Ward et al. (1994) reported a significant increase in hypoxanthine-guanine phosphoribosyltransferase (*hprt*) mutant frequency (measured in peripheral lymphocytes) among eight workers at a Texas 1,3-butadiene production plant working in the area of the plant where the highest 1,3-butadiene exposure occurred, as compared to levels in five workers in an area with low 1,3-butadiene exposures or in six controls who did not work at the 1,3-butadiene production plant. The mean area and personal 1,3-butadiene levels were 3.5 ppm (although most individual samples were <1 ppm) in the high-exposure area and 0.03 ppm in the

Table 3-2. Genotoxicity of 1,3-Butadiene *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium				
TA1530	Gene mutation	+	–	de Meester et al. 1980
TA100	Gene mutation	–	–	Victorin and Stahlberg 1988
TA1535	Gene mutation	+	–	Arce et al. 1989; Madhusree et al. 2002
Eukaryotic organisms:				
Chinese hamster ovary	SCE	+	–	Sasiadek et al. 1991
Human TK6 lymphoblastoid cells	*hprt* and *tk* loci mutations	NA	NA	Cochrane and Skopek 1993

– = negative result; + = positive result; NA = not applicable; SCE = sister chromatid exchange

Table 3-3. Genotoxicity of 1,3 Butadiene *In Vivo*

Species (test system)	End point	Results	Reference
B6C3F1 mice (inhalation)	Bone marrow: Dose-dependent increase in SCEs	+	Cunningham et al. 1986
Sprague-Dawley rats (inhalation)		-	
B6C3F1 mice (inhalation)	Bone marrow: increase in CAs, SCEs, and AGT, and depression of MI	+	Tice et al. 1987
Swiss mice (inhalation)	Peripheral blood erythrocytes: induction of micronuclei	+	Irons et al. 1986b
B6C3F1 mice (inhalation)	Bone marrow: alteration of hematopoietic stem cell development	+	Leiderman et al. 1986
B6C3F1 mice (inhalation)	Peripheral blood erythrocytes: induction of micronuclei	+	Jauhar et al. 1988
B6C3F1 mice (inhalation)	Induction of MN; induction of SCEs; CAs	+	Tice et al. 1988
B6C3F1 mice (inhalation)	Sperm abnormalities; dominant lethality	+	DOE/NTP 1988a
C57B1/6 mice (intraperitoneal injection)	Bone marrow increase in CAs and SCEs	+	Sharief et al. 1986
B6C3F1 mice (inhalation)	*lacZ*$^-$ mutant frequency in lung	+	Recio et al. 1992
B6C3F1 mice (inhalation)	*lacZ*$^-$ mutant frequency in liver and bone marrow	-	Recio et al. 1992
B6C3F1 mice and F344 rats (inhalation)	*hprt* loci mutations in splenic T lymphocytes	+	Cochrane and Skopek 1993; Meng et al. 1999, 2000, 2004, 2007
B6C3F1 mice (inhalation)	Peripheral blood erythrocytes and bone marrow: induction of micronuclei	+	Autio et al. 1994
Wistar rats	Peripheral blood erythrocytes and bone marrow: induction of micronuclei	-	Autio et al. 1994
C3H mice (inhalation)	Heritable spermatid chromosomal translocations; dominant lethality	+	Adler et al. 1998
C3H mice (inhalation)	Spermatocytes: induction of micronuclei	+	Xiao and Tates 1995
CD-1 mice (inhalation)	Dominant lethality	-	Brinkworth et al. 1998
CAST/EiJ, NOD/LTj, A/J, WSB/EiJ, PWK/PhJ, 129S/SvlmJ, C57BL/6J mice (inhalation)	DNA adduct formation	+	Koturbash et al. 2011a
C57BL/6J mice (inhalation)	DNA adduct formation	+	Koturbash et al. 2011b
Humans (inhalation)	CA; SCE	+	Sram et al. 1998
Humans (inhalation)	CA; SCE	-	Lovreglio et al. 2006
Humans (inhalation)	*hprt* loci in peripheral lymphocytes	-	Hayes et al. 1996, 2000
Humans (inhalation)	*hprt* loci in peripheral lymphocytes	-	Tates et al. 1996
Humans (inhalation)	*hprt* loci in peripheral lymphocytes	-	Albertini et al. 2001, 2007; HEI 2003

Table 3-3. Genotoxicity of 1,3 Butadiene *In Vivo*

Species (test system)	End point	Results	Reference
Humans (inhalation)	*hprt* loci in peripheral lymphocytes	-	Liu et al. 2008
	hprt exon deletion	+	Liu et al. 2008
Humans (inhalation)	*hprt* loci in peripheral lymphocytes	+	Ward et al. 1994
Humans (inhalation)	*hprt* loci in peripheral lymphocytes	+	Abdel-Rahman et al. 2001, 2003, 2005; Ma et al. 2000; Ward et al. 1996, 2001
Humans (inhalation)	*hprt* loci in peripheral lymphocytes	+	Wickliffe et al. 2009
C3H mice (inhalation)	Induction of spermatid micronuclei	+	Tommasi et al. 1998
B6C3F1 mice (inhalation)	H- and K-*ras* mutation frequency	+	Sills et al. 2001
NMRI mice (inhalation)	Bone marrow: induction of micronuclei	+	Vodicka et al. 2006

– = negative result; + = positive result; AGT = average generation time; CA = chromosomal aberration; MI = mitotic index; MN = micronucleated cell; SCEs = sister chromatid exchange

low-exposure area. A significant correlation between *hprt* variant frequency and urinary levels of the 1,3-butadiene-specific metabolite, M1, was also found. No significant correlations between *hprt* variant frequency and age or employment length were found. A subsequent study of these workers reported 2.5-fold higher *hprt* mutation frequencies in workers exposed to airborne concentrations of 0.3 ppm 1,3-butadiene as compared to workers exposed to mean levels of 0.12 ppm (Ward et al. 1996), indicating good correlation between exposure and mutation frequency. Unlike the earlier study, no correlation between *hprt* variant frequency and urinary metabolite levels were found. A follow-up study of the southeast Texas SBR workers found a 3-fold increase in *hprt* mutation frequency in workers exposed to 1.7 ppm, compared to workers exposed to 0.07 ppm (Ward et al. 2001). A re-analysis of the blood samples from the Ward et al. (1996) study using a cloning assay, rather than the autoradiographic assay used in the Ward analyses, confirmed the significant difference in *hprt* variant frequency between high-exposure workers and the outside controls and found a significant difference in *hprt* mutation frequency between the groups (Ma et al. 2000). However, the investigators did not examine the possible association between 1,3-butadiene exposure level and mutation frequency. Neither Ma et al. (2000) nor Ward et al. (1994, 1996) provided demographic information on the outside control group, which consisted of workers in the Department of Preventive Medicine and Community Health at the University of Texas Medical Branch; thus, the appropriateness of this comparison group to the SBR workers cannot be evaluated. A more recent study of these SBR workers did not find a significant association between 1,3-butadiene exposure level and *hprt* variant frequency, after removal of an outlier (Wickliffe et al. 2009). The current 1,3-butadiene exposure levels of the 30 subjects examined were low with only six subjects having levels of >0.1 ppm. A significant association between employment length and *hprt* mutant frequency was found. Another study conducted by this group (Ammenheuser et al. 2001) at a different SBR facility found a 3-fold increase in *hprt* mutation frequency in 22 workers in the high-exposure group (mean 1,3-butadiene exposure level was 1.48 ppm) compared to low-exposure workers (mean exposure level of 0.15 ppm). Significant correlations between *hprt* variant frequency and 1,3-butadiene levels and urinary M1 levels were found. Abdel-Rahman et al. (2001, 2003, 2005) examined the association between polymorphisms, 1,3-butadiene exposure, and *hprt* variant frequency among workers at the two Texas SBR facilities. Individuals with a polymorphism for microsomal epoxide hydrolase (EH), an enzyme important for the hydrolysis of epoxide metabolites of 1,3-butadiene (see Section 3.4.3), exhibited a 3-fold higher *hprt* mutation frequency than workers without the polymorphism (Abdel-Rahman et al. 2001). Further, polymorphisms in the glutathione-S-transferase (another important enzyme in epoxide metabolite metabolism) genotypes GSTM1 or GSTT1 did not impact *hprt* mutation rates, but a combination of EH and GST polymorphism did result in an increase in *hprt* mutation frequency (Abdel-Rahman et al. 2001, 2003, 2005). Several studies that examined the possible association between

1,3-butadiene exposure and the frequency of chromosomal aberrations and/or sister chromatid exchanges among Texas 1,3-butadiene workers have not found significant associations (Au et al. 1995; Hallberg et al. 1997; Kelsey et al. 1995).

Unlike the results of Texas cohorts, studies of Chinese rubber production workers have not found alterations in *hprt* mutation frequency. No significant difference in *hprt* gene mutation frequency was observed in male and female Chinese polybutadiene rubber production workers exposed to an average of 1.0–3.5 ppm (median of 2.0 ppm) and unexposed controls (Hayes et al. 1996, 2000, 2001). Additionally, no exposure-related significant associations between *hprt* mutation frequency and 1,3-butadiene exposure (as assessed by exposure levels, urinary metabolite levels, or hemoglobin adducts) were found. Similarly, no significant alterations in glycophorin A variant frequencies were observed (Hayes et al. 2000). In another Chinese study, *hprt* mutation frequencies in petrochemical workers exposed to mean levels of 10 ppm were higher, but were not significantly different than unexposed controls, while the percentage of workers exhibiting *hprt* exon deletions (27%) was significantly higher than levels found in controls (13%) (Liu et al. 2008).

Several investigators have examined *hprt* mutation frequency in cohorts of 1,3-butadiene workers in the Czech Republic. Tates et al. (1996) did not find significant alterations in *hprt* mutation frequency among male workers from a 1,3-butadiene production plant exposed to a mean concentration of 1.76 ppm, as compared to unexposed workers at the plant. However, a significant increase in the percentage of lymphocytes with chromosomal aberrations was observed in the exposed workers; increases in DNA damage (as assessed using the comet assay) and micronuclei frequency were observed in exposed smokers, as compared to unexposed smokers (Šrám et al. 1998; Tates et al. 1996). A significant increase in the frequency of sister chromatid exchanges was also observed (Šrám et al. 1998). When the exposed workers and unexposed workers were subdivided based on glutathione-*S*-transferase polymorphism for M1 gene (GSTM1) and glutathione-*S*-transferase polymorphism for T1 gene (GSTT1) genotypes, a significant increase in the frequency of chromosomal aberrations were observed in exposed workers with the GSTM1-positive genotype, as compared to exposed workers with GSTM1-null genotype; no effects was observed for the GSTT1 genotype. Multifactorial analysis (accounting for 1,3-butadiene exposure, smoking, GSTM1, GSTT1, and age) showed a significant association between the frequency of chromosomal aberrations and the number of cells with a high frequency of sister chromatid exchanges (Šrám et al. 1998). However, when 1,3-butadiene exposure was evaluated using N-1-(2,3,4-trihydroxy-butyl)adenine adduct levels, there were no significant associations between chromosomal aberration frequency, micronuclei formation, or sister chromatid exchange (Zhao et al. 2001). In another study of

Czech 1,3-butadiene workers, no alterations in chromosome aberrations, sister chromatid exchanges, or micronuclei formation were observed in 1,3-butadiene production workers or workers in 1,3-butadiene polymer production (Sorsa et al. 1994). However, when workers were subdivided based on GSTM1 and GSTT1 genotypes, a significantly higher frequency of chromosomal aberrations were observed in 1,3-butadiene workers lacking the GSTT1 gene (Sorsa et al. 1996). In a larger-scale study of 24 workers at a 1,3-butadiene production facility (mean exposure level of 0.64 mg/m^3 [0.29 ppm]) and 34 workers at a polymerization facility (mean exposure level of 1.76 mg/m^3 [0.79 ppm]), no significant association between 1,3-butadiene exposure (as assessed using air concentrations, urinary metabolites, or hemoglobin adducts) and *hprt* mutation frequency (assessed using cloning assay) were found; no associations were found when workers divided by a number of genotypes including GSTM1 or GSTT1 (Albertini et al. 2001; HEI 2003). No significant alterations in the frequency of chromosomal aberrations or sister chromatid exchanges were found. In a follow-up study at the polymerization facility, male and female workers were examined. The mean 1,3-butadiene exposure levels were 0.397 mg/m^3 (0.18 ppm) and 0.808 mg/m^3 (0.36 ppm) in the females and males, respectively (Albertini et al. 2007). No significant associations between 1,3-butadiene exposure and *hprt* mutation frequency were found; similarly, there were no significant associations between exposure and sister chromatid exchanges or chromosomal aberrations.

In a cohort of workers at an Italian petrochemical plant exposed to very low levels of 1,3-butadiene (mean concentration of 0.0115 mg/m^3 [0.005 ppm]), no significant relationship between 1,3-butadiene exposure and alterations in the frequency of chromosomal aberrations or sister chromatid exchanges was observed Fustinoni et al. 2004; Lovreglio et al. 2006).

In summary, studies of workers at Texas 1,3-butadiene production facilities or SBR facilities have found significantly higher frequencies of *hprt* variants in the lymphocytes in men working in areas of the facility with high 1,3-butadiene exposure levels (Ammenheuser et al. 2001; Ma et al. 2000; Ward et al. 1996, 2001) However, no significant associations were found in Czech cohorts (Albertini et al. 2001, 2007; HEI 2003; Tates et al. 1996) or Chinese cohorts (Hayes et al. 1996, 2000, 2001). The reasons for dissimilar outcomes in lymphocyte *hprt* gene mutation frequency between the different cohorts are not clear, but may be the result of varying exposure levels, experimental techniques in exposures assessment (active vs. passive sampling) and mutation analysis (autoradiography vs. cloning *hprt* assays). The mean exposure levels in the Texas cohort studies tended to be higher than other cohorts; the mean levels in the Chinese cohort are elevated due to intermittent high exposures rather than a high TWA level. This is supported by a study conducted by Wickliffe et al. (2009) that did not find significant increases in *hprt*

frequency in a Texas cohort with low 1,3-butadiene exposure levels (only six subjects were exposed to levels >0.1 ppm).

A number of rodent inhalation studies report genotoxic effects. Mice and rats exhibited increased *hprt* locus mutations in splenic T cells (Cochrane and Skopek 1993; Meng et al. 1999, 2000, 2004, 2007). Inhaled 1,3-butadiene also induce an increase in micronucleus induction in erythrocytes (Irons et al. 1986b; Jauhar et al. 1988; Tice et al. 1987; Vodicka et al. 2006), spermatocytes (Tommasi et al. 1998; Xiao and Tates 1995), and bone marrow cells (Autio et al. 1994), increased frequency of sister chromatid exchanges (Tice et al. 1987) and chromosomal aberration frequencies (Cunningham et al. 1986; Tice et al. 1987) in mice. Transgenic B6C3F1 mice exhibited an increased *lacZ*$^-$ mutant frequency in the lungs (Recio et al. 1992). Increased percentages of H- and K-*ras* proto-oncogene mutations were found in forestomach neoplasms from mice inhaling 1,3-butadiene for 2 years (83% in exposed mice compared to 24% in spontaneous neoplasms from controls) (Sills et al. 2001). Increases in N-7-(2,3,4-trihydroxybut-1-yl) guanine adduct formation in liver DNA were found in various mouse strains exposed to 1,3-butadiene (Koturbash et al. 2011a, 2011b); the increase DNA adduct formation was concentration-related (Koturbash et al. 2011b). No genotoxic effects (micronucleus induction, chromosomal aberrations, or sister chromatid exchanges) were found in bone marrow of rats or liver of mice exposed by inhalation to 1,3-butadiene (Autio et al. 1994; Cunningham et al. 1986; Recio et al. 1992).

In a dominant lethal study in which male CD-1 mice inhaled 1,3-butadiene for 5 days and were mated to nonexposed females, an increased number of dead implantations per pregnancy occurred at 200 and 1,000 ppm, but not at 5,000 ppm during the first 2 weeks postexposure (DOE/NTP 1988b). These results were considered to be inconclusive because of the lack of a strict dose-response relationship. Increased numbers of dead fetuses were also observed in offspring of CH3 males inhaling 130 ppm (Adler et al. 1998), but not in CD-1 mice exposed to 125 ppm (Brinkworth et al. 1998). Heritable spermatid chromosomal translocations occurred in F_1 offspring of male CH3 mice inhaling 1,3-butadiene (Adler et al. 1995a, 1998).

Although cytogenetic monitoring of 1,3-butadiene rubber workers for chromosomal aberrations revealed no or slight differences between exposed and control groups (Lovreglio et al. 2006; Sram et al. 1998; Zhou et al. 1986), 1,3-butadiene is clearly genotoxic in mice. As discussed in Section 3.4.3, species differences exist in the metabolism of 1,3-butadiene, and data suggest that humans may metabolize this compound at different metabolic rates than do rodents. If the genotoxic and clastogenic response of 1,3-butadiene requires activation to an active metabolite that is formed more slowly or deactivated more

rapidly in humans than in rats and mice, the genotoxicity observed in animals may only be observed after much higher exposures in humans. The data in humans are too limited, however, to rule out the possibility of a genotoxic potential in humans exposed to 1,3-butadiene.

3.4 TOXICOKINETICS

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

In human volunteers inhaling 2 ppm 1,3-butadiene for 20 minutes, the absorbed fraction varied from 18 to 74% (Lin et al. 2001). Neither sex nor age (30±8 years for males, 29±9 years for females) were factors in this variation. Fractional absorption in Asian volunteers was about 20% greater than in Caucasians, African-Americans, or Hispanics. Blood triglyceride levels may influence absorption, as blood:air partition coefficients increased 20–40% in humans having borderline higher triglyceride levels after ingestion of fat in the diet (Lin et al. 2002).

In male Sprague-Dawley rats and male B6C3F1 mice exposed to 20 ppm ^{14}C-radiolabeled 1,3-butadiene for 6 hours in a dynamic system, the total absorbed radioactivity, as estimated by the sum of ^{14}C in urine, feces, carcass, and expired air, was 2.2% in rats and 1.6% in mice (Swain et al. 2003). In close-chamber studies, the uptake of inhaled 1,3-butadiene in mice and rats was linear to 2,000 and 1,000 ppm, respectively, above which metabolism is saturated (Kohn and Melnick 2001). Absorption of 1,3-butadiene was demonstrated by measurements of the metabolite, 1,2-epoxy-3-butene (EB), in the test chamber (due to exhaled air) and measurement of 1,3-butadiene metabolites in blood of male Sprague-Dawley rats exposed 1–10,000 ppm and male B6C3F1 mice exposed to 1–6,000 ppm for 6–8 hours in closed chambers (Filser et al. 2007). In rats, EB concentrations in the test chamber reached a plateau at all exposure concentrations. In mice, chamber concentrations of EB were higher than for rats; EB levels reached a plateau at exposure concentrations up to 1,000 ppm, but no plateau was observed at exposure concentrations of 2,000–6,000 ppm. The study authors suggest that this concentration-dependence is due to “breakdown” of hepatic glutathione-S-transferase mediated 1,3-butadiene conjugation. Filsner et al. (2007) did not report an absorption fraction for either species. Absorption of 1,3-butadiene was also demonstrated by measurement of metabolites (butadiene monoepoxide and butadiene diepoxide) in blood and tissues of male Sprague-Dawley rats and male B6C3F1 mice exposed (nose only) to 62.5 ppm 1,3-butadiene for 2 or 4 hours (Thornton-Manning et al. 1995a). Similar results were observed in male and female rats exposed (nose only) to 62.5 ppm 1,3-butadiene for 6 hours (Thornton-Manning et al.

1995b). The distribution coefficient for 1,3-butadiene between rabbit blood and air was 0.603 *in vitro* and 0.654 *in vivo*, suggesting simple passive diffusion of the gas from the alveoli to the blood (Carpenter et al. 1944). After 9 minutes of exposure of rabbits to 250,000 ppm, the concentration of 1,3-butadiene was 0.26 mg/mL in the femoral artery and 0.18 mg/mL in the femoral vein. Pulmonary absorption, therefore, appears to be rapid. Distribution studies in rats and mice following inhalation exposure to 1,3-butadiene indicate that it is absorbed from the lungs in these species as well (see Section 3.4.2.1). When *Macaca fascicularis* monkeys were exposed to radioactively labeled 1,3-butadiene, the uptake was calculated as 16.40 µmol/hour/10 ppm of inhaled and 3.20 µmol/hour/10 ppm of retained 1,3-butadiene (Dahl et al. 1990).

3.4.1.2 Oral Exposure

No studies were located regarding absorption in humans or animals after oral exposure to 1,3-butadiene.

3.4.1.3 Dermal Exposure

No studies were located regarding absorption in humans or animals after dermal exposure to 1,3-butadiene.

3.4.2 Distribution

In vitro measurements of tissue:blood equilibrium partition coefficients suggest that 1,3-butadiene distributes to a variety of tissues. Partition coefficients in humans were highest in fat (18.4) and were similar in well- and poorly-perfused tissues (0.69 and 0.72, respectively) (Brochot et al. 2007). In rats, partition coefficients were highest for fat (21.9), similar for liver, kidney, muscle, and spleen (0.87–0.94), and lowest in brain (0.43) (Johanson and Filser 1993).

3.4.2.1 Inhalation Exposure

In volunteers inhaling 2 ppm 1,3-butadiene for 20 minutes, blood levels approached equilibrium by 5 minutes (Smith et al. 2001). In mice and rats inhaling up to 625 ppm 1,3-butadiene, equilibrium in blood concentrations was reached by 2 hours, with blood levels in mice being three- to 4-fold higher than in rats at all times (Himmelstein et al. 1994). The distribution of 1,3-butadiene in several tissues in rats was measured following a 1-hour inhalation exposure to 129,000 ppm (Shugaev 1969). Perinephric fat

contained 152 mg 1,3-butadiene/100 cc tissue, compared to levels of 36–51 mg 1,3-butadiene/100 mL in the brain, liver, septum, and kidney.

Species differences in the distribution of inhaled 1,3-butadiene were studied in Sprague-Dawley rats and B6C3F1 mice (Bond et al. 1986, 1987). When normalized for amount of inhaled ^{14}C-1,3-butadiene, molar tissue concentrations of radioactive material at 1 hour postexposure were 17-fold (thyroid) to 80-fold higher (lung) in mice than in rats. In blood, the normalized radioactivity concentration was 57-fold higher in mice than rats, while 110- to 120-fold more radioactivity was found in mouse intestine than in rat intestine.

3.4.2.2 Oral Exposure

No studies were located regarding distribution in humans or animals after oral exposure to 1,3-butadiene.

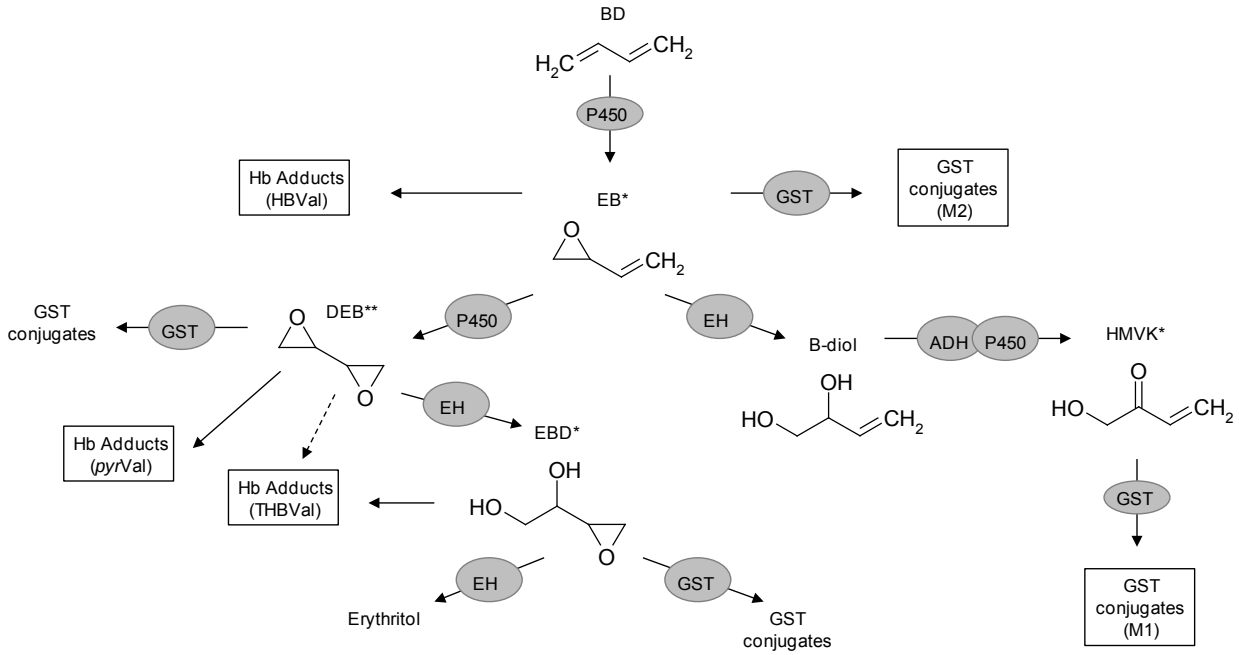
3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals after dermal exposure to 1,3-butadiene.

3.4.3 Metabolism

1,3-Butadiene is metabolized by oxidation, hydrolysis, and conjugation reactions, with oxidation and hydrolysis reactions leading to the formation of several reactive epoxide intermediates (Figure 3-2). Of the reactive intermediates formed, EB (formed by oxidation of 1,3-butadiene), 1,2:3,4-diepoxybutane (DEB; formed by oxidation of EB), and 1,2-dihydroxy-3,4-epoxybutane (EBD; formed by hydrolysis reactions of DEB) are reactive electrophilic compounds. Metabolism of 1,3-butadiene appears to follow the same enzymatic pathways in all species, including humans, with production of the same reactive intermediates. However, important species differences exist in the rates of formation and detoxification of reactive metabolites (Kirman et al. 2010a). As a result, rodents, particularly mice, have much higher tissue levels of reactive metabolites than nonhuman primates and humans. Evidence for species differences in metabolism of 1,3-butadiene is available from *in vitro* studies, studies using isolated perfused livers, and *in vivo* studies measuring tissue and urine metabolite levels and blood hemoglobin adduct levels. Metabolism of 1,3-butadiene exhibits nonlinear kinetics (Kirman et al. 2010a), with both dose- and exposure duration-dependent effects. Several processes have been proposed as sources of

Figure 3-2. Metabolism of 1,3-Butadiene



□ = boxes indicate biomarkers of exposure that have been measured in exposed workers (Albertini et al. 2003);
* = monofunctional alkylating agent; ** = bifunctional alkylating agent; ADH = alcohol dehydrogenase;
B-diol = butanediol; BD = 1,3-butadiene; DEB = diepoxybutane; EB = epoxybutene; EBD = epoxybutane diol;
EH = epoxide hydrolase; GST = glutathione S-transferase; HBVal = *N*-(2-hydroxy-3-butenyl)-valine;
HMVK = hydroxymethylvinyl ketone; M1 = 1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane (urinary metabolite);
M2 = 1-(*N*-acetylcysteinyl)-2-hydroxy-3-butene (urinary metabolite); P450 = cytochrome P450;
*pyr*Val = *N,N*-(2,3-dihydroxy-1,4-butadiyl)-valine; THBVal = *N*-(2,3,4-trihydroxybutyl)-valine

Source: Kirman et al. 2010

nonlinear kinetics; these include inhibition, induction, and saturation of various metabolizing enzymes and depletion of glutathione.

The metabolism of 1,3-butadiene has been observed in the liver, lung, and kidneys. The liver is the predominant site of 1,3-butadiene metabolism (Elfarra et al. 2001; Schmidt and Loeser 1985, 1986). 1,3-Butadiene is initially oxidized (Figure 3-2) by cytochrome P450 (CYP) to 2-butenal or EB (Bolt et al. 1983; Csanady et al. 1992; Duescher and Elfarra 1994; Himmelstein et al. 1994, 1995; Kirman et al. 2010; Malvoisin and Roberfroid 1982; Malvoisin et al. 1979; Thornton-Manning et al. 1995b, 1997). Metabolism of EB is mediated by three competing oxidative, hydrolytic, or conjugation pathways. The flux of 1,3-butadiene through the various pathways is concentration- and species-dependent. Successive oxidation steps of EB result in DEB and 3,4-epoxy-1,2-diol (EBdiol). EB can also be conjugated to glutathione by glutathione-S-transferase (GST) to form 1-glutathionyl-3-buten-2-ol, or can be hydrolized via epoxide hydrolase (EH) to 3-butene-1,2-diol (BDdiol). BDdiol is metabolized via CYP or aldehyde dehydrogenase (ADH) to the ketone 1-hydrozy-3-buten-2-one (hydroxymethylvinyl ketone, or HMVK) or EBdiol. GST can conjugate glutathione to HMVK and EBdiol to form 4-glutathionyl-1-hydroxy-2-butanone and 4-glutathionylbutane-1,2,3-triol. Several isoforms of CYP have been implicated in the oxidative metabolism of 1,3-butadiene and resulting epoxides in various tissues. In human liver microsomes, CYP2E1 dominates metabolism at low concentrations (i.e., 0.16 mM), while CYP2A6 dominates at higher concentrations (i.e., 4.4 mM) (Elfarra et al. 1996). In mice, CYP2E1 and 2A5 are active in 1,3-butadiene oxidation in lung and liver microsomes, but CYP4B1 dominates metabolism in the kidneys (Elfarra et al. 2001).

Examination of blood levels of 1,3-butadiene metabolites in male Sprague-Dawley rats exposed to 1–10,000 ppm and male B6C3F1 mice exposed to 1–6,000 ppm for 6–8 hours in closed chambers indicates species differences in the predominance of metabolic pathways (Filsner et al. 2007). In rats and mice, EB, EBD, and 3-butene-1,2-diol concentrations in blood increased with increasing exposure concentrations. Ratios of mouse:rat EB blood levels ranged from 2.0 to 8.6 over 1,3-butadiene exposure concentrations of 1–1,250 ppm. DEB was detected in blood of mice, but not in rats. Similar results were observed in male Sprague-Dawley rats and male B6C3F1 mice exposed (nose only) to 62.5, 625, or 1,250 ppm 1,3-butadiene for 6 hours; DEB was detected in mouse, but not rat, blood, and higher levels of butadiene monoepoxide were present in mouse blood, compared to rat blood (Himmelstein et al. 1994). Results of these studies are consistent with enhanced formation and/or lower metabolism of DEB in mice compared to rats. Differences also were noted between rodents and monkeys in 1,3-butadiene metabolism (Dahl et al. 1990; Sun et al. 1989a). At 10 ppm, blood levels of EB, DEB, and EBdiol were

lower in monkeys inhaling 10 ppm than in rodents. The difference was not so great at 8,000 ppm (Sun et al. 1989a). Similar exposures of 10 ppm 1,3-butadiene resulted in blood concentrations of total 1,3-butadiene metabolites in monkeys that were about 5–50 times lower than in mice and about 4–14 times lower than in rats (Dahl et al. 1991). The results indicated possible lower susceptibility to toxic effects of low levels of 1,3-butadiene in primates.

Species differences in metabolism are also supported by results of studies examining tissue levels of metabolites (Filser et a;. 2007; Himmelstein et al. 1995; Thorton-Manning et al. 1995a, 1995b). Comparison of butadiene epoxide levels in livers and lungs of male Sprague-Dawley rats and male B6C3F1 exposed (nose only) to 62.5, 625, 1,250, or 8,000 (rats only) ppm 1,3-butadiene shows higher levels of butadiene monoepoxide in mice compared to rats, and the presence of butadiene diepoxide in mice, but not rats (Himmelstein et al. 1995). In male Sprague-Dawley rats and male B6C3F1 mice exposed to 62.5 ppm 1,3-butadiene for 4 hours, tissue levels of butadiene monoepoxide were higher in tissues (blood, heart, lung, liver, fat, spleen, and bone marrow) of mice compared to rats, with mouse:rat ratios ranging from 3.0 (heart) to 11.5 (bone marrow); butadiene diepoxide was not detected in lung or liver of rats (Thorton-Manning et al. 1995a). In mice, butadiene diepoxide levels were similar to monoepoxide levels in blood, heart, thymus and bone marrow; diepoxide levels in mouse lung, liver, and spleen were higher than monoepoxide levels, but were lower in fat. Butadiene diepoxide levels in rats were very low compared to levels in mice, and diepoxide was not detected in liver or bone marrow. Comparison of butadiene epoxide levels in tissues of male and female Sprague-Dawley rats exposed to 1,3-buradiene for 6 hours (nose only) suggests gender differences in metabolism (Thorton-Manning et al. 1995b). Butadiene monoepoxide levels in lung were approximately 5-fold higher in males compared to females; whereas similar monoepoxide levels for males and females were observed for blood, femur, and fat.

Results of studies using isolated perfused livers provide additional evidence of species differences in metabolism of 1,3-butadiene. Following single pass, isolated perfusion of livers from male Sprague-Dawley rats and male B6C3F1 mice with 1,3-butadiene (at concentrations that approached saturation of 1,3-butadiene metabolism), differences were observed in 1,3-butadiene metabolites in liver effluent (Filser et al. 2001). In mice, three epoxides (EB, DEB, and EBD) and 3-butane-1,2-diol (B-diol) were detected, whereas only EB and B-diol were detected in effluent from rat livers. Furthermore, the concentration of EB in effluent from rat livers was approximately 8.5-fold less than that in effluent from mouse livers. Additional species differences were observed in a study evaluating single-pass perfusion of the 1,3-butadiene metabolites, EB, DEB and B-diol (Filser et al. 2010). For perfusion with EB, EBD,

DEB, and B-diol were formed in rats and mice, with an approximately 4-fold higher percentage of DEB in mice compared to rats. The major metabolite of DEB in rats and mice was EBD. For perfusion with B-diol, EBD was detected in effluent of rats, but not in mice. Results of studies using hepatic microsomes from mice and rats indicate differences in stereochemistry of 1,3-butadiene metabolites (Nieusma et al. 1997). Mouse microsomes form more (*S*)-EB than (*R*)-EB; rat microsomes initially formed more (*S*)-EB than (*R*)-EB, although the *S*:*R* fell below 1.0 as incubation time increased to 30 minutes. For DEB formation, mice microsomes formed more DEB when starting with (*S*)-EB compared to when starting with (*R*)-EB; the opposite was observed with rat microsomes.

In vitro studies indicate that mouse lung and liver have a higher capacity than other species, including humans, to oxidize 1,3-butadiene to EB and DEB, but have much less ability to detoxify the epoxides via the EH pathway (Jackson et al. 2000b). Female mouse tissue homogenates resulted in higher EB generation than in males or in rat, human, or monkey tissues, while human and monkey tissues hydrolyzed the epoxides to diols approximately 20-fold more extensively than rodents (Schmidt and Loeser 1985, 1986). In studies of liver microsomes, mice had intrinsic clearance (V_{max}/K_m) values of 57.5 and 0.77 $minute^{-1}$ for oxidation of 1,3-butadiene to EB and EB to DEB, respectively. These values are 3–4-fold higher than the respective rat values of 1,637 and 0.21 $minute^{-1}$ (Elfarra et al. 2001). Conversely, intrinsic clearance via EH-mediated hydrolysis of EB was 34.4 $minute^{-1}$ in rats, compared to 12.4 $minute^{-1}$ in mice. Clearance by EB conjugation with GSH was similar (21.0 and 22.0 $minute^{-1}$) in both species. These *in vitro* findings comport with metabolic differences observed between rats and mice after inhalation exposure to EB (Kreiling et al. 1987; Laib et al. 1990). A limited rate of EB removal and its subsequent accumulation was observed in mice at 500 ppm exposure, but not in rats at exposures up to 5,000 ppm. This may partially account for the differing levels of toxicity and carcinogenicity between rats and mice in long-term studies. For additional information, see Section 3.5 (Mechanisms of Action).

Based on evaluation of hemoglobin adduct biomarkers (adducts formed by interaction of 1,3-butadiene metabolites with hemoglobin), mice appear to have higher DEB levels than rats and much higher levels than humans (Swenberg et al. 2007). Analysis of hemoglobin adducts in mice and rats exposed to 1,3-butadiene by inhalation show much higher levels of the hemoglobin adducts, *pyr*-Val and THB-Val (formed by interaction of DEB with hemoglobin), in mice than in rats (Boysen et al. 2004). Results are consistent with results of studies showing higher levels of DEB in tissues of mice compared to rats. In polymerization workers exposed to 0.81 ppm 1,3-butadiene, THB-Val was the predominant hemoglobin adduct, comprising 99.6% of the total; the mean percent of HB-Val was 0.33% and the mean percent of *pyr*-Val was 0.05% (Boysen et al. 2012). In monomer workers exposed to lower concentrations of

1,3-butadiene (0.29 ppm), lower levels of HB-Val (0.26%) and higher levels of *pyr*-Val (0.11%) were found; the decrease in *pyr*-Val levels was significantly related to the increasing 1,3-butadiene concentrations. The lower percentage of *pyr*-Val formed in the workers exposed to higher 1,3-butadiene levels may be suggestive of saturation of the formation of *pyr*-Val in humans (Boysen et al. 2012).

As noted in the introduction to Section 3.4.3, metabolism of 1,3-butadiene exhibits nonlinear kinetics (Kirman et al. 2010a), with both dose- and exposure duration-dependent effects. Glutathione deletion and saturable kinetics have been proposed as possible sources of nonlinear kinetics. Studies evaluating effects of hepatic glutathione levels show that gluthione depletion exhibited concentration-dependence and was greater in mice than in rats (Deutschman and Laib, 1989; Kreiling et al. 1988). Glutathione depletion was also observed in livers and lungs of rats and mice exposed to 1,3-butadiene, with more extensive depletion in mice than in rats (Himmelstein et al. 1995). Regarding saturable kinetics, blood levels of EB reached a plateau in rats, but not mice (Filser et al. 2007). The study authors suggested that the results are consistent with competitive inhibition of CYP450 isozymes.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

The monoepoxide metabolite, EB, can be conjugated to glutathione by GST to form monohydroxybutenylmercaptic acid (MHBMA, or M2), a mixture of N-acetyl-S-([1-hydroxymethyl]-2-propenyl)cysteine and N-acetyl-S-([2-hydroxymethyl]-3-propenyl)cysteine. EBdiol, formed by hydrolysis of EB, may also be conjugated by GST to glutathione to form N-acetyl-S-(3,4-dihydroxy-butyl)cysteine (DHBMA, or M1). Both mercaptic acids are excreted in the urine (Boogaard et al. 2001a; McDonald et al. 2004). These excretion products have been used as biomarkers of 1,3-butadiene exposures in both environmental and occupational settings (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Boogaard et al. 2001a) (see Section 3.8). The relative abundance of MHBMA and DHBMA in urine indicates the flux of EB through the competing GST and EH metabolic pathways. In humans, >97% of urinary mercaptic acid measured following 1,3-butadiene inhalation is DHBMA, indicating that most EB proceeds to hydrolysis via EH rather than to formation of the diepoxide (Henderson et al. 1996). Albertini et al. (2007) showed that women excrete lower levels of both mercaptic acids than men per unit of 1,3-butadiene exposure; however, they maintain the ratio of M1 and M2, suggesting sex differences in metabolic activity, but not pathway flux.

In rats exposed to 1,3-butadiene, 1,2-epoxybutene-3 and acetone were exhaled as suspected metabolites of the administered compound (Bolt et al. 1983). The pharmacokinetic profile of inhaled 1,3-butadiene was studied in mice (Kreiling et al. 1986b) and in rats (Bolt et al. 1984; Filser and Bolt 1984). Following exposure of mice and rats to ^{14}C-1,3-butadiene, the elimination of radioactivity was rapid, and 77–99% of the initial tissue amount was eliminated with half-lives of between 2 and 10 hours (Bond et al. 1987). At concentrations of approximately ≤1,000 ppm, the elimination of 1,3-butadiene followed first-order kinetics in both species. The first-order metabolic clearance of inhaled 1,3-butadiene per kg body weight was 4,500 mL/hour for rats (Laib et al. 1988) and 7,300 mL/hour for mice (Kreiling et al. 1986b). The maximal metabolic elimination rate was calculated as 220 μmol/hour/kg for rats (Laib et al. 1988) and 400 μmol/hour/kg for mice (Kreiling et al. 1986b). With increasing concentrations of ^{14}C-1,3-butadiene, exhalation of radiolabeled carbon was a major pathway for elimination of ^{14}C in mice and rats (Bond et al. 1986). Similar results were observed in mice and rats following inhalation of 62.5 ppm 1,3-butadiene for 6 hours (Himmelstein et al. 1996). Blood 1,3-butadiene concentrations in mice fell from about 3 μM at the end of exposure to 0.03 μM 15 minutes later. Rat elimination of 1,3-butadiene from blood was slower, falling from a post-exposure maximum of about 1.5–0.1 μM 30 minutes later.

About 2% of the total inhaled amount of 1,3-butadiene was excreted as metabolites in Cynomolgus monkeys (Sun et al. 1989a). Carbon dioxide was the major exhalation product at 10 ppm, while epoxy-metabolites (specific compounds not determined) were predominant in exhaled breath at 300 and 8,000 ppm. Urinary excretion of total metabolites was not influenced by exposure levels. In *Macaca fascicularis* monkeys, about 39% of metabolite radioactivity (specific compounds not determined) were eliminated in the urine, 0.8% in feces, and 56% were exhaled as carbon dioxide during the first 70 hours postexposure (Dahl et al. 1990).

3.4.4.2 Oral Exposure

No studies were located regarding excretion in humans or animals after oral exposure to 1,3-butadiene.

3.4.4.3 Dermal Exposure

No studies were located regarding excretion in humans or animals after dermal exposure to 1,3-butadiene.

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

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

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

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

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for

many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

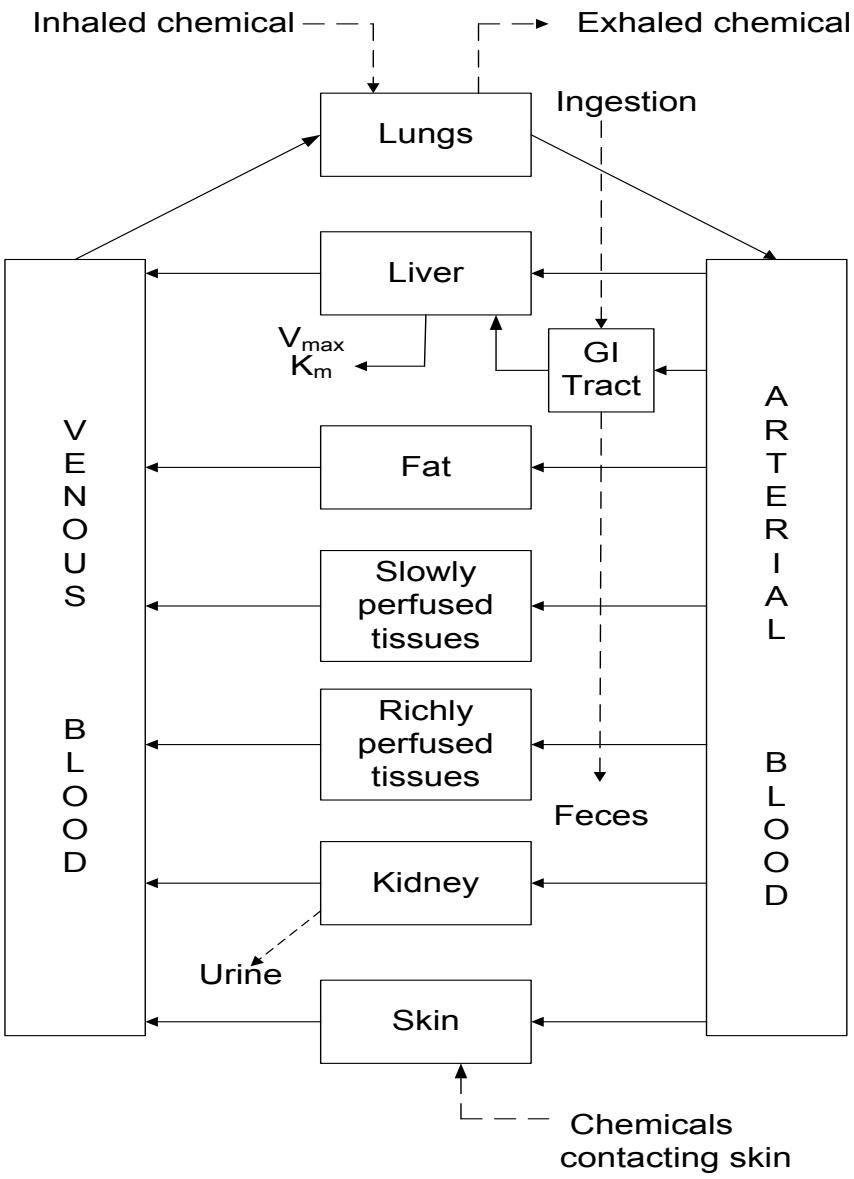
PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in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

Literature on PBPK modeling of 1,3-butadiene is extensive (Beaudouin et al. 2010; Bois et al. 1999; Bond et al. 1996; Brochot et al. 2007; Csanady et al. 1996; Evelo et al. 1993; Filser et al. 1993; Johanson and Filser 1993, 1996; Kohn 1997; Kohn and Melnick 1993, 1996, 2000, 2001; Leavens and Bond 1996, Péry and Bois 2009; Seilken et al. 1996; Smith et al. 2001; Sweeney et al. 1996, 1997, 2001). Models have been developed to simulate 1,3-butadiene kinetics in mice (Bond et al. 1996; Csanady et al. 1996; Johanson and Filser 1993, 1996; Kohn and Melnick 1993, 1996, 2000, 2001; Leavens and Bond 1996; Sweeney et al. 1996, 1997, 2001), rats (Bond et al. 1996; Csanady et al. 1996; Johanson and Filser 1993, 1996; Kohn and Melnick 1993, 1996, 2000, 2001; Sweeney et al. 1996, 1997, 2001), and humans (Beaudouin et al. 2010; Bond et al. 1996; Brochot et al. 2007; Csanady et al. 1996; Evelo et al. 1993; Johanson and Filser 1996; Péry and Bois 2009). Model structures differ with respect to the number of physiological compartments simulated, the extent to which secondary and tertiary metabolites are simulated, and in which tissue compartments metabolism is assumed to occur. Selected examples are described in greater detail in the sections that follow.

Johanson and Filser 1996

Description of the Model. The Johanson and Filser model (Filser et al. 1993; Johanson and Filser 1993, 1996) model simulates absorption and disposition of 1,3-butadiene and the metabolite, 3,4-epoxy-1-butene, in the mouse, rat, and human. The hepatic conjugation of 3,4-epoxy-1-butene to GSH is also simulated. Tissue compartments include the blood/lung, liver, fat, and muscle/richly-perfused tissues.

Figure 3-3. 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

Model parameters are presented in Table 3-4. Both the exchange of 1,3-butadiene and 3,4-epoxy-1-butene between blood and tissue or lung air is assumed to be first-order and flow-limited. *In vitro* derivation of tissue:air and tissue:blood partition coefficients was performed by the model authors and reported in the study. Michaelis-Menten expressions were included for 1,3-butadiene oxidation, 3,4-epoxy-1-butene hydrolysis, and 3,4-epoxy-1-butene conjugation to GSH, all occurring in the liver compartment. Values for physiological (alveolar, pulmonary, and tissue perfusion rates, organ weights) and metabolic parameters (V_{max}, K_m, and GSH content and elimination rates) were taken from the literature, except for the affinity constant (K_m) for 1,3-butadiene oxidation, which was fit to chamber air 1,3-butadiene timecourse data from rat and mouse closed chamber experiments. Elimination of 1,3-butadiene and 3,4-epoxy-1-butene was represented as either metabolism or as exchange passage back to the lung air.

Risk Assessment. This model has not been used in risk assessment. The model predicts that steady-state blood concentrations of 3,4-epoxy-1-butene resulting from continuous inhalation exposures to 1,3-butadiene would be higher in mice compared to rats or humans. At non-saturating conditions (e.g., exposures <1,000 ppm), the ratio of blood 3,4-epoxy-1-butene concentrations predicted from the model were: 1.6:1.0:0.3 for mouse:rat:human (Johanson and Filser 1996).

Validation of the Model. The K_m for 1,3-butadiene oxidation and 3,4-epoxy-1-butene hydrolysis were the only parameters optimized against close-chamber gas uptake data for 1,3-butadiene or 3,4-epoxy-1-butene (1,000–5,000 ppm) in rats (Filser and Bolt 1984) and mice (Kreiling et al. 1987). Model predictions were evaluated against the concentration of 3,4-epoxy-1-butene appearing in the chambers (due to metabolism and exhalation from the animals) during the 1,3-butadiene exposures, and were found to predict 3,4-epoxy-1-butene levels that were similar to observations.

Target Tissues. The model simulates concentrations of 1,3-butadiene and 3,4-epoxy-1-butene in liver, a target tissue for 1,3-butadiene metabolites, as well as in blood, fat, and a lumped compartment for muscle and richly-perfused tissues.

Species Extrapolation. The model has been developed for simulations of rats, mice, and humans. Extrapolation to other species would require species-specific physiological and metabolism parameter values and blood-tissue partition coefficients.

Table 3-4. Physiological and Chemical Parameters Used in the Johanson and Filser (1993) PBPK Model for 1,3-Butadiene

		Mouse	Rat
Physiological data			
Body weight (bw) (g)	Standard animal	25	250
	Simulations	27.5	157.5–217.5
Alveolar ventilation (mL/minute)	Standard animal	15	70.2
	Simulations	proportional to $bw^{2/3}$	proportional to $bw^{2/3}$
Cardiac output	Standard animal	17	83
	Simulations	proportional to $bw^{2/3}$	proportional to $bw^{2/3}$
Blood flows (percent of cardiac output)	Muscle and vessel-rich group (VRG)	66	66
	Fat	9	9
	Liver	25	25
Compartment volumes[a] (percent of body weight)	Lung and arterial	1	1
	Muscle and VRG	75	80
	Fat	10	7
	Liver	5.5	4
Tissue:air partition coefficients			
Butadiene	Lung and arterial, muscle and VRG, liver	0.76	0.76
	Fat	21.9	21.9
	Blood	3.03	3.03
Epoxybutene	Lung and arterial, muscle and VRG, liver	58.9	58.9
	Fat	155	155
	Blood	83.4	83.4
	Water	43.0	43.0
Metabolic constants			
Butadiene oxidation	Microsomal protein (mg/g liver)	30	30
	V_{max} (nmol·minute^{-1}·mg^{-1})	3.22	2.17
	K_m (µmol/L air)	5	5
Epoxybutene hydrolysis	Microsomal protein (mg/g liver)	30	30
	V_{max} (nmol·minute^{-1}·mg^{-1})	19	17
	Apparent K_m (mmol/L)	1.5	0.7
	Intrinsic K_m (percent of apparent K_m)	20%	20%
Epoxybutene conjugation	Cytosolic protein (mg/g liver)	95	95
	V_{max}/K_m of epoxybutene (µL·minute^{-1}·mg^{-1})	15	11
	K_m towards epoxybutene (mmol/L)	100	100
	K_m towards glutathione (mmol/L)	0.1	0.1
Glutathione kinetics	Initial steady-state concentration (mmol/L)	5.5	4.2
	Elimination rate constant (hour^{-1})	0.15	0.15

[a]Density was set to 1 for all organs

Source: Johanson and Filser 1993 (although simulations from a human model were reported in Johanson and Filser 1996, parameter values were not reported)

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in mice and rats ranging from 500 to 5,000 ppm.

Interroute Extrapolation. The model simulates inhalation exposures only and would require additional parameterization to simulate exposures by other routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition and clearance of inhaled 1,3-butadiene, as well as production and clearance of 3,4-epoxy-1-butene, the major oxidative metabolite formed in rodents. Limitations include: (1) the model has not been evaluated for inhalation exposures below 500 ppm; (2) the model does not simulate the appearance and disposition of other metabolites, such as the diepoxide, diols, and GSH-conjugate products eliminated in the urine; and (3) the model does not simulate 1,3-butadiene disposition in humans.

Kohn and Melnick 2001

Description of the Model. The Kohn and Melnick model (Kohn and Melnick 1993, 1996, 2000, 2001) simulates absorption of 1,3-butadiene and the disposition of 1,3-butadiene and the metabolite, 3,4-epoxy-1-butene, in the mouse and rat. Model parameters are presented in Tables 3-5, 3-6, and 3-7. The body is represented by discrete compartments for venous and arterial blood, lung, liver, kidney, fat, the gastrointestinal tract, and lumped compartments for richly- and poorly perfused tissues (viscera and muscle, respectively). Values for physiological flow rates, tissue volumes, and tissue:blood partition coefficients were taken from the literature. 1,3-Butadiene is eliminated by exhalation to lung air or oxidative metabolism to 3,4-epoxy-1-butene and its oxidation metabolites (3,4-epoxy-1-butene, DEB, 3,4-epoxy-1,2-diol, 3-butene-1,2-diol) and glutathione conjugates. All of these pathways are described as saturable Michaelis-Menten processes, with V_{max} and K_m values optimized against published closed-chamber 1,3-butadiene and 3,4-epoxy-1-butene uptake data (Bolt et al. 1984; Filser and Bolt 1984; Kreiling et al. 1986b, 1987). Epoxide hydrolysis was modeled such that dissociation of the diepoxide from P450 in the microsome is followed by preferential binding of the epoxide hydrolase, replicating the so-called privileged access model for epoxide hydrolysis. The model simulates production and utilization of glutathione in kidney, liver, and lung, with the assumption that glutathione production is limited by availability of cysteine for the first step in glutathione synthesis (γ-glutamylcysteine synthetase).

Risk Assessment. This model has not been used in risk assessment.

Table 3-5. Physiological Parameter Values Used in the Kohn and Melnick (2001) PBPK Model for 1,3-Butadiene

	Mouse (percent)	Rat (percent)
Tissue compartment volumes, percent body weight		
Liver	5.5	3.7
Lung	0.6	0.52
Alveolar	0.5	0.515
Kidney	1.67	1.48
Gastrointestinal tract	7.5	7.5
Viscera	3.93	14.3
Fat	6	7
Muscle and skin	64.5	54.2
Blood	6	5.4
Capillary blood volume, percent tissue volume		
Liver	11	13.8
Lung	11	18
Kidney	10.2	16
Gastrointestinal tract	2.9	2.65
Viscera	7.1	7.1
Fat	3	2
Muscle and skin	1.3	2
Blood flow rate, percent cardiac output		
Liver (hepatic artery only)	4.4	3.9
Kidney	16.3	13.3
Gastrointestinal tract	18.1	18.1
Viscera	22.4	24.8
Fat	5	6.5
Muscle and skin	33.8	33.4

Source: Kohn and Melnick 2001

Table 3-6. Chemical Partition Coefficients Parameter Values Used in the Kohn and Melnick (2001) PBPK Model for 1,3-Butadiene

	Butadiene	Epoxybutane	Butenediol	Epoxybutanediol	Diepoxybutane
Blood:air	1.95	56.8	–	–	–
Liver	0.595	0.984	1.04	0.903	1.41
Lung	0.615	0.977	1.107	0.958	1.41
Kidney	0.472	0.842	0.962	0.833	1.54
Gastrointestinal tract	0.446	0.908	1.22	1.06	1.41
Viscera	0.446	0.908	1.22	1.06	1.41
Fat	10.8	2.25	0.573	0.496	2.19
Muscle and skin	0.564	0.736	1.139	0.986	1.82

Source: Kohn and Melnick 2001

Table 3-7. Chemical Metabolism Parameter Values Used in the Kohn and Melnick (2001) PBPK Model for 1,3-Butadiene[a]

	Liver	Lung	Kidney
Butadiene metabolism			
V_{max}^{P450}	155, **130**	139, **9.6**	1,430, **30**
K_m^{P450}	0.002, **0.00375**	0.00501, **0.00775**	0.00501, **0.00216**
Epoxybutene metabolism			
V_{max}^{P450}	45.1, **24.3**	*10.2*, ***9.84***	*48.6*, ***12.6***
K_m^{P450}	0.0156, **0.145**	0.0156, **0.145**	0.0156, **0.145**
V_m^{EH}	347, **584**	34.8, **42.8**	*113*, ***14.7***
K_m^{EH}	1.59, **0.26**	1.59, **0.7**	1.59, **0.7**
V_{max}^{GST}	6,420, **4,260**	720, **196**	960, **494**
Adjusted K_{mx}^{GST}	3.59, **2.59**	3.59, **4.94**	3.59, **4.39**
Butenediol metabolism			
V_{max}^{P450}	*16.3*, *67.1*	*1.0*, *31.5*	*1.0*, ***85.0***
K_m^{P450}	0.0156, **0.145**	0.0156, **0.145**	0.0156, **0.145**
V_{max}^{GST}	*3,480*, *1,230*	*491*, *276*	*1,070*, ***658***
K_{mx}^{GST}	34, **34**	34, **34**	34, **34**
Epoxybutanediol metabolism			
V_{max}^{EH}	*363*, *1,150*	*69.5*, **169**	*10.0*, ***152***
K_m^{EH}	8.1, **2.76**	7.5, **7.1**	7.5, **7.1**
V_{max}^{GST}	*2,260*, **271**	*50.0*, **100**	*50.0*, ***138***
K_{mx}^{GST}	6.40, **4.17**	6.40, **4.17**	6.40, **4.17**
Diepoxybutane metabolism			
V_{max}^{EH}	1,920, **3,170**	10.0, **1,160**	*35.2*, ***1,000***
K_m^{EH}	8.1, **2.76**	7.5, **7.1**	7.5, **7.1**
V_{max}^{GST}	9,720, **1,940**	*100*, *100*	*100*, *100*
Adjusted K_{mx}^{GST}	6.40, **4.17**	6.40, **4.17**	6.40, **4.17**
Cysteine metabolism			
Tissue cysteine	0.193, **0.195**	0.171, **0.127**	0.280, **0.326**
$V_{max}^{\gamma\text{-GCS}}$	420, **396**	54, **50**	7,920, **6,080**

EH = epoxide hydrolase; γ-GCS = gamma-glutamylcysteine synthesase; GST = glutathione S-transferase

[a]Non-bold values for mouse; bold values for rat; values in italics were estimated by formal optimization; entries in italics indicate optimized parameter values; V_{max} values in nmol/hour/mg of protein; K_m values and tissue cysteine concentrations in mM.

Source: Kohn and Melnick 2001

Validation of the Model. The model predicted similar profiles of 1,3-butadiene and 3,4-epoxy-1-butene uptake data (100–4,000 ppm) in mice and rats against which it was optimized. Further, it predicted single time point concentrations of 1,3-butadiene in mice and rats similar to observations from nose-only exposures to 7–1,250 ppm (Bond et al. 1986; Himmelstein et al. 1994). Additionally, it predicted similar percentages of GSH depletion in mouse and rat lung and liver observed following 7-hour 1,3-butadiene exposures of 50–2,000 ppm (Deutschmann and Laib 1989).

Target Tissues. The model simulates concentrations of 1,3-butadiene and 3,4-epoxy-1-butene in lung, liver, and kidney, all target tissues for 1,3-butadiene metabolite intoxication, as well as in blood, fat, gastrointestinal tract, and lumped compartments for muscle and viscera.

Species Extrapolation. The model has been developed for simulations of rats and mice. Extrapolation of predictions to humans would require additional species-specific values for physiology and metabolism, as well as human data to verify the accuracy of the predictions.

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures ranging from 7 to 4,000 ppm.

Interroute Extrapolation. The model simulates inhalation exposures only and would require additional parameterization to simulate exposures by other routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition and clearance of inhaled 1,3-butadiene, as well as production and clearance of the major oxidative metabolites of 1,3-butadiene in kidney, liver, and lung, including 3,4-epoxy-1-butene, 1,2:3,4-diepoxybutane, 3,4-epoxy-1,2-diol, and 3-butene-1,2-diol. It also simulates production and utilization of GSH in the kidney, lung, and liver, which allows prediction of glutathione depletion resulting from 1,3-butadiene metabolism. Limitations include: (1) the model has not been evaluated against data for inhalation exposures in humans, and (2) the model does not simulate the appearance and disposition of other metabolites, such as the diepoxide, diols, and GSH-conjugate products eliminated in the urine.

Brochot et al. 2007

Description of the Model. The Brochot model (Brochot and Bois 2005; Brochot et al. 2007) simulates absorption of 1,3-butadiene and the disposition of 1,3-butadiene, 1,2-epoxy-3-butene, and 1,2:2,3-diepoxybutane in the blood, fat, and lumped compartments for richly- and poorly-perfused tissues in humans. Model parameters are presented in Table 3-8. In addition, the disposition and clearance of 3-butene-1,2-diol and 3,4-epoxy-1,2-butanediol was modeled for the blood and richly- and poorly-perfused tissues. Tissue:blood partition coefficients were taken from the literature. 1,3-Butadiene is eliminated by exhalation to lung air or oxidative metabolism, epoxide hydrolysis, or GSH conjugation in the richly-perfused tissues to mono- and diepoxide and the two diols. Since the model was intended to direct further study design for low-ppm human exposures, all of the metabolic steps are described as 1^{st}-order processes, governed by a rate constant for each pathway. The metabolic rate constants and physiological parameters were optimized using Bayesian techniques against 133 datasets from individual subjects inhaling 2 ppm 1,3-butadiene for 20 minutes (Lin et al. 2001). Several extensions of the Brochot et al. (2007) model have been reported. A 23-compartment model was developed for simulating 1,3-butadiene and 1,2-epoxy-3-butene kinetics in humans (Péry and Bois 2009). The model simulates flow-limited distribution to each tissue with partitioning assumed to occur predominantly into tissue fat (estimated from the fat:blood partition coefficient and fat content of each tissue). Beaudouin et al. (2010) further extended this model to a generic human lifetime PBPK model that included growth and transplacental transfer to the fetus.

Risk Assessment. This model has not been used in risk assessment.

Validation of the Model. The model fit well against the human data from which it was calibrated. Its performance has not been evaluated against other independent human inhalation data.

Target Tissues. The model predicts parent compound, mono- and diepoxide, and viscinal thiol (including the epoxydiol) concentrations in the blood, but not in other tissues.

Species Extrapolation. The model was optimized for humans. Extrapolation of predictions to animals would require additional species-specific values for physiology and metabolism, as well as animal data (available in the literature) to verify the accuracy of the predictions.

Table 3-8. Physiological and Chemical Parameters Used in the Brochot et al. 2007 PBPK Model for 1,3-Butadiene Humans

Parameter	Mean[a]	Standard deviation[a]
Body weight (kg)	70	
Sex	M	
Metabolic activity in liver (mg protein/kg liver)		
Microsomal protein	14,500	
Cytosolic protein	45,000	
Relative weight (percent of body weight)		
Liver	0.026	
Relative volumes (percent of body weight)		
Well-perfused tissues	0.10	0.02
Fat	0.21	0.05
Relative flows (percent of total blood flow)		
Poorly perfused tissues	0.26	0.06
Fat	0.05	0.01
Pulmonary characteristics		
Minute volume (L/minute)	7.5	1.87
Ventilation perfusion ratio	1.0	0.25
Dead space fraction	0.33	0.08
Partition coefficients for BD		
Blood:air	1.22	0.30
Poorly perfused tissues:blood	0.72	0.18
Well-perfused tissues:blood	0.69	0.17
Fat:blood	18.4	4.6
Partition coefficients for EB		
Blood:air	93.3	23.3
Poorly perfused tissues:blood	0.49	0.12
Well-perfused tissues:blood	0.59	0.15
Fat:blood	1.80	0.45
Partition coefficients for DEB		
Poorly perfused tissues:blood	1.98	0.49
Well-perfused tissues:blood	1.53	0.38
Fat:blood	2.20	0.55
Partition coefficients for BDD		
Poorly perfused tissues:blood	1.00	0.25
Well-perfused tissues:blood	1.00	0.25
Partition coefficients for EBD		
Poorly perfused tissues:blood	1.00	0.25
Well-perfused tissues:blood	1.00	0.25
Metabolic constants ($minute^{-1}$)		
BD → EB	0.119	0.06

Table 3-8. Physiological and Chemical Parameters Used in the Brochot et al. 2007 PBPK Model for 1,3-Butadiene Humans

Parameter	Mean[a]	Standard deviation[a]
Metabolic constants		
EB → DEB	0.020	0.01
EB → BDD	0.511	0.25
DEB → EBD	0.471	0.23
BDD → EBD	0.008	0.01
Other transformations (L/kg liver/minute)		
EB by GSH	0.195	0.10
DEB by GSH	0.113	0.06
EBD by GSH	0.056	0.03
EBD by hydrolysis	0.235	0.12
BDD by ADH	0.045	0.02

[a]Mean and standard deviation of lognormal distributions, reflecting variability in the human population.

ADH = alcohol dehydrogenase; BD = 1,3-butadiene; BDD = 3-butene-1,2-diol; DEB = 1,2:3,4-diepoxybutane; EB = 1,2-epoxy-3-butene; EBD = 3,4-epoxy-1,2-butanediol; GSH = glutathione

Source: Brochot et al. 2007

High-low Dose Extrapolation. The model was calibrated for low (2 ppm) exposures in humans. Extrapolation to higher doses will require that the metabolic expression be modified to account for saturation of the oxidative, hydrolytic, and conjugating pathways described.

Interroute Extrapolation. The model was designed to simulate inhalation exposures. Additional parameters and absorption expressions must be added (and optimized with oral or dermal data) in order to extrapolate internal dosimetry from inhalation exposures across other routes of exposure. Parameters for gastrointestinal absorption of 1,3-butadiene were reported for use in a generic lifetime model (Beaudouin et al. 2010).

Strengths and Limitations. The model is the first PBTK model to simultaneously predict blood levels of 1,3-butadiene and its epoxide and viscinal diol metabolites in humans. Limitations include: (1) the model has not been evaluated against data for inhalation exposures in animals, and (2) the model does not account for saturation of metabolic pathways that may occur in humans (and have been observed in rodents) exposed to higher inhalation concentrations of 1,3-butadiene.

Sweeney et al. 2001

Description of the Model. The Sweeney et al. (1996, 1997, 2001) model simulates inhalation absorption of 1,3-butadiene and the disposition of 1,3-butadiene, 1,2-epoxy-3-butene, and 1,2:2,3 diepoxybutane in mice, rats, and humans. Blood, fat, liver, and lumped compartments for richly- and poorly-perfused tissues are simulated. Model parameters are presented in Table 3-9, 3-10, 3-11, 3-12, 3-13, 3-14, and 3-15. 1,3-Butadiene is eliminated by exhalation to lung air or oxidative metabolism to 1,3-epoxide-3-butene or to other products (e.g., aldehydes) in liver. The monoepoxide (1,3-epoxide-3-butene) undergoes further oxidative metabolism to the diepoxide (DEB) and both the mono- and diepoxide undergo epoxide hydrolysis, GSH conjugation, or nonenzymative degradation. Enzymatic reactions are simulated as saturable reactions (K_m, V_{max}) and non-enzymatic elimination reactions are simulated as first-order reactions. Production and utilization of glutathione in liver are simulated, which allows prediction of glutathione depletion resulting from 3,4-epoxy-1-butene metabolism.

Risk Assessment. This model has not been used in risk assessment.

Table 3-9. Physiological Parameters Used in Sweeney et al. (1997) 1,3-Butadiene PBPK Model

Parameter	Rat	Mouse
Alveolar ventilation[a] (Q_{pu}) (L/hour/kg)	17	41
Cardiac output[a] (Q_t) (L/hour/kg)	17	41
Body weight (BW) (kg)	0.215–0.475	0.028–0.035
Blood flow[b] (Q_i) (fraction of cardiac output) (F_i) (dimensionless)		
Lung	1.0	1.0
Fat	0.09	0.09
Slowly perfused tissues	0.15	0.15
Richly perfused tissues	0.51	0.51
Liver	0.25	0.25
Organ volumes[c] (V_i) (fraction of body weight) (dimensionless)		
Lung	0.0053	0.005
Fat	0.09	0.10
Slowly perfused tissues	0.71	0.7
Richly perfused tissues	0.0347	0.0226
Liver	0.05	0.0624

[a]Alveolar ventilation and cardiac output are given for a hypothetical 1-kg animal. In the model simulations, the parameter is multiplied by the body weight of the animal (in kg) to calculate the ventilation rate and cardiac output (in L/hour) for that individual animal.
[b]Tissue blood flows are calculated by multiplying the total cardiac output by the fractional flow: $Q_i = F_i \times Q_t$.
[c]Tissue volumes are calculated by multiplying the body weight by the fractional volume: $V_i = F_i \times BW$.

Source: Sweeney et al. 1997

Table 3-10. Partition Coefficients Used in Sweeney et al. (1997) PBPK Model for 1,3-Butadiene

	Butadiene[a]		Epoxybutene[a]		Diepoxybutane[b]
Tissue	Rat	Mouse	Rat	Mouse	Mouse
Blood	1.49	1.34	50.4	36.6	0.437
Liver	1.19	1.35	72.0	42.1	0.615
Lung	0.92	1.47	54.7	56.3	ND
Kidney	ND	ND	ND	ND	ND
Muscle	1.47	4.01	19.8	23.6	0.795
Fat	22.2	19.2	138.0	91.2	0.959
Saline	0.088		44.3		0.723
Oil	23.2		164		ND

[a]Tissue:air partition coefficients.
[b]Tissue:hexane partition coefficient.

ND = not determined

Source: Sweeney et al. 1997

Table 3-11. Nonenzymatic Reaction Rate Constants Used in Sweeney et al. (1997) PBPK Model for 1,3-Butadiene

	Epoxybutene		Diepoxybutane	
Tissue	Rat	Mouse	Rat	Mouse
Blood	0.582	0.558	ND	0.189
Liver	4.94	4.14	ND	3.15
Lung	6.07	2.70	ND	4.1
Fat	1.72	1.56	ND	2.8
Muscle	0	0	ND	0

ND = not determined

Source: Sweeney et al. 1997

Table 3-12. Metabolism Rate Constants Used in the Sweeney et al. (1997) PBPK Model for 1,3-Butadiene

				Parameter value	
Substrate	Tissue	Pathway	Units	Rat	Mouse
Butadiene	Liver	Oxidation	µmol/kg/hour	62	338
		(to all products)	µmol/L	3.75	2.0
		Oxidation	µmol/kg/hour	8.2	97
		(to epoxybutene only)	µmol/L	1.54	0.88
		Oxidation	µmol/kg/hour	54	243
		(to other volatiles)	µmol/L	4.36	2.72
	Lung	Oxidation	µmol/kg/hour	1.01	21.6
		(to all products)	µmol/L	7.75	5.01
		Oxidation	µmol/kg/hour	0.13	6.4
		(to epoxybutene only)	µmol/L	3.18	1.6
		Oxidation	µmol/kg/hour	0.88	16.1
		(to other volatiles)	µmol/L	9.14	9.5
Epoxybutene	Liver	Oxidation	µmol/kg/hour	57.1	176.6
		(one enzyme)	µmol/L	141	145
		Oxidation	µmol/kg/hour	10	32.5
		(two enzymes)	µmol/L	141	15.6
			µmol/kg/hour	47.1	144.1
			µmol/L	141	145
		Hydrolysis	µmol/kg/hour	260	754
			µmol/L	260	1,590
		Glutathione conjugation	µmol/kg/hour	78,100	154,000
			µmol/L	13,800	35,300
			µmol/L	100	100
	Lung	Glutathione conjugation	µmol/kg/hour	819	4,088
			µmol/L	17,400	36,500
Diepoxybutane	Liver	Hydrolysis	µmol/kg/hour	5,555	4,193
			µmol/L	2,700	8,100
		Glutathione conjugation	µmol/kg/hour	60,264	50,342
			µmol/L	24,000	6,400
	Lung	Hydrolysis	µmol/kg/hour	122.7	466.1
			µmol/L	7,100	7,500
		Glutathione conjugation	µmol/kg/hour	332	577
			µmol/L	4,170	1,700

Source: Sweeney et al. 1997

Table 3-13. Physiological Parameters Used in the Sweeney et al. (2010) PBPK Model for 1,3-Butadiene in Humans

Parameter	Value	Units	Comment
Alveolar ventilation rate (QPC)	4.3	L/hour/kg body weight	QP = QPC x BW
Cardiac output (QCC)	4.5	L/hour/kg body weight	QC = QCC x BW
Fractional blood flow to liver	0.227	Dimensionless	QR = 0.76 x QC−QL
Fractional blood flow to fat	0.052	Dimensionless	QS = 0.24 x QC−QF
Fractional weight of liver	0.027	Dimensionless	
Fractional weight of lung	0.0076	Dimensionless	VR = 0.09−VL−VLU
Fractional weight of fat	0.2142	Dimensionless	VS = 0.81−VF
Body weight	70	kg	
Cytosolic protein content of liver	89,000	mg protein/kg liver	Rat value
Microsomal protein content of liver	77,000	mg protein/kg liver	Human value

Source: Sweeney et al. 2001

Table 3-14. Chemical Partition Coefficients Used in the Sweeney et al. (2010) PBPK Model for 1,3-Butadiene in Humans

Parameter	Value	Comment
	1,3-Butadiene	
Blood:air	1.22	Average of individual values for humans
Liver:air	0.68	Human tissue
Fat:air	22.5	Human tissue
Lung:air	0.48	Human tissue
Slowly perfused tissue:air	0.88	Value for human muscle
Richly perfused tissue:air	0.84	Values for human kidney, brain, and liver weighted by contribution to body weight
	Butadiene monoepoxide	
Blood:air	93.3	Human tissue
Liver:air	55.3	Human tissue
Fat:air	168	Human tissue
Lung:air	55.3	Value measured for human liver
Slowly perfused tissue:air	45.8	Value measured for human muscle
Richly perfused tissue:air	55.3	Value measured for human liver
	Butene diol	
Liver:blood	1	Volume of distribution=0.87 L/kg in mouse; mouse model has perfused tissues=0.9 kg/kg total body weight
Rest of body:blood	1	Volume of distribution=0.87 L/kg in mouse; mouse model has perfused tissues=0.9 kg/kg total body weight
	Butadiene diepoxide	
Liver:blood	1.53	Rat tissue
Fat:blood	2.2	Rat tissue
Lung:blood	1.53	Rat liver value
Slowly perfused tissue:air	1.82	Rat muscle value
Richly perfused tissue:air	1.41	Rat kidney value
	Epoxybutane diol	
Liver:blood	1	Assumption based on butene diol and butadiene diepoxide partition coefficients
Rest of body:blood	1	Assumption based on butene diol and butadiene diepoxide partition coefficients

Source: Sweeney et al. 2001

Table 3-15. Chemical Metabolism Parameters Used in the Sweeney et al. (2010) PBPK Model for 1,3-Butadiene in Humans

Parameter (units)	Baseline value[a]
V_{max} for epoxidation of 1,3-butadiene to butadiene monoepoxide (µmol/mg protein/hour)	0.0132
K_m for epoxidation of 1,3-butadiene to butadiene monoepoxide (µM)	0.7
V_{max} for epoxidation of butadiene monoepoxide to butadiene diepoxide (µmol/mg microsomal protein/hour)	0.031
K_m for epoxidation of butadiene monoepoxide to butadiene diepoxide (µM)	880
V_{max} for hydrolysis of butadiene monoepoxide to butene diol	1.4
K_m for hydrolysis of butadiene monoepoxide to butene diol	540
V_{max} for conjugation of butadiene monoepoxide and glutathione (µmol/mg cytosolic protein/hour)	2.7
K_m for conjugation of butadiene monoepoxide and glutathione (µM)	10,400
V_{max} for conjugation of butadiene diepoxide and glutathione (µmol/mg cytosolic protein/hour)	0.4
K_m for conjugation of butadiene diepoxide and glutathione (µM)	3,390
V_{max} for epoxidation of butene diol to epoxybutane diol (µmol/mg microsomal protein/hour)	0.031
K_m for epoxidation of butene diol to epoxybutane diol (µM)	880
V_{max} for hydrolysis of butadiene diepoxide to epoxybutane diol (µmol/mg microsomal protein/hour)	9.2
K_m for hydrolysis of butadiene diepoxide to epoxybutane diol (µM)	4,605
V_{max} for metabolism of butene diol by alcohol dehydrogenase (µmol/mg cytosolic protein/hour)	0.64
K_m for metabolism of butene diol by alcohol dehydrogenase (µM)	10,600
V_{max} for hydrolysis of epoxybutane diol to erythritol (µmol/mg microsomal protein/hour)	4.6
K_m for hydrolysis of epoxybutane diol to erythritol (µM)	4,605
V_{max} for conjugation of epoxybutane diol and glutathione (µmol/mg cytosolic protein/hour)	0.2
K_m for conjugation of epoxybutane diol and glutathione (µM)	3,390

[a]Median or average parameter values.

Source: Sweeney et al. 2001

Validation of the Model. The model was calibrated and evaluated against data from intravenous studies in rats and inhalation exposures of mice and rats (Sweeney et al. 1997) and humans (Sweeney et al. 2001).

Target Tissues. The model has been used to predict parent compound, monoepoxide, and diepoxide concentrations in the blood.

Species Extrapolation. Mouse, rat, and human models have been developed. Extrapolation of predictions to animals would require additional species-specific values for physiology and metabolism, as well as animal data (available in the literature) to verify the accuracy of the predictions.

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in mice and rats ranging from 60 to 1,250 ppm in rodents and 5 ppm in humans.

Interroute Extrapolation. The model was designed to simulate inhalation exposures. Additional parameters and absorption expressions must be added (and optimized with oral or dermal data) in order to extrapolate internal dosimetry from inhalation exposures across other routes of exposure.

Strengths and Limitations. Strengths of this model include simulation of an alternative oxidative pathway for 1,3-butadiene (other than leading to 1,2-epoxy-3-butene); simulations of both enzymatic and nonenzymatic elimination of 1,3-butadiene metabolites; and simulation of production and utilization of glutathione.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Formation of Reactive Metabolites. The role of metabolism of 1,3-butadiene to reactive metabolites and the importance of species differences in metabolism of 1,3-butadiene to human health risk assessment were recently reviewed by Kirmam et al. (2010a). As discussed in Section 3.4.3 (Toxicokinetics/ Metabolism; also see Figure 3-2), 1,3-butadiene is metabolized by oxidation, hydrolysis, and conjugation reactions, with oxidation and hydrolysis reactions leading to the formation of several reactive epoxide intermediates. Of the reactive intermediates formed, EB (formed by oxidation of 1,3-butadiene), DEB (formed by oxidation of EB), and EBD (formed by hydrolysis reactions of DEB) are reactive electrophilic

compounds that have been shown to interact with DNA. The order of genotoxic potency of the epoxide metabolites is DEB >> EB > EBD. The higher genotoxic potency of DEB may be due to its ability to bind to two molecules or two places in the molecule at the same time (e.g., DNA-protein cross-links or DNA cross-links) (see discussion below in Section 3.5.2 on mechanisms of genotoxicity) (Albertini et al. 2010). Thus, the formation of reactive metabolites is critical to the genotoxic activity of 1,3-butadiene. Although little information was identified regarding the role of reactive metabolites in the development of other adverse effects of 1,3-butadiene (e.g., nongenotoxic, noncancer), given their reactive nature, it is likely that reactive epoxide metabolites play an important role in the development of other adverse effects.

Species Differences in Metabolism. Metabolism of 1,3-butadiene appears to follow the same enzymatic pathways in all species, including humans, with production of the same reactive intermediates. However, as discussed below, important species differences exist in the rates of formation and detoxification of reactive metabolites (Bond et al. 1993; Csanády et al. 1992; Dahl et al. 1991; Filser et al. 2001, 2007, 2010; Himmelstein et al. 1997; Henderson et al. 1996, 2001; Kirman et al. 2010a; Krause and Elfarra 1997; Schmidt and Loeser 1985; Thornton-Manning et al. 1995a). As a result, rodents, particularly mice, have much higher tissue levels of reactive metabolites than nonhuman primates and humans. Therefore, based on the assumption that the same mechanism of action is involved in the development of 1,3-butadiene-induced toxicity (i.e., interaction of the reactive metabolites with DNA and other cellular macromolecules) is the same for all species, mice are expected to be much more sensitive to 1,3-butadiene than other rats, nonhuman primates, and humans.

As reviewed by Kirman et al. (2010a), evidence for species differences in metabolism of 1,3-butadiene is available from *in vitro* studies, studies using isolated perfused livers, and *in vivo* studies measuring tissue and urine metabolite levels and blood hemoglobin adduct levels. Results of *in vitro* studies using hepatic microsomal fractions isolated from mice, rats, and humans show that conversion of EB to DEB in mice is 3.3-fold greater than in rats and 2.4–61-fold greater than in humans. Studies in isolated perfused livers show differences in metabolism of 1,3-butadiene in mice and rats. In livers perfused with 1,3-butadiene, three epoxide metabolites (EB, DEB, and EBD) were identified in perfusion effluent in mice, whereas only one epoxide metabolite (EB) was identified in rats. Effluent concentrations of EB in mice were 8.5-fold greater than in rats. For perfusion studies with EB (which is oxidized to form DEB), DEB formation was greater in mice than in rats. Metabolite levels in tissues following inhalation exposure of mice and rats to 1,3-butadiene also provide evidence of species differences. Compared to rats, EB and DEB levels in blood and tissues of mice were from approximately 2–15- and >100-fold higher,

respectively, with levels exhibiting dose- and time-dependence (see discussion below on nonlinear kinetics) (Filser et al. 2007). Based on evaluation of hemoglobin adduct biomarkers (adducts formed by interaction of 1,3-butadiene metabolites with hemoglobin), mice appear to have higher DEB levels than rats and much higher levels than humans (Swenberg et al. 2011). However, quantitative measurements of differences in hemoglobin adduct profiles show considerable variability, possibly due to differences in exposure conditions. Comparison of urinary excretion profiles of 1,3-butadiene metabolites in mice, rats, and humans also shows that species differences exist in the detoxification pathways. Findings suggest that humans and rats are more "efficient" at detoxification of reactive metabolites than mice. Taken together, results of *in vitro* and *in vivo* studies showing that mice have higher levels of reactive metabolites, particularly the highly reactive DEB, than other species suggest that mice may be uniquely sensitive to toxic effects of 1,3-butadiene and, therefore, may not be an appropriate animal model for use in human health risk assessment of 1,3-butadiene.

Nonlinear Toxicokinetics (Metabolism). Metabolism of 1,3-butadiene exhibits nonlinear kinetics (Kirman et al. 2010a), with both dose- and duration-dependent effects (see discussion in Section 3.4.3 Toxicokinetics/Metabolism). Because the formation of reactive metabolites are critical in the development of 1,3-butadiene toxicity, nonlinearity in metabolic processes has the potential to affect dose-response extrapolation from animals to humans. Several processes have been proposed as sources of nonlinear kinetics; these include inhibition, induction and saturation of various metabolizing enzymes and depletion of glutathione (as reviewed by Kirman et al. 2010a).

3.5.2 Mechanisms of Toxicity

Genotoxicity. The genotoxicity of 1,3-butadiene and its electrophilic metabolites have been extensively studied. A comprehensive review of the genotoxicity of 1,3-butadiene metabolites, focusing primarily on EB, DEB, and EDB, was recently published (Albertini et al. 2010). The weight of evidence strongly suggests that 1,3-butadiene metabolites, rather than 1,3-butadiene itself, are responsible for genotoxic effects, due to their highly reactive nature. Of these metabolites, the order of potency for mutagenicity is DEB >> EB > EDB. Results of *in vitro* studies in bacterial and mammalian cells (including human cells) show that the electrophilic metabolites of 1,3-butadiene form DNA adducts, induce DNA strand breaks, increase unscheduled DNA synthesis and DNA excision repair, induce sister-chromatid exchange, induce micronucleus formation, and produce mutations, chromosome aberrations (including breaks), and aneuploidy (as reviewed by Albertini et al. 2010). Results of *in vivo* studies in animals show that 1,3-butadiene metabolites form adducts with DNA (Koturbash et al. 2011a, 2011b). Other studies evaluating genotoxicity following inhalation exposure to 1,3-butadiene provide indirect evidence of

genotoxicity of 1,3-butadiene metabolites (Cunninham et al. 1986; Jauhar et al. 1988; Lovreglio et al. 2006; Sharief et al. 1986; Sram e tal. 1998; Tice et al. 1987).

Carcinogenicity. As discussed in Section 3.2.1.7, chronic exposure to 1,3-butadiene is associated with an increased risk of mortality due to leukemia in styrene-butadiene workers and the development of multisite cancers in laboratory rodents. The mode of action for carcinogenicity of 1,3-butadiene was recently assessed by Kirman et al. (2010b). As discussed above (Mechanism of Toxicity, Genotoxicity), the genotoxicity of electrophilic metabolites of 1,3-butadiene (DEB, EB, and EBD) have been extensively studied. Results show that DEB, EB, and EBD react with DNA and are mutagenic. Based on the weight of evidence for genotoxicity, it is likely that the carcinogenic mode of action of 1,3-butadiene is mutagenic activity of the electrophilic 1,3-butadiene metabolites.

Ovarian atrophy. Intraperitoneal studies in mice suggest that 1,3-butadiene metabolites are the causative agent for the ovarian effects observed in intermediate- and chronic-duration inhalation studies (NTP 1993). Dose-related decreases in ovarian and uterine weights, number of small (primordial) ovarian follicles, and number of growing (primary to pre-antral) ovarian follicles were observed following a 30-day intraperitoneal exposure to EB or DEB (Doerr et al. 1996). The ED_{50} (i.e., the effective dose that reduces the number of follicles to 50% of controls) values for small and growing follicles were 0.29 and 0.40 mmol/kg, respectively, for EB and 0.10 and 0.14 mmol/kg, respectively, for DEB. Similarly, decreases in ovarian or uterine weight and the number of ovarian follicles were also observed in rats similarly exposed to DEB (Doerr et al. 1996). However, no alterations were observed in rats administered EB at doses as high as 1.43 mmol/kg. These data strongly suggest that DEB is the causative agent of the ovarian atrophy.

Although similar effects were observed in rats and mice administered DEB, mice appear to be more sensitive to its toxicity than rats. Administration of 0.14 mmol/kg resulted in 83 and 52% depletion of small and growing follicles, respectively, in mice and only 31 and 40% reductions in rats. When the dose-response plot for ovarian weight is based on area under the blood EB or DEB concentration-time curve (estimated using PBPK modeling), the curves are similar for both species (Sweeney et al. 2001). Sweeney et al. (2001) also used PBPK modeling to estimate the blood area under the DEB concentration-response curve using the data from the NTP (1993) study, which found ovarian effects in mice exposed to ≥6.25 ppm, and from the Owen study (Owen and Glaister 1990; Owen et al. 1987), which did not find ovarian effects in rats exposed to ≤8,000 ppm. In mice, the blood area under the DEB curve was consistent with the results of the Doerr et al. 1996) intraperitoneal study. The blood DEB area under the

curve for rats exposed to 8,000 ppm 6 hours/day, 5 days/week for 105 weeks would be 189 µM-hour; this value lies between the predicted NOAEL and LOAEL for ovarian effects in rats administered DEB via intraperitoneal injection.

3.5.3 Animal-to-Human Extrapolations

Comparison of rat and mouse data identify large differences in sensitivity to 1,3-butadiene, which are due to metabolic differences between species. Humans, rats, and mice metabolize 1,3-butadiene using the same enzymatic pathways resulting in the production of the same reactive metabolites, in particular, EB, DEB, and EBD. However, quantitative differences in the rate of formation and detoxification of reactive metabolites have been found that result in higher tissue levels of reactive metabolites in rodents, particularly mice, than in humans (Bond et al. 1993; Csanády et al. 1992; Dahl et al. 1991; Filser et al. 2001, 2007, 2010; Henderson et al. 1996, 2001; Himmelstein et al. 1997; Kirman et al. 2010a; Krause and Elfarra 1997; Schmidt and Loeser 1985; Thornton-Manning et al. 1995b). *In vitro* and perfusion data show that mice are more efficient than rats at oxidizing 1,3-butadiene to form EB, and the conversion of EB to DEB in mice is 3.3-fold greater than in rats and 2.4–61-fold greater than in humans (Kirman et al. 2010a). In addition, mice have a higher ratio of 1,3-butadiene activation to detoxification than rats or humans; the ratio of activation to detoxification was 74:1 in mouse, 6:1 in rat, and 6:1 in human liver tissues (Bond et al. 1993).

Following inhalation exposure to 1,3-butadiene, blood and tissue levels of EB and DEB were 2–15- and >100-fold higher, respectively, in mice as compared to rats (Filser et al. 2007). At equivalent inhalation concentrations, the total amount of 1,3-butadiene metabolites were 5–50 times lower in cynomolgus monkeys than in mice and 4–14 times lower in monkeys compared to rats (Dahl et al. 1991). Swenberg et al. (2011) estimated DEB blood levels measured 1,3-butadiene-derived hemoglobin adducts levels in rats, mice, and humans exposed to approximately 1 ppm 1,3-butadiene. The estimated DEB doses were 0.02, 0.42, and 24 nM-hour/ppm-hour in humans, rats, and mice, respectively. Thus, the extrapolation of rodent data to humans would require the use of an internal dose metric to account for these species differences in the metabolism of 1,3-butadiene. Although PBPK models have been developed in rodents (Johanson and Filser 1993; Kohn and Melnick 1993, 1996, 2000) and a preliminary model has been developed in humans (Brochot et al. 2007), the models are limited in their ability to predict internal doses for key metabolites (Kirman and Grant 2012). An alternative approach to using PBPK models would be to use a biomarker of exposure to the reactive metabolites. Several biomarkers of exposure have been identified for reactive 1,3-butadiene metabolites including MHB-Val hemoglobin adducts, *N*-(2,3,4-tri-

hydroxybutyl)valine (THB-Val) hemoglobin adducts, and *pyr*-Val hemoglobin adducts, which have been shown to be good surrogate biomarkers for EB, EBD, and DEB, respectively (Georgieva et al. 2010; Slikker et al. 2004).

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 vivo* or *in vitro* studies were located regarding endocrine disruption in humans and/or animals after exposure to 1,3-butadiene.

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 human data are available to determine whether children are more sensitive than adults to 1,3-butadiene toxicity. Mechanistic data in animals suggest that the ratio of 1,3-butadiene oxidation:epoxide hydrolysis may be a significant determinant of 1,3-butadiene sensitivity (see Sections 3.5.1 and 3.5.2). It is not known if the ratio of 1,3-butadiene oxidation:epoxide hydrolysis is different in children than adults. Unborn children may be more sensitive to 1,3-butadiene toxicity than adults, as changes in fetal body weight and developmental effects have been identified at the lowest LOAELs in mice exposed to acute- and intermediate-duration inhalation exposures (Anderson et al. 1996; DOE/NTP 1987b). Several studies have associated the development of childhood leukemia to close proximity of birthplace to industrial point sources of 1,3-butadiene (and other high-volume industrial chemicals, including benzene) (Knox et al. 2005, 2006; Reynolds et al. 2003; Whitworth et al. 2008). Although these study authors suggest that *in utero* exposure to 1,3-butadiene may have significantly contributed to cancer risks in these populations, there are no estimates of actual prenatal or postnatal exposures of mothers or children, respectively.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 1,3-butadiene 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 1,3-butadiene 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 1,3-Butadiene

Two urinary metabolites of 1,3-butadiene have been identified in tollbooth workers. Sapkota et al. (2006) measured 258–378 ng/mL 1,2-dihydroxy-4-(N-acetylcysteinyl)-butane and 6–9.7 ng/mL of the isomeric mixture of 1-hydroxy-2-(N-acetylcysteinyl)-3-butene and 1-(N-acetylcysteinyl)-2-hydroxy-3-butene in

workers' urine following average ambient 1,3-butadiene exposures of 0.4–1 ppb. These biomarkers are quite specific to 1,3-butadiene exposure, but the measured levels were not significantly associated with exposure, although this may have been due to the small sample size used or the low (0.0005 ppm) exposure levels studied. N-acetyl-S-((1-hydroxymethyl)-2-propenyl)cysteine and N-acetyl-S-((2-hydroxymethyl)-3-propenyl)cysteine, the isomeric mixture known as MHBMA (or M2), are the GST conjugation products of EB found in human urine following 1,3-butadiene exposure. Another urinary metabolite, N-acetyl-S-(3,4-dihydroxybutyl)cysteine, or DHBMA (M1), is formed by EBdiol conjugation with glutathione via GST (Boogaard et al. 2001a; McDonald et al. 2004). These urinary metabolites have been used as biomarkers of 1,3-butadiene exposures in several human studies (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Boogaard et al. 2001a; Fustinoni et al. 2004).

Protein adducts have been widely used to monitor the formation of alkylating metabolites and can be used as dose metrics to compare species differences in metabolism. Hemoglobin adducts accumulate over the lifespan of the erythrocyte and they represent cumulative exposure since they are not removed by enzymatic repair systems (Georgieva et al. 2010). Three N-terminal valine hemoglobin adducts have been identified for 1,3-butadiene: MHB-Val, THB-Val, and *pyr*-Val. MHB-Val, THB-Val, and *pyr*-Val are formed when EB, DEB, and EB-diol, respectively, react with hemoglobin (Georgieva et al. 2010; Slikker et al. 2004). A linear accumulation of MHB-Val and THB-Val was observed in B6C3F1 mice and Sprague-Dawley rats after intraperitoneal (Sun et al. 1989b) and inhalation (HEI 2000) exposures. Species differences have been detected in the amount of adducts formed at a given 1,3-butadiene concentration. Higher levels of MHB-Val and *pyr*-Val adducts were found in mice compared to rats (Albrecht et al. 1993; Boysen et al. 2004). Concentration-response studies have shown that the formation of *pyr*-Val hemoglobin adducts is saturable. In rats, the formation of *pyr*-Val adducts plateaus at inhalation exposures of ≥200 ppm (Georgieva et al. 2010). In mice, the formation of *pyr*-Val adducts did not plateau; however, the rate of formation decreased below 1.5 ppm (Georgieva et al. 2010). MHB-Val and THB-Val hemoglobin adduct levels were well correlated with 1,3-butadiene exposure in 1,3-butadiene monomer workers (Albertini et al. 2001, 2007; Begemann et al. 2001a, 2001b; Osterman-Golkar et al. 1996) and Chinese polymer workers (Hayes et al. 2000; HEI 2000). *Pyr*-Val was not detected in blood of male and female Czech workers exposed to 0.2–0.4 ppm (Albertini et al. 2007). *Pyr*-Val adducts were detected in the blood of workers not occupationally exposed to 1,3-butadiene, in monomer workers and polymerization workers (Boysen et al. 2012); the levels in the polymerization workers (mean 1,3-butadiene exposure level of 0.81 ppm) were significantly higher than in the control and monomer workers (mean 1,3-butadene exposure levels of 0.01 and 0. 29 ppm, respectively). When the three groups of workers were combined, a significant association between *pyr*-Val adduct levels and

individual 1,3-butadiene exposure levels was found. Using levels of these hemoglobin adducts, Swenberg et al. (2011) estimated metabolite levels in rats, mice, and humans, which allowed for a species comparison of reactive metabolite levels at a given 1,3-butadiene concentration.

Zhao et al. (2000) found a significant linear relationship of DNA adduction and 1,3-butadiene exposure between 1,3-butadiene workers and controls. The levels of N1-(2,3,4-trihydroxybutyl)adenine adduct in lymphocytes of 1,3-butadiene workers (mean exposure: 0.3 ppm; range: <0.005–7.7 ppm) were 5-fold higher than controls (mean exposure: 0.01 ppm; range: <0.001–0.07 ppm). 1,3-Butadiene-specific urinary metabolites and hemoglobin and DNA adducts have also been observed in animals. Excretion of 1,3-butadiene metabolites was reported to be high in the urine of exposed monkeys (Dahl et al. 1990). DNA adducts were detected in the livers of mice and rats exposed to radiolabeled 1,3-butadiene (Kreiling et al. 1986b). In mice exposed to 1,3-butadiene by nose-only inhalation, the N7-guanine adduct (*N*7-(1-(hydroxymethyl)-2,3-dihydroxypropyl)guanine) from interaction with 3,4-epoxy-1,2-diol, was the major DNA adduct measured (Boogaard et al. 2001b).

1,3-Butadiene has been measured in expired air of forestry workers living in mountain villages (Perbellini et al. 2003); however, the levels measured (median of 1.2 ng/L) were not correlated with any exposure to 1,3-butadiene.

3.8.2 Biomarkers Used to Characterize Effects Caused by 1,3-Butadiene

Dermal, ocular, and/or upper respiratory irritation can occur following 1,3-butadiene exposure (NIOSH 2005) and may alert the exposed individual. However, the effects are not specific for 1,3-butadiene exposure and may be caused by several other chemicals.

Given the genotoxic (Section 3.3) and carcinogenic (Section 3.2.1.7) activity of 1,3-butadiene, a useful biomarker of effect would correlate a quantifiable measure of genetic mutation with 1,3-butadiene exposure. 1,4-Bis-(guan-7-yl)-2,3-butanediol (bis-N7G-BD) and 1-(guan-7-yl)-4-(aden-1-yl)-2,3-butanediol (N7G-N1A-BD) are DEB-specific DNA-DNA cross-links identified in rats and mice inhaling up to 625 ppm 1,3-butadiene (Goggin et al. 2009, 2011). Mice exhibited 4–10-fold higher levels than rats of these cross-links. Further, higher levels of bis-N7G-BD were measured in females, compared to males. The sensitivity of female mice to these biomarkers and to 1,3-butadiene-induced carcinogenicity suggests that: (1) DEB is the putative carcinogenic metabolite of 1,3-butadiene, and

(2) bis-N7G-BD and N7G-N1A-BD levels may quantitatively inform on genotoxicity leading to tumor development.

Another biomarker of genetic change is the mutation frequency of the *hprt* gene locus in human peripheral lymphocytes, which has been used in multiple studies of 1,3-butadiene SBR, monomer, and polymer workers in the United States, China, and Czech Republic. These studies are discussed in detail in Section 3.3. Several of these studies demonstrated good correlation between the increase in *hprt* mutation frequency and 1,3-butadiene exposure (Abdel-Rahman et al. 2001, 2003, 2005; Ammenheuser et al. 2001; Ma et al. 2000; Ward et al. 1994, 1996, 2001), as well as correlation with urinary and hematological biomarkers of exposure. Others did not find a significant correlation between exposure and mutation frequency (Albertini et al. 2001, 2007; Hayes et al. 1996, 2000; HEI 2003; Liu et al. 2008; Tates et al. 1996). The reasons for the differences in sensitivity of the *hprt* mutation as a biomarker may include the very low exposures that were studied and differences in assay procedures (as discussed in Section 3.3). It is unclear at this time which *hprt* mutation frequency assay is most adequate for risk assessment.

Two biomarkers for carcinogenic effect have been consistently found in multiple tumors sites in rodent chronic bioassays. A number of malignant gliomas and neuroblastomas in mice chronically inhaling 1,3-butadiene exhibited mutations of the p53 gene and H- and K-*ras* oncogenes (Kim et al. 2005), which have also been observed in forestomach tumors (Sills et al. 2001), hemagiosarcomas (Hong et al. 2000), and lymphomas (Zhuang et al. 1997) of chronically exposed mice.

3.9 INTERACTIONS WITH OTHER CHEMICALS

In addition to 1,3-butadiene, workers in the rubber industry are exposed to other chemicals, including styrene and its mutagenic metabolite, styrene oxide (Loprieno et al. 1978; Norppa et al. 1980; Pohlova et al. 1985; Watabe et al. 1978), as well as dithiocarbamates (Irons and Pyatt 1998). It is unclear whether these other chemicals or their active metabolites have a synergistic harmful effect in humans, but a multivariate analysis of an SBR worker cohort (HEI 2006) did not detect interactive effects of co-exposure to 1,3-butadiene, styrene, and dimethyldithiocarbamate (DMDTC). Animal studies have found that DMDTC can qualitatively and quantitatively effect the metabolism of 1,3-butadiene. *In vitro* studies show that DMDTC treatment decreases the metabolism of 1,3-butadiene to EB and the metabolism of epoxybutene to DEB in rats and mice (Green et al. 2001). Styrene has been shown to inhibit the metabolism of 1,3-butadiene in rats simultaneously exposed to both compounds (Laib et al. 1992;

Leavens et al. 1996). The inhibition was only observed at 1,000 ppm 1,3-butadiene concentration and not at 100 ppm (Leavens et al. 1996). However, blood levels of EB increased and the blood levels of DEB were unaffected by styrene co-exposure, as compared to exposure to 1,3-butadiene only (Leavens et al. 1996). Thus, co-exposure to styrene may not affect the toxicity of 1,3-butadiene. Inhalation exposure to 1,3-butadiene and styrene did not affect the genotoxic potential of 1,3-butadiene in mice (Leavens et al. 1997).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to 1,3-butadiene than will most persons exposed to the same level of 1,3-butadiene 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 1,3-butadiene, or compromised function of organs affected by 1,3-butadiene. Populations who are at greater risk due to their unusually high exposure to 1,3-butadiene are discussed in Section 6.7, Populations with Potentially High Exposures.

The human and animal data do not identify a gender-specific susceptibility to 1,3-butadiene. Human *in vivo* data do not identify specific populations that may be sensitive to the effects of 1,3-butadiene. Polymorphisms in metabolic enzymes may affect the toxicokinetics of 1,3-butadiene and render some individuals more sensitive to toxicity, based on increased sensitivity to genetic changes seen in these groups. Lymphocytes from GSTT1-null 1,3-butadiene workers in Texas had higher induction of sister chromatid exchange following *in vitro* DEB exposure (Kelsey et al. 1995), while GSTT1-null Czech workers exhibited higher rates of chromosomal aberrations (Sorsa et al. 1996). However, no such effects were observed in other Czech (Sram et al. 1998) or Chinese (Hayes et al. 2000) workers that were GSTT1 or GSTM1 deficient. Increased *hprt* mutation frequencies have been reported in U.S. 1,3-butadiene workers with various polymorphisms in EH (Abdel-Rahman et al. 2001, 2003, 2005). In *in vitro* studies with human lymphocytes, EB induced higher levels of sister chromatid exchanges in GSTM1-null samples, as compared to GSTM1 samples (Uusküla et al. 1995), suggesting that the clastogenic change may the result of less EB being detoxified via GSTM1-mediated glutathione conjugation. Similarly, EB induced higher levels of sister chromatid exchanges in lymphocytes from GSTT1-null individuals, as compared to GSTT1-positive individual (Bernardini et al. 1998).

The relationship between polymorphisms and the urinary excretion of M1 and M2 metabolites has been exposed in studies of Czech 1,3-butadiene workers. GST polymorphisms resulted in shifts in the mean

ratio of M2/(M1 + M2) (indicative of the activity of EB glutathione conjunction pathway), which was significantly lower in GSTM1-null workers, as compared to GSTM1-positive workers (HEI 2003); a lower ratio was also found in the GSTT1-null workers, but it was not significantly different from the GSTT1-positive workers. No significant alterations in M1 or M2 concentrations or the ratio were found for workers genotyped for CYP2E1, EH, and ADH polymorphisms. Regression analysis revealed that the slopes of the 1,3-butadiene concentration-M2 levels and concentration-M2/(M1+M2) was significantly different in CYO2E1 D/D intron 6 polymorphism compared to workers who were heterozygous for C/D (HEI 2003). In a subsequent study by this group, a significant difference in M2/(M1+M2) ratio was observed in the GSTT1-null workers (Albertini et al. 2007). When different EH genotypes were combined into high, intermediate, and low activity phenotypes, significantly higher M2 levels were observed in the low activity genotype group (Albertini et al. 2007).

Animals studies indicate that the predominant factor in species sensitivity is related to the toxicokinetics of 1,3-butadiene, specifically, the ratio of P450-mediated oxidation:epoxide hydrolase activity (see Section 3.4.3). Human populations that have a higher ratio of 1,3-butadiene oxidation:hydrolysis metabolism may also be more sensitive, although such populations have not been identified. In terms of absorption capacity, Asian volunteers had about 20% greater fractional absorption of inhaled 1,3-butadiene than did Caucasians, African-Americans, or Hispanics (Lin et al. 2001). However, it is not known if this results in higher internal doses of the putative epoxide toxicants.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 1,3-butadiene. 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 1,3-butadiene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

3.11.1 Reducing Peak Absorption Following Exposure

No specific antidotes for 1,3-butadiene are available; however, recommendations have been made for general treatment of intoxicated persons (Bronstein and Currance 1988; Stutz and Janusz 1988). First, the exposed individual should be removed from the contaminated area and contaminated clothing should be taken away (Currance et al. 2007; Leikin and Paloucek 2002). It has been suggested that exposed skin should be washed with soapy water and contaminated eyes should be flushed with water. Inhalation

exposure to high 1,3-butadiene concentrations may result in narcosis leading to respiratory paralysis and death. Data have shown that neurological effects, including anesthesia have been observed in animals exposed to 250,000 ppm (Carpenter et al. 1944). Therefore, administration of oxygen has been used and ventilation has been assisted as needed in cases of 1,3-butadiene poisoning.

3.11.2 Reducing Body Burden

No information is available regarding displacing or removing absorbed 1,3-butadiene prior to metabolism to reactive metabolites.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Toxicity studies found mice to be extremely sensitive (DOE/NTP 1987b; Melnick et al. 1989, 1990b; NTP 1993). Studies on the metabolism of 1,3-butadiene demonstrated that the chemical is converted to its epoxy derivatives by P450 isoforms in lung, liver, kidney (see Section 3.4.3), and possibly other tissues. The epoxides may be responsible for most toxic and carcinogenic effects caused by 1,3-butadiene exposure. The epoxides are detoxified by hydrolysis or conjugation with glutathione (Kreiling et al. 1988). A higher rate of epoxide formation and a greater depletion of hepatic nonprotein sulfhydryl content in mice is probably responsible for their higher susceptibility to 1,3-butadiene toxicity. Since the macromolecular covalent binding is enhanced only after a substantial decrease of glutathione levels (Kreiling et al. 1988), sufficient availability of glutathione should mitigate the effects of 1,3-butadiene exposure.

Interference with the oxidative metabolism of 1,3-butadiene may also be effective in allowing pulmonary clearance of the parent compound to occur before reactive epoxides are formed. In mice, a 30–56% reduction in induction of erythrocyte micronuclei was observed in mice pretreated with various P450 inhibitors (Jackson et al. 2000b). However, the side effects of systemically inhibiting P450s in humans are unknown.

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 1,3-butadiene 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 1,3-butadiene.

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 1,3-Butadiene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 1,3-butadiene are summarized in Figure 3-4. The purpose of this figure is to illustrate the existing information concerning the health effects of 1,3-butadiene. 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.

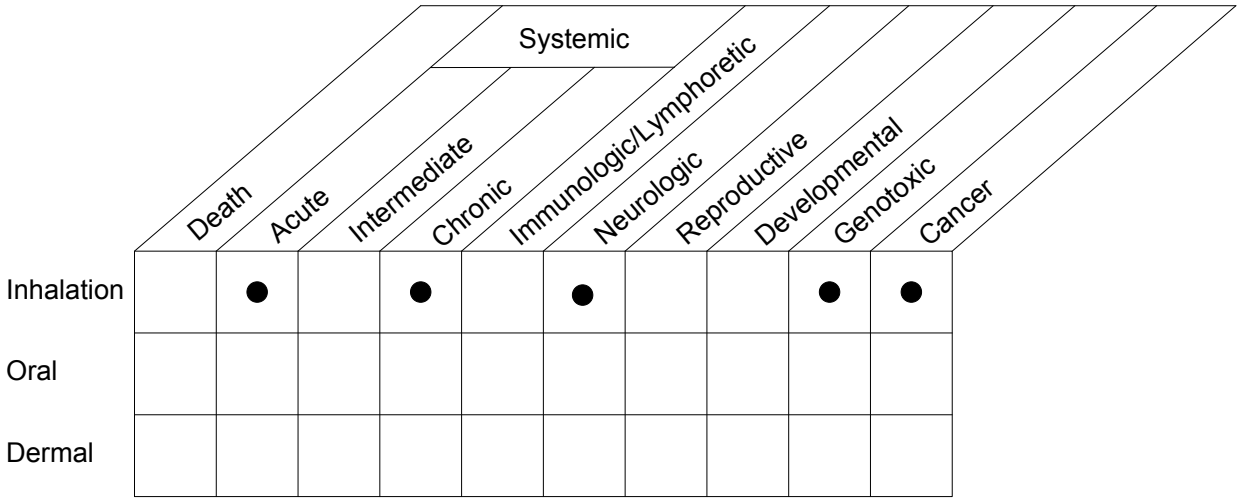
As seen from Figure 3-4, information regarding acute systemic effects (respiratory tract irritation and narcotic effect), chronic systemic effects (respiratory irritation), genotoxicity, and cancer exists for inhalation exposure in humans. No information was located regarding oral or dermal exposure of humans to 1,3-butadiene.

Inhalation studies in animals provide data on death, systemic effects, immunologic effects, neurologic effects, reproductive and developmental effects, genotoxicity, and carcinogenicity. No information was located regarding effects in animals after oral or dermal exposure to 1,3-butadiene.

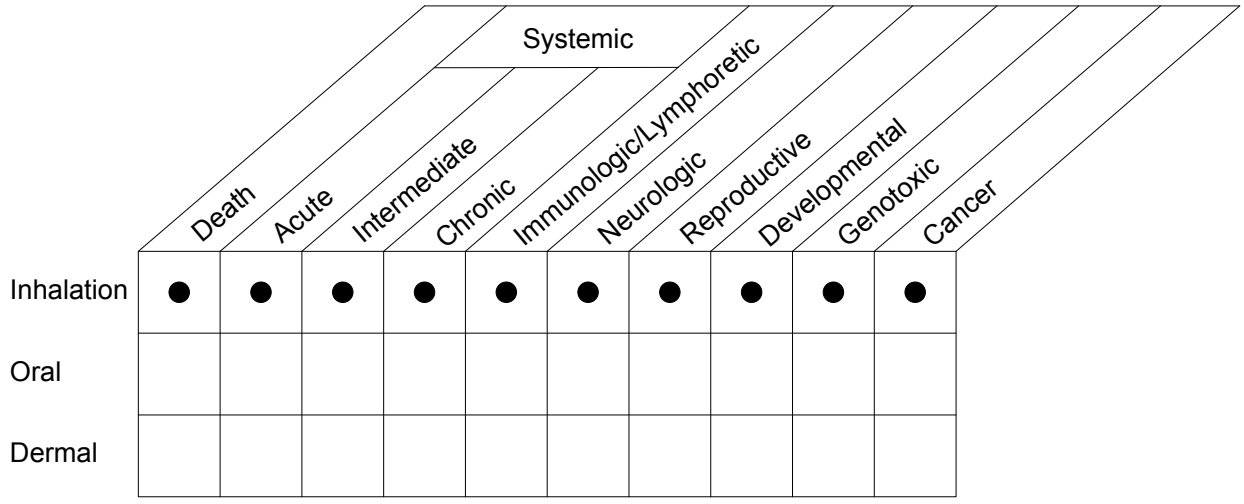
3.12.2 Identification of Data Needs

No studies were located regarding effects following oral or dermal exposure, and no pharmacokinetic studies by the oral or dermal routes were located; therefore, it is not possible to predict if effects

Figure 3-4. Existing Information on Health Effects of 1,3-Butadiene



Human



Animal

● Existing Studies

following oral or dermal exposure would be similar to those observed after inhalation exposure. Because 1,3-butadiene exists primarily as a gas and has been detected in soil off-gases at hazardous waste sites, inhalation exposure appears to be the greatest concern. However, it is not known if 1,3-butadiene is present in groundwater or soil at these hazardous waste sites because it is difficult to analyze these media for the compound. 1,3-Butadiene has been detected in industrial waste water and drinking water, and is poorly soluble in water (735 ppm). Therefore, oral and dermal routes of exposure cannot be ruled out. Information concerning 1,3-butadiene toxicity by these routes of exposure would be useful.

Acute-Duration Exposure. Acute inhalation exposure to very high concentrations (>25,000 ppm) of 1,3-butadiene may lead to narcosis and death by respiratory paralysis in animals (Carpenter et al. 1944). Data in humans are limited to a study of two men exposed to various concentrations of 1,3-butadiene for 6–8 hours that examined clinical signs and psychomotor function (Carpenter et al. 1944). No studies were located that correlated the level of exposure with the first signs of toxicity in humans or animals. Developmental effects were seen in mice after exposure to concentrations as low as 40 ppm (DOE/NTP 1987b; Irvine 1981). Although the available animal data identify critical targets of toxicity following acute-duration inhalation exposure, the database lacks adequate toxicokinetic and PBPK modeling data, which could be used to account for species differences in the metabolism of 1,3-butadiene; thus, an acute-duration MRL was not derived. Additionally, there are limited data on the potential effects of the parent compound, such as neurotoxicity, which may be a more sensitive target of toxicity in humans. Because people living at or near these hazardous waste sites may be exposed for brief periods of time, more dose-response data for acute exposures by oral and inhalation routes is considered to be important.

Intermediate-Duration Exposure. No information is available regarding effects of 1,3-butadiene during intermediate-duration exposure in humans. No studies were located regarding effects in humans or animals following oral or dermal exposure to 1,3-butadiene, and pharmacokinetic data for these routes of exposure are insufficient to predict whether the disposition or toxicity of 1,3-butadiene following oral or dermal exposure would be similar to that following inhalation exposure. Therefore, information regarding the toxicity of 1,3-butadiene by the oral route of exposure would be useful. Several studies on intermediate-duration inhalation exposure to 1,3-butadiene have been conducted in animals (Anderson et al. 1996, 1998; Crouch et al. 1979; Irons et al. 1986a; NTP 1984, 1993; Thurmond et al. 1986). Atrophy of reproductive organs, anemia, precancerous hyperplasia in multiple organs, and cancer occurred in mice exposed to ≥200 ppm (Irons et al. 1986a; NTP 1984, 1993). The observed hematological changes (macrocytic megaloblastic anemia) were similar to those found in human preleukemic syndrome (Biemer 1983), suggesting that 1,3-butadiene exposure might interfere with normal bone marrow cell

development. Further investigation of this topic could be valuable since epidemiological studies in humans indicate that hematopoietic tissue may be a possible target for 1,3-butadiene toxicity (Checkoway and Williams 1982). Several studies have identified potential targets of toxicity following intermediate-duration exposure; however, the available toxicokinetic and PBPK modeling data do not allow for adequate adjustment for species differences in the metabolism of 1,3-butadiene. Thus, intermediate-duration inhalation MRLs were not derived.

Chronic-Duration Exposure and Cancer. Possible risk for hematological disorders was reported in humans after chronic inhalation exposure to 1,3-butadiene in occupational settings, but exposure levels are lacking, and exposure to other chemicals occurs in these settings (Checkoway and Williams 1982). However, other studies (Cowles et al. 1994; Tsai et al. 2005) that monitored 1,3-butadiene levels have not found hematological alterations. Well-conducted inhalation studies identified respiratory effects, liver necrosis, gonadal atrophy, multi-site cancer, and increased mortality in mice at exposures as low as 6.25 ppm (Melnick et al. 1989, 1990a; NTP 1984, 1993), while renal pathology, cancer, and increased mortality were observed in rats exposed to ≥1,000 ppm (Owen et al. 1987). Because a serious LOAEL of severe ovarian atrophy (with complete destruction of oocytes, follicles, and corpora lutea) was found at 6.25 ppm, with no associated NOAEL, no chronic MRL has been derived. Chronic-duration studies are needed that identify a NOAEL in mice for gonadal atrophy. Oral studies are lacking, and toxicokinetic data are insufficient to predict toxicity across routes of exposure. Therefore, information concerning the possible toxicity of 1,3-butadiene by this route would be useful to identify the target organs and the thresholds for toxic effects.

Epidemiological studies in humans indicate a possible increase in risk of leukemia from occupational exposure to 1,3-butadiene (Cheng et al. 2007; Delzell et al. 1996; Divine 1990; Divine and Hartman 2001; Divine et al. 1993; Downs et al. 1987; Macaluso et al. 1996; Matanoski and Schwartz 1987; Matanoski et al. 1982, 1989a, 1989b, 1990; McMichael et al. 1974, 1975, 1976; Meinhardt et al. 1982; Ward et al. 1995). This is supported by the information about mutagenic activity of 1,3-butadiene metabolites (de Meester 1988) and by well-conducted chronic inhalation studies that provide information on carcinogenic effects of 1,3-butadiene in mice and rats (Melnick et al. 1989; NTP 1984, 1993; Owen et al. 1987). IARC (2009) and EPA (EPA 2002; IRIS 2012) concluded that there is sufficient evidence for the carcinogenicity of 1,3-butadiene in animals. IARC has classified 1,3-butadiene in group 1, carcinogenic to humans. EPA has classified 1,3-butadiene as a human carcinogen. The Department of Health and Human Services (NTP 2011) also identified 1,3-butadiene as a “known human carcinogen”. Further epidemiological investigations that examined additional potential targets of 1,3-butadiene toxicity

would be useful. Also, data are needed on 1,3-butadiene exposure to urban populations living in close proximity to major roadways and intersections, as well as on long-term follow-up of health effects, particularly the detection of diminishment in reproductive capability and prevalence of lympho-hematopoietic cancers.

No chronic oral or dermal carcinogenicity studies in animals were located, and pharmacokinetic data are insufficient to predict a carcinogenic potential of 1,3-butadiene by these routes.

Genotoxicity. Studies of chromosomal aberrations, sister chromatid exchanges, and *hprt* mutation frequencies among petrochemical and 1,3-butadiene monomer workers exposed to low levels (≤2 ppm) provide conflicting results (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Hayes et al. 1996, 2000; HEI 2003; Lovreglio et al. 2006; Sram et al. 1998; Tates et al. 1996; Ward et al. 1996, 2001; Zhou et al. 1986). 1,3-Butadiene has caused increases in micronuclei induction, chromosomal aberration frequency, and mutations of proto-oncogenes in *in vivo* studies of rats and mice following inhalation exposure (Autio et al. 1994; Cochrane and Skopek 1993; Cunningham et al. 1986; Irons et al. 1987b; Jauhar et al. 1988; Meng et al. 1999, 2000, 2004, 2007; Recio et al. 1992; Sills et al. 2001; Tice et al. 1987; Vodicka et al. 2006). Information on the genotoxic effects of 1,3-butadiene was also obtained from *in vitro* studies in prokaryotic (Arce et al. 1989; de Meester 1988, de Meester et al. 1980; Victorin and Stahlberg 1988) and eukaryotic (Cochrane and Skopek 1993; Sasiadek et al. 1991) organisms. These data sufficiently characterize the mutagenic potential of 1,3-butadiene metabolites.

Reproductive Toxicity. The atrophy of gonads in mice after chronic inhalation exposures as low as 6.25 ppm 1,3-butadiene was reported (Melnick et al. 1989; NTP 1984, 1993). The fertility of rats, guinea pigs, or rabbits was reported to be unaltered by acute- and intermediate-duration inhalation exposure to 1,3-butadiene (Anderson et al. 1996, 1998; Carpenter et al. 1944). Sperm head abnormalities were found in male mice exposed to 1,3-butadiene by inhalation (DOE/NTP 1988b). Further information regarding the reproductive effects of 1,3-butadiene in animals such as multigeneration studies would be useful to estimate the possible risk for reproductive effects in humans. An epidemiological study among exposed populations concentrating on reproductive effects would be useful.

No studies were located regarding reproductive toxicity of 1,3-butadiene by the oral or dermal routes in humans or animals, and pharmacokinetic data were insufficient to suggest the potential for 1,3-butadiene to cause reproductive effects by these routes of exposure. The potential for exposure of humans by the oral and dermal routes, however, is not known.

Developmental Toxicity. No information on developmental toxicity in humans was located. A developmental study by the inhalation route indicated growth retardation in rat fetuses and an increase in major skeletal abnormalities at a concentration of 1,000 ppm of 1,3-butadiene (Irvine 1981). Furthermore, fetotoxicity was observed in mice at acute-duration exposures of 40 ppm and intermediate-duration exposures of 12.5 ppm 1,3-butadiene (DOE/NTP 1987b). More data on developmental toxicity in other species (at least one of them nonrodent) would be useful to identify the possible developmental risk for humans. The developmental effects following other routes of exposure have not been studied, and pharmacokinetic data are insufficient to predict that responses would be similar to those by the inhalation route. Therefore, studies of oral exposures in animals to determine the possible developmental effects of 1,3-butadiene and the thresholds for these effects would be useful.

Immunotoxicity. Reduction in thymus weight and lymphoid histopathology was seen after the intermediate-duration exposure of mice to ≥625 ppm 1,3-butadiene (NTP 1993; Thurmond et al. 1986). The indications of disturbances in hemato- and lymphatopoietic stem cell regulations were observed after inhalation exposure of mice to 1,3-butadiene (Liederman et al. 1986). The high incidence of lymphoma among mice after the chronic exposure (NTP 1984, 1993) also indicates that the immune system is a target. A battery of immune function tests has not been performed in humans or animals. More data regarding humans and animals would be useful for determining potential human immunotoxicity of 1,3-butadiene. Studies regarding skin sensitization with 1,3-butadiene are lacking.

Neurotoxicity. Narcosis has been demonstrated in animals after acute inhalation exposure to very high levels of 1,3-butadiene (250,000 ppm) (Carpenter et al. 1944). No reliable information was located regarding neurotoxicity due to chronic inhalation exposure or to oral or dermal exposure for any duration. Information regarding early, subtle signs of possible neurological effects with correlation to the exposure levels is lacking. A battery of neurological and neurobehavioral tests would be useful to better define the neurological end points.

Epidemiological and Human Dosimetry Studies. Several epidemiological studies on health effects of 1,3-butadiene have been conducted (Case and Hosker 1954; Fox et al. 1974; Matanoski and Schwartz 1987; Matanoski et al. 1989a, 1989b; McMichael et al. 1974, 1975, 1976; Meinhardt et al. 1982). The limitation of these studies is that the cohorts of exposed workers were recruited from the rubber industry, in which the people were exposed to a mixture of various chemicals. Some genotoxicity studies have been conducted among 1,3-butadiene manufacturing workers and petrochemical workers

exposed to low (≤2 ppm) exposures, but have reported conflicting results (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Hayes et al. 1996, 2000; HEI 2003; Kelsey et al. 1995; Lovreglio et al. 2006; Tates et al. 1996; Ward et al. 1996, 2001; Wickliffe et al. 2009; Zhou et al. 1986). Reliable dosimetry data on the exposed populations would be useful for good epidemiological comparisons. Efforts to improve estimates of past exposures and to more accurately define current exposure levels to 1,3-butadiene would be valuable. Epidemiological studies should concentrate on the possible carcinogenic effect of 1,3-butadiene in humans and on changes in hemato- and lymphatopoietic systems as possible targets for 1,3-butadiene induced toxicity. The data obtained from workers exposed occupationally to low concentrations of 1,3-butadiene could possibly be extrapolated to populations living near hazardous waste sites.

Biomarkers of Exposure and Effect.

Exposure. The determination of 1,3-butadiene-derived urinary metabolites (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Bechtold et al. 1994; Dahl et al. 1990; Hayes et al. 1996, 2000; Sapkota et al. 2006; Ward et al. 1994, 1996), DNA adducts (Kreiling et al. 1986b; Sun et al. 1989b; Zhao et al. 2001), and hemoglobin adducts (Albertini et al. 2001, 2007; Begemann et al. 2001a, 2001b; Boogaard et al. 2001b; Hayes et al. 2000; HEI 2000; Osterman-Golkar et al. 1996) of rats, mice, and humans exposed to 1,3-butadiene has been performed. Data are needed that accurately correlate the level of biomarkers measured in the body, particularly for *pyr*-Val hemoglobin adducts with the exposure to 1,3-butadiene, as well as the variability in this correlation between sexes and ethnicity.

Effect. Conflicting data exist for the sensitivity of human lymphocyte *hprt* mutation frequencies resulting from occupational exposures (Albertini et al. 2001, 2007; Ammenheuser et al. 2001; Hayes et al. 1996, 2000; HEI 2003; Kelsey et al. 1995; Lovreglio et al. 2006; Tates et al. 1996; Ward et al. 1996, 2001; Wickliffe et al. 2009; Zhou et al. 1986). In mice and rats, levels of DEB-specific DNA-DNA cross-links correlated well with 1,3-butadiene inhalation and relative sensitivity of female mice to toxicity (Goggin et al, 2009). Two biomarkers for carcinogenic effect have been consistently found in multiple tumors sites in rodent chronic bioassays. A number of malignant gliomas and neuroblastomas in mice chronically inhaling 1,3-butadiene exhibited mutations of the p53 gene and H- and K-ras oncogenes (Kim et al. 2005), which have also been observed in forestomach tumors (Sills et al. 2001), hemagiosarcomas (Hong et al. 2000), and lymphomas (Zhuang et al. 1997) of chronically exposed mice. Data for reliable and specific biomarkers indicating onset of developmental effects in animals would be useful to determine if comparable exposure may lead to these effects in humans.

Absorption, Distribution, Metabolism, and Excretion. The timecouse of 1,3-butadiene and its primary metabolite, EB, in human blood has been investigated in volunteers inhaling 2 ppm for 20 minutes (Lin et al. 2001). *In vitro* studies have characterized some of the metabolism dynamics of 1,3-butadiene in animals (Bolt et al. 1983; Csanady et al. 1992; Duescher and Elfarra 1994; Elfarra et al. 1996, 2001; Himmelstein et al. 1994, 1995; Jackson et al. 2000a; Kreiling et al. 1987; Laib et al. 1990; Malvoisin and Roberfroid 1982; Malvoisin et al. 1979; Schmidt and Loeser 1985, 1986; Thornton-Manning et al. 1995b, 1997). Several toxicokinetic studies on 1,3-butadiene metabolism *in vivo* have been conducted in rats and mice following inhalation exposure (Bolt et al. 1983; Bond et al. 1987; Himmelstein et al. 1994; Kohn and Melnick 2001; Kreiling et al. 1986b; Shugaev 1969), but not following exposure by other routes. Thus, further studies in animals by the oral route to determine possible target organs by this route could be useful. Ethical considerations limit the testing of humans, but the development of methods to determine urinary and breath excretion of 1,3-butadiene and its metabolites by humans with known exposure to 1,3-butadiene may provide a means of monitoring humans for exposure.

Comparative Toxicokinetics. The study by Schmidt and Loeser (1985) indicated that there is a difference between the capability of mouse and rat liver postmitochondrial fractions to produce 1,2-epoxybutene-3 after incubation with 1,3-butadiene. Furthermore, monkey and human postmitochondrial liver preparations catalyzed the formation of only a small amount of the epoxide. Higher levels of the toxic epoxides were found in blood of mice following 1,3-butadiene exposure as compared to monkeys (Bolt et al. 1983; Csanady et al. 1992; Dahl et al. 1990; Duescher and Elfarra 1994; Elfarra et al. 1996, 2001; Jackson et al. 2000a; Sun et al. 1989a). Species differences in the toxicokinetics of a chemical may account for differences in toxic responses. Analysis of the blood, breath, and urine of humans exposed to 1,3-butadiene for parent compound and metabolites over time would provide a greater knowledge of the human metabolic pathways. Qualitative and quantitative comparison of human metabolites with those of animals could help identify the most appropriate species to serve as a model for predicting toxic effects and mechanisms of action in humans.

Methods for Reducing Toxic Effects.

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.

The sensitivity of children to 1,3-butadiene toxicity, if any, is unknown. In mice, acute- and intermediate-duration inhalation exposures resulted in *in utero* fetal effects at exposure levels of 21.5–40 ppm (Anderson et al. 1996; DOE/NTP 1987b). No information is available for interfering with effects of 1,3-butadiene toxicity *in utero*.

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

The following ongoing studies were identified in the Federal Research in Progress database (FEDRIP 2009).

E. Struble is being funded by EPA National Health and Environmental Effects Research Laboratory (HEERL) to use environmental irradiation chambers (smog chambers) to induce the natural photochemically stimulated transformations of environmental pollutants. The synthetic urban smog mixture is composed of 55 hydrocarbon species representative of the ambient air of an average city in the United States. In this study, A549 cells were exposed simultaneously to irradiated and non-irradiated chamber mixtures for 5 hours. Post exposure, adverse health effects were determined by measures of increased cellular stress cytokine release and cytotoxicity. Exposure to the photochemically-generated products of 1,3-butadiene, toluene, and methanol induced increases in both cytotoxicity and IL-8 gene expression compared to 1,3-butadiene, toluene, or methanol alone. The exposure design was used to investigate the toxicity of chemicals after photochemical reactions and interactions with the urban atmosphere on healthy and susceptible individuals using representative *in vitro* samples. This research informs the toxicity from exposures to multiple environmental pollutants found in urban settings.

James A. Swenberg is being funded by the National Institute of Environmental Health Sciences (NIEHS) to examine the molecular dose of previously unexplored DNA adducts in rodents exposed to 1,3-butadiene and 3-butene-1,2-diol (BD-diol). The data will be compared with mutation frequencies and mutational spectra to determine (1) if a particular adduct could be used as a mutagenic indicator, and (2) to determine the effects of exposure on gene expression. First, it will be determined if hydroxymethylvinyl ketone (HMVK) is formed in vivo during exposure to 1,3-butadiene and BD-diol in a sex-, species-, and exposure concentration-dependent manner, resulting in mutagenicity. Secondly,

promutagenic N1 adenine adducts will be observed to see if they are converted to more stable inosine adducts, which may accumulate in tissues during chronic exposures. Several specific aims will be accomplished while addressing these questions. Specific Aim 1 is to examine the formation of potentially mutagenic DNA adducts (specifically 1, N2-propanodeoxyguanosine) by HMVK *in vivo*. Specific Aim 2 is to determine the utility of the N-terminal valine adduct of HMVK (HMVK-Val) as a biomarker of HMVK formation by BD and BD-diol. Specific Aim 3 is to develop methods for detecting N1- inosine, N1- and N6-adenine adducts derived from BD metabolites in vivo. Specific Aim 4 is to determine the mutagenic responses induced by BD exposures and characterize the impact of BD-diol-derived metabolites on the spectra of mutations induced by BD exposure in the B6C3F1 mouse and F344 rat to identify which adducts studied in Aims 1 and 3 are quantitative indicators of mutagenesis. Specific Aim 5 will examine the effects of exposure to BD and BD-diol on gene expression and DNA repair pathways. Collectively, these experiments have been designed to inform on adduct formation, DNA repair, mutagenicity, and genomic alterations in rodents exposed to 1,3-butadiene and BD-diol, as well as the impact of glutathione depletion and DNA repair deficiency.

Elaine Symanski is being funded by the National Cancer Institute (NCI) to evaluate an association between lymphohematapoietic (LH) cancer incidence and air pollution in the Houston metropolitan area, with particular emphasis on three compounds: benzene, 1,3-butadiene, and styrene. Data from the Texas Cancer Registry (TCR) from 1995 to 2005 and the Texas Commission on Environmental Quality (TCEQ) from 1992 to 2003 will be analyzed to: (1) investigate the spatial and temporal distribution of LH cancer incidence in Harris and surrounding counties; (2) evaluate the association between distance from industrial sources and LH cancer incidence; (3) identify optimal methods for assessing ambient levels of benzene, 1,3-butadiene, and styrene using existing TCEQ monitoring data; and (4) evaluate the association between ambient levels of benzene, styrene, and 1,3-butadiene and LH cancer incidence using Poisson regression in single and multi-pollutant models. This will be the first study to examine this association in Harris and seven surrounding counties (Texas) and is among the first to utilize monitored levels of HAPs to assess increased risks of LH cancer associated with air pollution. This work will also correlate cancer rates with proximity to industrial facilities as well as ambient levels of benzene, styrene, and 1,3-butadiene. This study will address a gap in the literature by examining the association between HAPs and cancer incidence in Harris County, Texas and will further explore innovative methods to evaluate this association utilizing existing data sources.

Natalia Y. Tretyakova is being funded by the National Cancer Institute to evaluate the role of DNA-DNA cross-linking in the genotoxicity of diepoxybutane and 1,3-butadiene. DEB-DNA cross-links will be

structurally characterized and formation sequence preferences will be determined. The hydrolytic stability of DEB-DNA cross-links will be evaluated for their recognition by the *E. coli* UvrABC repair complex. DEB-DNA cross-links in rodent tissues will be quantified by capillary high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) methods. This research will provide valuable information on the molecular mechanisms underlying the genotoxic activity of diepoxybutane.