

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 benzene. 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

3. HEALTH EFFECTS

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 benzene are indicated in Tables 3-1 and 3-2 and Figures 3-1 and 3-2. Because cancer effects could occur at lower exposure levels, Figures 3-1 and 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), 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

Although occupational or environmental exposure to benzene or benzene-containing materials may include inhalation, oral, and dermal exposure routes, the inhalation and dermal routes are usually of primary concern in such scenarios. Data regarding occupational or environmental exposure in which inhalation is considered to have been the primary exposure route are summarized in this section (Section 3.2.1). Information regarding adverse health effects following oral or dermal exposure to benzene or benzene-containing materials is summarized in Sections 3.2.2 and 3.2.3, respectively.

3.2.1.1 Death

Case reports of fatalities due to acute benzene exposures have appeared in the literature since the early 1900s (Cronin 1924; Greenburg 1926; Hamilton 1922). Deaths occurred suddenly or within several hours after exposure (Avis and Hutton 1993; Cronin 1924; Greenburg 1926; Hamilton 1922; Winek et al. 1967). The benzene concentrations encountered by the victims were not often known. However, it has been estimated that 5–10 minutes of exposure to 20,000 ppm benzene in air is usually fatal (Flury 1928). Lethality in humans has been attributed to asphyxiation, respiratory arrest, central nervous system

3. HEALTH EFFECTS

depression, or suspected cardiac collapse (Avis and Hutton 1993; Hamilton 1922; Winek and Collom 1971; Winek et al. 1967). Cyanosis, hemolysis, and congestion or hemorrhage of organs were reported in the cases for which there were autopsy reports (Avis and Hutton 1993; Greenburg 1926; Hamilton 1922; Winek et al. 1967). No studies were located regarding noncancer-related mortality in humans following long-term inhalation exposure to benzene. Cancer-related mortality data for chronic-duration human occupational exposure to benzene are presented in Section 3.2.1.7.

In animals, acute inhalation exposure to high concentrations of benzene has caused death. An inhalation LC₅₀ value for rats was calculated as 13,700 ppm for a 4-hour exposure (Drew and Fouts 1974). Additionally, 4 of 6 rats died following a 4-hour exposure to 16,000 ppm benzene (Smyth et al. 1962). However, in a study by Green et al. (1981b), male CD-1 mice exposed by inhalation to doses of benzene up to 4,862 ppm, 6 hours/day for 5 days showed no lethality. Lower doses (up to 400 ppm) for longer periods of time (2 weeks) did not cause death in mice (Cronkite et al. 1985). Lethality in monkeys and cats exposed to unspecified concentrations has been ascribed to ventricular fibrillation due to increased release of adrenaline (Nahum and Hoff 1934). Exposure of rabbits to 45,000 ppm of benzene for approximately 30 minutes caused narcosis that was followed by the death of all exposed animals (Carpenter et al. 1944). Furthermore, early deaths of rats and mice have occurred from intermediate and chronic exposure to air concentrations of 200 or 300 ppm of benzene in cancer studies (Cronkite et al. 1989; Farris et al. 1993; Maltoni et al. 1982a, 1983). Intermediate exposures (6 hours/day, 5 days/week for 50 days) of male CD-1 mice to benzene at doses of 9.6 ppm caused no increase in mortality, although mice exposed to 302 ppm benzene under the same regimen for a total of 26 weeks showed mortality approaching 50% (Green et al. 1981b). Mortality was observed in 97% of the CBA/Ca mice exposed to 300 ppm benzene for 16 weeks, as compared to 20% mortality in sham-exposed mice (Cronkite 1986). In Sprague-Dawley rats that received 300 ppm benzene vapor for 6 hours/day, 5 days/week for 691 days, the calculated median survival time was shown to be 51 weeks as compared to 65 weeks for controls (Snyder et al. 1978a). However, Snyder et al. (1984) reported a median survival time of 546 days for male Sprague-Dawley rats exposed to 100 ppm benzene for 5 days/week, 6 hours/day for life, compared to 560 days for air-exposed controls. It is not clear whether the difference in survival was significant or due to benzene exposure since both controls and exposed rats experienced early mortality from respiratory infections. Companion studies were also conducted with AKR and C57BL mice exposed to 300 ppm benzene (Snyder et al. 1978a, 1980). The calculated median survival time for AKR and C57BL mice exposed to 300 ppm benzene was shown to be 11 and 41 weeks compared to 39 and 75 weeks, respectively, for controls. For AKR mice exposed to 100 ppm, the calculated median survival time was 39 weeks (number of deaths not shown) as compared to 41 weeks for controls (Snyder et al. 1980).

3. HEALTH EFFECTS

The LC₅₀ value and all reliable LOAEL values for each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No studies were located regarding endocrine, metabolic, or body weight effects in humans or gastrointestinal, musculoskeletal, endocrine, metabolic, or dermal effects in animals following inhalation exposure to benzene. Available data pertaining to systemic effects are presented below.

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

Respiratory Effects. Respiratory effects have been reported in humans after acute exposure to benzene vapors (Avis and Hutton 1993; Midzenski et al. 1992; Winek and Collom 1971; Winek et al. 1967; Yin et al. 1987b). Fifteen male workers employed in removing residual fuel from shipyard tanks were evaluated for benzene exposure (Midzenski et al. 1992). Mucous membrane irritation was noted in 80% and dyspnea was noted in 67% of the workers at occupational exposures of >60 ppm for up to 3 weeks. Nasal irritation and sore throat were reported by male and female workers exposed to 33 and 59 ppm benzene, respectively, for more than 1 year (Yin et al. 1987b). After a fatal occupational exposure to benzene vapors on a chemical cargo ship for only minutes, autopsy reports on three victims revealed hemorrhagic, edematous lungs (Avis and Hutton 1993). Acute granular tracheitis, laryngitis, bronchitis, and massive hemorrhages of the lungs were observed at autopsy of an 18-year-old male who died of benzene poisoning after intentional inhalation of benzene (Winek and Collom 1971). Similarly, acute pulmonary edema was found during the autopsy of a 16-year-old who died after sniffing glue containing benzene (Winek et al. 1967).

Snyder et al. (1978a, 1984) reported no treatment-related effects on lung tissue in male Sprague-Dawley rats exposed to 0, 100, or 300 ppm benzene 5 days/week, 6 hours/day for life. In addition, no adverse histopathological effects on lung tissue were observed in AKR/J or C57BL/65 mice exposed to 300 ppm benzene for life (Snyder et al. 1978a, 1980).

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

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
ACUTE EXPOSURE								
Death								
1	Human	1 d 5-10 min				20000	(death)	Flury 1928
2	Rat (Sprague-Dawley)	4 hr				13700	(LC50)	Drew and Fouts 1974
3	Rat (NS)	4 hr				16000	(4/6 died)	Smyth et al. 1962
4	Rabbit (NS)	3.7-36.2 min				45000	(death in 36.2 min)	Carpenter et al. 1944
Systemic								
5	Human	1-21 d 2.5-8 hr/d	Resp		60 M (mucous membrane irritation, dyspnea)			Midzenski et al. 1992
			Hemato			60 M	(leukopenia, anemia, thrombocytopenia, MCV elevation)	
			Dermal		60 M (skin irritation)			
6	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d	Bd Wt	300 F	2200 F (decreased maternal body weight)			Green et al. 1978
7	Rat (Sprague-Dawley)	Gd 6-15 7 hr/d	Bd Wt	10 F	50 F (decreased maternal body weight and weight gain)			Kuna and Kapp 1981

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
8	Rat (Wistar)	7 d 8 hr/d	Hemato	50 F	100 F (leukopenia)		Li et al. 1986	
9	Rat (Wistar)	15 min	Cardio			3526 M (ventricular arrhythmia)	Magos et al. 1990	
10	Rat (CFY)	Gd 7-14 24 hr/d	Hepatic	125 F			Tatrai et al. 1980a	
			Bd Wt		125 F (decreased maternal weight gain of <22.08% of controls)			
11	Rat (CFY)	Gd 7-14 24 hr/d	Hepatic	47 F	141 F (increased relative liver weight)		Tatrai et al. 1980b	
			Bd Wt		47 F (decreased maternal weight gain)			
12	Rat (Sprague-Dawley)	2 wk 5 d/wk 6 hr/d	Hemato	30	300 (decrease in leukocytes, males; decrease in lymphocytes)		Ward et al. 1985	
13	Mouse (BALB/c)	7 d 6 hr/d	Hemato	47 M	211 M (depressed WBC count)		Aoyama 1986	
			Bd Wt	47 M	211 M (16% decrease in body weight)			

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
14	Mouse (BALB/c)	14 d 6 hr/d	Hemato		48 M (depressed WBC count)		Aoyama 1986	
			Bd Wt	48 M	208 M (18% decrease in body weight)			
15	Mouse (DBA/2)	2 wk 6 hr/d 5 d/wk	Hemato			300 M (hematocrit decreased by 26%, leukocytes decreased by 80%, bone marrow cellularity decreased by 93%)	Chertkov et al. 1992	
			Bd Wt		300 M (15% decrease)			
16	Mouse (Hale- Stoner)	11 d 5 d/wk 6 hr/d	Hemato			400 M (decreased erythrocytes and leukocytes)	Cronkite et al. 1982	
17	Mouse (C57B1/6 BNL)	2 wk 5 d/wk 6 hr/d	Hemato	25		100 (decreased hematocrit, hemolytic anemia)	Cronkite et al. 1985	
18	Mouse (Hale- Stoner)	2 d 5 d/wk 6 hr/d	Hemato		400 M (decreased CFU-E cells)		Cronkite et al. 1989	
19	Mouse (C57BL/6BNL)	8 d 6 hr/d	Hemato		3000 F (decreased marrow cellularity)		Cronkite et al. 1989	

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
20	Mouse (DBA/2J)	5 d 6 hr/d	Hemato		10 M (50% decrease in CFU-E numbers)		Dempster and Snyder 1991	
21	Mouse (C57B1/6)	2-8 d 24 hr/d	Hemato			100 M (leukopenia; decrease in marrow cellularity)	Gill et al. 1980	
22	Mouse (CD-1)	5 d 6 hr/d	Hemato	9.9 M		103 M (decreased marrow cellularity; granulocytopenia, lymphocytopenia; decreased polymorphonucleocytes)	Green et al. 1981b	
			Bd Wt	4862 M				
23	Mouse (Swiss Webster, C57B1/6J)	2 wk 4 d/wk 6 h/d	Hemato			300 M (reduced bone marrow cellularity and CFU-E development)	Neun et al. 1992	
24	Mouse (Hybrid)	5 d 5 d/wk 6 hr/d	Hemato	300 F	900 F (CFU-E depression)		Plappert et al. 1994a	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
25	Mouse (C57Bl/6J)	6 d 6 hr/d	Hemato		10.2 M (depressed lymphocyte counts; elevated RBCs)		Rozen et al. 1984	
26	Mouse (NMRI)	1-10 d 24 hr/d	Hemato			21 M (reduced bone marrow cellularity; increased polychromatic erythrocytes; decreased granulopoietic stem cells)	Toft et al. 1982	
27	Mouse (NMRI)	1 wk	Hemato	14 M			Toft et al. 1982	
28	Mouse (NMRI)	2 wk 5 d/wk 8 hr/d	Hemato	10.5 M	21 M (increased micronucleated polychromatic erythrocytes; decreased granulopoietic stem cells)		Toft et al. 1982	
29	Mouse (CD-1)	2 wk 5 d/wk 6 hr/d	Hemato	30		300 (anemia, decreased hemoglobin, erythrocytes and hematocrit; hypoplasia of bone marrow; leukopenia)	Ward et al. 1985	
			Bd Wt	300				

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
30	Mouse (Swiss-Webster)	5 d 6 hr/d	Hemato	3 M	25 M (decrease in WBC count)		Wells and Nerland 1991	
31	Rabbit	Gd 7-20 24 hr/d	Bd Wt	156.5 F	313 F (reduced maternal weight gain)		Ungvary and Tatrai 1985	
Immuno/ Lymphoret								
32	Rat (Wistar)	7 d 8 hr/d		50 F	100 F (leukopenia; increased leukocyte alkaline phosphatase)		Li et al. 1986	
33	Rat (Sprague-Dawley)	2 wk 5 d/wk 6 hr/d		200 M	400 M (29% reduction in total splenic cells, 28% lower thymus weight)		Robinson et al. 1997	
34	Mouse (BALB/c)	7 d 6 hr/d			47 M (depressed T- and B-lymphocytes; decreased spleen weight and WBC count)		Aoyama 1986	
35	Mouse (BALB/c)	14 d 6 hr/d			48 M (depressed T- and B-lymphocytes; decreased spleen and thymus weights and WBC count)		Aoyama 1986	

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments	
					Less Serious (ppm)	Serious (ppm)			
36	Mouse (DBA/2)	2 wk 6 hr/d 5 d/wk					300 M (leukocytes decreased by 80%, bone marrow cellularity decreased by 93%)	Chertkov et al. 1992	
37	Mouse (CBA/Ca)	2 wk 5 d/wk 6 hr/d		10	25 (lymphopenia)			Cronkite 1986	
38	Mouse (Hale-Stoner)	11 d 5 d/wk 6 hr/d					400 M (decreased bone marrow cellularity)	Cronkite et al. 1982	
39	Mouse (C57B1/6 BNL)	2 wk 5 d/wk 6 hr/d		10	25 (lymphopenia)			Cronkite et al. 1985	
40	Mouse (CBA/Ca BNL)	2 d 5 d/wk 6 hr/d			3000 M (decreased lymphocytes, CFU-S content in marrow)			Cronkite et al. 1989	
41	Mouse (C57B1/6)	2-8 d 24 hr/d					100 M (leukopenia; decrease in marrow cellularity)	Gill et al. 1980	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
42	Mouse (CD-1)	5 d 6 hr/d		9.9 M		103 M (decreased femoral marrow and splenic cellularities; reduced splenic granulocytes)	Green et al. 1981a	
43	Mouse (Swiss Webster, C57B1/6J)	2 wk 4 d/wk 6 h/d				300 M (reduced bone marrow cellularity)	Neun et al. 1992	
44	Mouse (Hybrid)	5 d 5 d/wk 6 hr/d		100 F	300 F (increased helper lymphocytes)		Plappert et al. 1994a	
45	Mouse (C57BL/6)	1-12 d 6 hr/d		10 M	30 M (Listeria infection, T and B lymphocyte depression)		Rosenthal and Snyder 1985	
46	Mouse (C57Bl/6J)	6 d 6 hr/d			10.2 ^b M (decreased circulating lymphocytes and mitogen-induced blastogenesis of femoral T and B-lymphocytes)		Rozen et al. 1984	
47	Mouse (NMRI)	2 wk 5 d/wk 8 hr/d		10.5 M	21 M (decreased granulopoietic stem cells)		Toft et al. 1982	

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
48	Mouse (NMRI)	1-10 d 24 hr/d			21 M (decreased granulopoietic stem cells)		Toft et al. 1982	
49	Mouse (CD-1)	2 wk 5 d/wk 6 hr/d		30		300 (leukopenia; lymphopenia; bone marrow hypoplasia; histopathological lesions in spleen, thymus, selected lymph nodes)	Ward et al. 1985	
50	Mouse (Swiss-Webster)	5 d 6 hr/d		3 M	25 M (decrease in spleen weight and WBC count)		Wells and Nerland 1991	
Neurological								
51	Human	30 min			300 (drowsiness, dizziness, headaches)		Flury 1928	
52	Human	1-21 d 2.5-8 hr/d			60 M (dizziness, nausea, headache, peculiar or strong odor, chemical taste, fatigue)		Midzenski et al. 1992	
53	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		300 F	2200 F (lethargy)		Green et al. 1978	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
54	Mouse (C57BL)	1-14 d 5 d/wk 6 hr/d			100 M (increased milk licking: behavioral index)	3000 M (tremors; decreased grip strength)	Dempster et al. 1984	
55	Mouse (CD1, C57BL/6J)	5 d 6 hr/d			300 (hyperactivity)	900 (narcosis)	Evans et al. 1981	
56	Rabbit (NS)	3.7-36.2 min				45000 (narcosis, tremors, excitement, chewing, loss of pupillary and blink reflex; pupillary contraction and involuntary blinking)	Carpenter et al. 1944	
Reproductive								
57	Rat (Sprague- Dawley)	Gd 6-15 6 hr/d		100 F			Coate et al. 1984	
58	Rat (Sprague- Dawley)	Gd 6-15 6 hr/d		2200 F			Green et al. 1978	
59	Rat (CFY)	Gd 7-14 24 hr/d		125 F			Tatrai et al. 1980a	
60	Mouse (CF-1)	Gd 6-15 7 hr/d		500 F			Murray et al. 1979	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
61	Rabbit (New Zealand)	Gd 6-18 7 hr/d		500 F			Murray et al. 1979	
62	Rabbit	Gd 7-20 24 hr/d		156.5 F		313 F (increased abortions and resorptions)	Ungvary and Tatrai 1985	
Developmental								
63	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		40 F	100 F (decreased fetal weight)		Coate et al. 1984	
64	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d			100 F (increased incidence of missing sternebrae)		Green et al. 1978	
65	Rat (Sprague-Dawley)	Gd 6-15 7 hr/d		10 F	50 F (decreased fetal weight)		Kuna and Kapp 1981	
66	Rat (CFY)	Gd 7-14 24 hr/d			125 F (decreased mean fetal weight; increased fetal weight retardation; skeletal retardation; 17% decrease in mean placental weight)		Tatrai et al. 1980a	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
67	Rat (CFY)	Gd 7-14 24 hr/d			47 F (decreased fetal weight; signs of skeletal retardation)	141 F (significant increase in fetal mortality)	Tatrai et al. 1980b	
68	Mouse (Swiss-Webster)	Gd 6-15 6 hr/d		10 F	20 F (decreased circulating erythroid precursors, elevation of granulocytic precursor cells in neonates and 6-week old offspring)		Keller and Snyder 1988	
69	Mouse (CF-1)	Gd 6-15 7 hr/d			500 F (decreased fetal body weight; delayed skeletal ossifications)		Murray et al. 1979	
70	Mouse (CFLP)	Gd 6-15 12 hr/d			156.5 F (decreased fetal body weight and skeletal retardation)		Ungvary and Tatrai 1985	
71	Rabbit (New Zealand)	Gd 6-18 7 hr/d			500 F (increased minor skeletal variants)		Murray et al. 1979	
72	Rabbit (New Zealand)	Gd 7-20 24 hr/d		156.5 F	313 F (decreased fetal weight; increased minor anomalies)		Ungvary and Tatrai 1985	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
INTERMEDIATE EXPOSURE								
Death								
73	Rat (Sprague-Dawley)	15 wk 4-5 d/wk 4-7 hr/d				200	(death)	Maltoni et al. 1983, 1985
74	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M	(97% mortality)	Cronkite 1986
75	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				300	(deaths in males during exposure; deaths in females shortly after exposure)	Cronkite et al. 1989
76	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M	(11/125 died during first 9 months after initiation of exposure)	Farris et al. 1993
77	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d				302 M	(50% mortality)	Green et al. 1981b
Systemic								
78	Human	4 mo- 1 yr (occup)	Hemato			150	(pancytopenia)	Aksoy and Erdem 1978
79	Human	4 mo -1 yr (occup)	Hemato			210	(pancytopenia, hypocellular to hypercellular bone marrow)	Aksoy et al. 1972

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
80	Human	1 yr (occup)	Hemato		40	(decrease in WBC counts in first 4 months)		Cody et al. 1993
81	Human	3.5 mo- 19 yr (occup)	Hemato			29	(aplastic anemia)	Yin et al. 1987c
82	Rat (Sprague-Dawley)	3 wk 5 d/wk 6 hr/d	Hemato			500	(decreased WBC and lymphocytes; increased RBC and hemoglobin)	Dow 1992
83	Rat (Sprague-Dawley)	10 wk Gd 0-20 Ld 5-20 5 d/wk 6 hr/d	Bd Wt	300 F				Kuna et al. 1992
84	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d	Hemato	30		300	(decrease in leukocytes, slight decrease in marrow cellularity)	Ward et al. 1985
			Bd Wt	300				
85	Rat (Wistar)	204 d 5 d/wk 7 hr/d	Hemato		88 M	(leukopenia)		Wolf et al. 1956

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
86	Mouse (C57BL)	24 wk 5 d/wk 6 hr/d	Hemato		10 M (depressed peripheral red blood cells, CFU-E)		Baarson et al. 1984	The use of one dose precludes a dose-response assessment.
87	Mouse (C57BL)	24 wk 5 d/wk 6 hr/d	Hemato		10 M (depressed splenic red cells)		Baarson et al. 1984	The use of one dose precludes a dose-response assessment.
88	Mouse (Hale- Stoner)	9.5 wk 5 d/wk 6 hr/d	Hemato			400 M (decreased erythrocytes and leukocytes, decreased bone marrow cellularity)	Cronkite et al. 1982	
89	Mouse (C57B1/6BNL)	4-16 wk 5 d/wk 6 hr/d	Hemato		300 (stem cell depression in bone marrow, reversible after 2-4 weeks)		Cronkite et al. 1985	
90	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d	Hemato	25 M	316 M (decreased stem cells in bone marrow)		Cronkite et al. 1989	
91	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d	Hemato		300 M (granulocytic hyperplasia in bone marrow)		Farris et al. 1993	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
92	Mouse (B6C3F1)	up to 8 wk 5 d/wk 6 hr/d	Hemato	10 M	100 M (decreased numbers of differentiating and maturing hematopoietic bone marrow cells)		Farris et al. 1997a	
93	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d	Hemato			302 M (decreased WBC, RBC; altered RBC morphology)	Green et al. 1981b	
			Bd Wt	302 M				
94	Mouse (CD-1)	50 d 5 d/wk 6 hr/d	Hemato	9.6 M			Green et al. 1981b	
			Bd Wt	9.6 M				
95	Mouse (Kunming)	30 d 6 d/wk 2 hr/d	Hepatic	12.52 M			Li et al. 1992	Exposures conducted under static conditions; measured only on first 3 of 30 days.
			Renal	12.52 M				
			Bd Wt	12.52 M				
96	Mouse (DBA/2, B6C3F1, C57B1/6)	13 wk 3 or 5 d/wk 6 hr/d	Hemato			300 M (depressed rate of erythropoiesis, increased frequency of MN-PCE and MN-NCE)	Luke et al. 1988b	

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
97	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d	Hemato	100 F	300 F (slight anemia; BFU-E and CFU-E depression in bone marrow)		Plappert et al. 1994a	
98	Mouse (Hybrid)	8 wk 5 d/wk 6 hr/d	Hemato			300 F (decreased Hgb, Hct, erythrocyte counts)	Plappert et al. 1994b	
99	Mouse (BDF1)	8 wk 5 d/wk 6 hr/d	Hemato		100 F (BFU-E and CFU-E depression)		Seidel et al. 1989b	
100	Mouse (NMRI)	8 wk	Hemato	14 M			Toft et al. 1982	
101	Mouse (Hybrid)	6 or 7 wk 5 d/wk 6 h/d	Hemato			300 F (decreased CFU-C, BFU-E and CFU-E)	Vacha et al. 1990	
102	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d	Hemato	30		300 (pancytopenia, bone marrow hypoplasia)	Ward et al. 1985	
			Bd Wt	300				
103	Gn Pig (NS)	32 or 269 d 5 d/wk 7 hr/d	Hemato		88 (leukopenia)		Wolf et al. 1956	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments	
					Less Serious (ppm)	Serious (ppm)			
104	Rabbit (NS)	243 d 5 d/wk 7 hr/d	Hemato		80	(leukopenia)		Wolf et al. 1956	
105	Pig (Duroc-Jersey)	3 wk 5 d/wk 6 hr/d	Hemato	20	100	(decreased peripheral WBC, and increased erythroid cells)		Dow 1992	
Immuno/ Lymphoret									
106	Human	4 mo- 1 yr (occup)					210	(pancytopenia, hypoplastic to hyperplastic bone marrow, enlarged spleen)	Aksoy et al. 1972
107	Human	1 yr (occup)			40	(decreased lymphocytes)			Cody et al. 1993
108	Rat (Sprague-Dawley)	3 wk 5 d/wk 6 hr/d					500	(decreased myeloid and lymphoid cells)	Dow 1992
109	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d		200 M	400 M	(29% reduction in total splenic cells, 28% lower thymus weight)			Robinson et al. 1997
110	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		30	300	(leukopenia and lymphopenia)			Ward et al. 1985

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments	
					Less Serious (ppm)	Serious (ppm)			
111	Rat (Wistar)	204 d 5 d/wk 7 hr/d			88	(leukopenia, increased spleen weight)		Wolf et al. 1956	
112	Rat (NS)	20 wk 6 d/wk 4 hr/d			4570	(increased leukocyte alkaline phosphatase, decreased white blood cell count)		Yin et al. 1982	
113	Mouse (C57B1)	24 wk 5 d/wk 6 hr/d			10 M	(decreased number of splenic lymphocytes)		Baarson et al. 1984	The use of one dose precludes a dose-response assessment.
114	Mouse (C57B1/6BNL)	4-16 wk 5 d/wk 6 hr/d					300	(reduced bone marrow cellularity; stem cell depression, reversible after 2-4 weeks)	Cronkite et al. 1985
115	Mouse (CBA/Ca BNL)	20 d 5 d/wk 6 hr/d						316 M (decreased lymphocytes, CFU-S content in marrow)	Cronkite et al. 1989

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency/ (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
116	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d			300 M (granulocytic hyperplasia)		Farris et al. 1993	
117	Mouse (B6C3F1)	up to 8 wk 5 d/wk 6 hr/d		10 M	100 M (reduced numbers of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, peripheral blood leukocytes and RBCs)		Farris et al. 1997a	
118	Mouse (B6C3F1)	8 wk 5 d/wk 6 hr/d		10 M	100 M (reduced lymphocyte and total nucleated cell counts)		Farris et al. 1997b	
119	Mouse (C57B1/6)	6 wk 5 d/wk 6 hr/d				1000 (leukopenia, granulocytopenia, lymphocytopenia)	Gill et al. 1980	
120	Mouse (CD-1)	50 d 6 hr/d 5 d/wk			9.6 M (increased splenic CFU-S)		Green et al. 1981a	The use of one exposure level precludes usefulness for dose-response assessment.

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments	
					Less Serious (ppm)	Serious (ppm)			
121	Mouse (CD-1)	26 wk 6 hr/d 5 d/wk					302 M (reduced marrow and spleen cellularity; decreased spleen weight)	Green et al. 1981a	
122	Mouse (CD-1)	50 d 5 d/wk 6 hr/d			9.6 M (increased spleen weight, total splenic nucleated cellularity and NRBC)			Green et al. 1981b	The use of one exposure level precludes usefulness for dose-response assessment.
123	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d					302 M (lymphocytopenia, anemia, decreased spleen weight, decreased spleen and marrow cellularities)	Green et al. 1981b	
124	Mouse (Kunming)	30 d 6 d/wk 2 hr/d		3.13 M	12.52 M (26% decrease in relative spleen weight; decrease in myelocytes, premyelocytes, myeloblasts, and metamyelocytes in the bone marrow)			Li et al. 1992	Exposures conducted under static conditions; measured only on first 3 of 30 days.

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
125	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d		100 F	300 F (increased T4/T8 ratio)		Plappert et al. 1994a	
126	Mouse (C57B1/6)	20 d 5 d/wk 6 hr/d			^c 10 M (delayed splenic lymphocyte reaction to foreign antigens evaluated in in vitro mixed lymphocyte reaction)		Rosenthal and Snyder 1987	
127	Mouse (C57B1/6)	100 d 5 d/wk 6 hr/d				100 M (death in 9/10 mice due to depressed cell-mediated immunity)	Rosenthal and Snyder 1987	
128	Mouse (Hale- Stoner)	4-5 wk 5 d/wk 6 hr/d		50 F	200 F (suppressed antibody response to fluid tetanus toxoid)		Stoner et al. 1981	
129	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d		30		300 (leukocyte & lymphocyte depression; bone marrow hypoplasia; histopathologic lesions in spleen and selected lymph nodes)	Ward et al. 1985	
130	Gn Pig (NS)	32 or 269 d 5 d/wk 7 hr/d			88 (leukopenia, increased spleen weight)		Wolf et al. 1956	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
131	Rabbit (NS)	243 d 5 d/wk 7 hr/d			80 (leukopenia)		Wolf et al. 1956	
132	Pig (Duroc-Jersey)	3 wk 5 d/wk 6 hr/d		20 F	100 F (T-cell depression; decreased peripheral WBC; decreased total lymphocytes)		Dow 1992	
Neurological								
133	Rat (Wistar)	3 wk 3-4 x 4 hr				929 M (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994	
134	Mouse (H)	3 wk 3-4 x 2 hr				856 F (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994	
135	Mouse (Kunming)	30 d 6 d/wk 2 hr/d			0.78 M (increased rapid response)		Li et al. 1992	Exposures conducted under static conditions; measured only on first 3 of 30 days.
Reproductive								
136	Rat (Sprague-Dawley)	10 wk Gd 0-20 Ld 5-20 5 d/wk 6 hr/d		300 F			Kuna et al. 1992	

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
137	Rat (Wistar)	93 d 5 d/wk 7-8 hr/d			6600 M (testicular weight increase)		Wolf et al. 1956	
138	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d		30		300 (bilateral cyst in ovaries; atrophy/degeneration of testes; decrease in spermatozoa; increase in abnormal sperm)	Ward et al. 1985	
139	Gn Pig (NS)	32 or 269 d 5 d/wk 7-8 hr/d			88 M (testicular weight increase)		Wolf et al. 1956	
140	Rabbit (NS)	243 d 5 d/wk 7-8 hr/d			80 M (degeneration of germinal epithelium in testes)		Wolf et al. 1956	
Cancer								
141	Human	3.5 mo- 19 yr (occup)				29 (CEL: humanlymphocytic leukemia)	Yin et al. 1987c	
142	Rat (Sprague-Dawley)	15 wk 4-5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1982a, 1983, 1985	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments	
					Less Serious (ppm)	Serious (ppm)			
143	Rat (Sprague-Dawley)	15 wk 5 d/wk 4-7 hr/d				200	(CEL: hepatomas; onset of Zymbal gland carcinoma)	Maltoni et al. 1982b, 1983, 1985	
144	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				100 M	(CEL: leukemia)	Cronkite 1986	
145	Mouse (C57BL/6BNL)	4-16 wk 5 d/wk 6 hr/d				300	(CEL: thymic and non-thymic lymphoma)	Cronkite et al. 1984, 1985	
146	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				300	(Harderian and Zymbal gland, squamous cell and mammary carcinoma, papillary adenocarcinoma of the lung)	Cronkite et al. 1989	
147	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				100 M	(CEL: hepatomata, lymphomatous and myelogenous neoplasms)	Cronkite et al. 1989	
148	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M	(CEL: lymphoma in 12%)	Farris et al. 1993	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
149	Mouse (C57BL, CD-1)	10 wk 5 d/wk 6 hr/d				1200 M (CEL: 46% lung adenoma on CD-1 mice)	Snyder et al. 1988	
CHRONIC EXPOSURE								
Death								
150	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (61% died, versus 46% in controls)	Maltoni et al. 1982a	
151	Rat (Sprague-Dawley)	691 d 5 d/wk 6 hr/d				300 M (median lifespan 51 weeks versus 65 weeks in controls)	Snyder et al. 1978a	
152	Mouse (AKR/J, C57Bl)	lifetime 5 d/wk 6 hr/d				300 M (median lifespan 11-41 weeks versus 39-75 weeks in controls)	Snyder et al. 1978a, 1980	
153	Mouse (CD-1)	222 d 5 d/wk 6 hr/d				300 M (decreased survival)	Snyder et al. 1982	
Systemic								
154	Human	4 mo- 15 yr (occup)	Hemato			150 (pancytopenia)	Aksoy and Erdem 1978	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
155	Human	14 yr (Occup)	Hemato	0.55			Collins et al. 1997	The NOAEL is for abnormal hematological values, not significantly altered values that would still fall within the range of normal values.
156	Human	1-3 yr (occup)	Hemato			3 (anemia, lymphocytosis, thrombocytopenia, leukopenia, leukocytosis)	Doskin 1971	
157	Human	NS (occup)	Hemato			24 (pancytopenia, hypoplastic bone marrow)	Erf and Rhoads 1939	
158	Human	3-29 yr (occup)	Hemato		25 M (increased mean corpuscular volume)		Fishbeck et al. 1978	
159	Human	0.5-5 yr (occup)	Hemato			11 (anemia, macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939	
160	Human	1-25 yr (occup)	Hemato	20		75 (anemia and leukopenia)	Kipen et al. 1989	

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
161	Human	6.1 yr (avg) (Occup)	Hemato		0.57 ^d	(reduced WBC and platelet counts, approximately 7-18% lower than control values)	Lan et al. 2004a	
162	Human	4.5-9.7 yr (mean duration)	Hemato		2.26	(reduced neutrophils and RBC counts, approximately 12% lower than controls)	Qu et al. 2002, 2003	
163	Human	6.3 yr (avg) (Occup)	Hemato		7.6	(reduced absolute lymphocyte count, approximately 16% lower than controls)	Rothman et al. 1996a, 1996b	
164	Human	1-21 yr (occup)	Hemato	0.53 M			Tsai et al. 1983	
165	Human	>1 yr (occup)	Hemato		0.69	(leukopenia)	Xia et al. 1995	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
166	Human	>1 yr (occup)	Resp		33 ^e M (sore throat; nasal irritation)		Yin et al. 1987b	
					59 F (sore throat; nasal irritation)			
			Hemato	33 ^e M				
					59 F			
			Renal	33 ^e M				
					59 F			
Ocular		33 ^e M (eye irritation)						
		59 F (eye irritation)						
167	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d	Resp	300 M			Snyder et al. 1978a, 1984	
			Hemato			100 M (anemia, leukopenia)		
			Hepatic	300 M				
			Renal	300 M				
			Bd Wt	100 M	300 M (unspecified decreased body weight gain relative to controls)			

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
168	Mouse (AKR/J, C57BL/6J)	lifetime 5 d/wk 6 hr/d	Resp	300 M			Snyder et al. 1978a, 1980	
			Hemato			100 M (anemia, increased neutrophil levels; pancytopenia; bone marrow hypoplasia)		
			Hepatic	300 M				
			Renal	300 M				
			Bd Wt			300 M (59% decrease in weight gain)		
169	Mouse (CD-1)	222 d 5 d/wk 6 hr/d	Hemato		300 M (decreased RBCs and lymphocytes)		Snyder et al. 1982	
			Bd Wt		300 M (reduced body weight gain)			
Immuno/ Lymphoret								
170	Human	3-5 yr (occup)				11 (macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939	
171	Human	1-25 yr (occup)		20	75 (leukopenia)		Kipen et al. 1989	

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
172	Human	>1 yr (occup)			0.69	(leukopenia)		Xia et al. 1995
173	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d			100 M	(decreased lymphocyte counts, splenic hyperplasia)		Snyder et al. 1978a, 1984
174	Mouse (AKR/J, C57BI)	lifetime 5 d/wk 6 hr/d			100 M	(lymphocytopenia, bone marrow hypoplasia)		Snyder et al. 1978a, 1980
175	Mouse (C57BL, CD-1)	lifetime 6 hr/d 5 d/wk			300 M	(lymphopenia)		Snyder et al. 1988
Cancer								
176	Human	4-15 yr (occup)					150	(CEL: leukemia) Aksoy and Erdem 1978
177	Human	28 mo- 40 yr					1	(CEL: leukemia, lymphoma) Aksoy et al. 1987
178	Human	1-10 yr (occup) (occup)					10 M	(CEL: leukemia) Infante 1978; Infante 1977
179	Human	18 mo (occup)					0.3 M	(CEL: leukemia) Ott et al. 1978

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
180	Human	1-14 yr (occup)				16 M (CEL: leukemia)	Rinsky et al. 1981; Infante et al. 1977a	
181	Human	1-30 yr (occup)				200 (CEL: leukemia)	Vigliani and Forni 1976	
182	Human	>1 yr (occup)				2 (CEL: chronic erythroid leukemia)	Yin et al. 1989	
183	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1982a	
184	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1983, 1985	
185	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d				100 M (CEL: Zymbal gland carcinoma, myelogenous leukemia, liver tumors)	Snyder et al. 1984	
186	Mouse (AKR/J, C57BL6J)	lifetime 5 d/wk 6 hr/d				300 M (CEL: hematopoietic neoplasms [8/40], including 6 thymic lymphomas)	Snyder et al. 1978a, 1980	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (ppm)	LOAEL		Reference Chemical Form	Comments
					Less Serious (ppm)	Serious (ppm)		
187	Mouse (C57BL, CD-1)	lifetime every 3rd wk 7 d/wk				300 M (CEL: 35% increase of Zymbal gland carcinomas in C57BL mice)	Snyder et al. 1988	

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

b Used to derive an acute-duration inhalation minimal risk level (MRL) of 0.009 ppm for benzene. Concentration was adjusted for intermittent exposure by multiplying by 6 hours/24 hours and converted to a human equivalent concentration, which was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability) (see Appendix A).

c Used to derive an intermediate-duration inhalation minimal risk level (MRL) of 0.006 ppm for benzene. Concentration was adjusted for intermittent exposure by multiplying by 6 hours/24 hours and 5 days/7 days and converted to a human equivalent concentration, which was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability) (see Appendix A).

d Study results used to derive a chronic-duration inhalation minimal risk level (MRL) of 0.003 ppm for benzene, as described in detail in Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts to select a point of departure, which was adjusted for intermittent exposure and divided by an uncertainty factor of 10 for human variability. Study results also used to derive a chronic-duration oral minimal risk level (MRL) of 0.0005 mg/kg/day based on route-to-route extrapolation, as described in detail in Chapter 2 and Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts in benzene-exposed workers to select a point of departure, which was adjusted for intermittent exposure. An equivalent oral dose was estimated based on route-to-route extrapolation to determine a point of departure for deriving a chronic-duration oral MRL for benzene, which was divided by an uncertainty factor of 30 (10 for human variability and 3 for uncertainty in route-to-route extrapolation).

e Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

AChe = acetylcholinesterase; Bd Wt = body weight; BFU-E = burst-forming units - erythroid; Cardio = cardiovascular; CEL = cancer effect level; CFU-E = colony-forming units - erythroid progenitor cells; CFU-G = colony-forming units - granulopoietic stem cells; CFU-GM = colony-forming units - macrophages; CFU-S = colony-forming units - spleen; CNS = central nervous system; d = day(s); F = female; Gd = gestational day; Hct = hematocrit; Hemato = hematological; Hgb = hemoglobin; hr = hour(s); LC50 = lethal concentration, 50% kill; Ld = lactational day(s); LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); MN-NCE = micronucleated normochromatic erythrocytes; MN-PCE = micronucleated polychromatic erythrocytes; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NRBC = nucleated red blood cells; NS = not specified; occup = occupational exposure; RBC = red blood cell; WBC = white blood cell; wk = week(s); yr = year(s)

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

Acute (≤14 days)

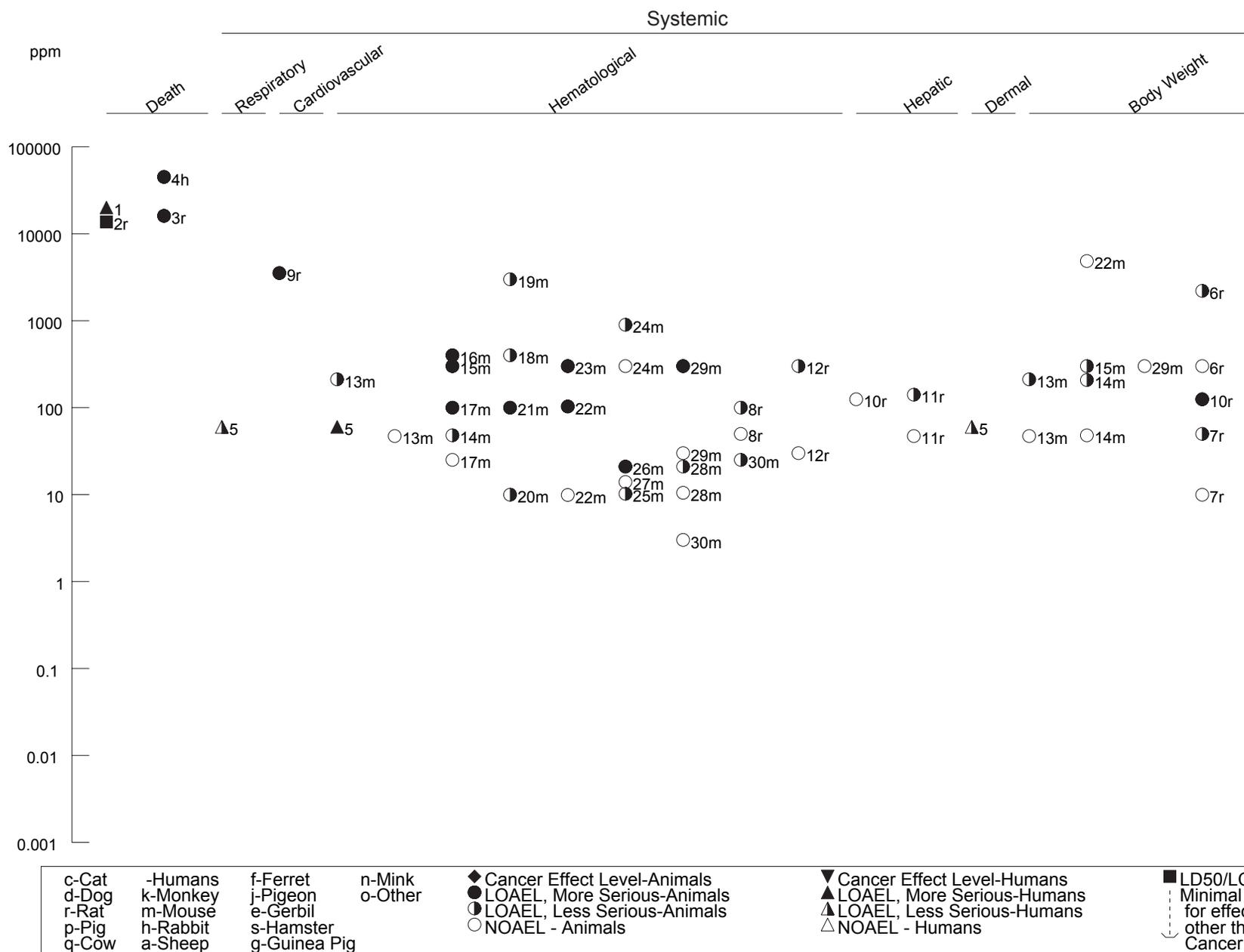


Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation (Continued)

Acute (≤14 days)

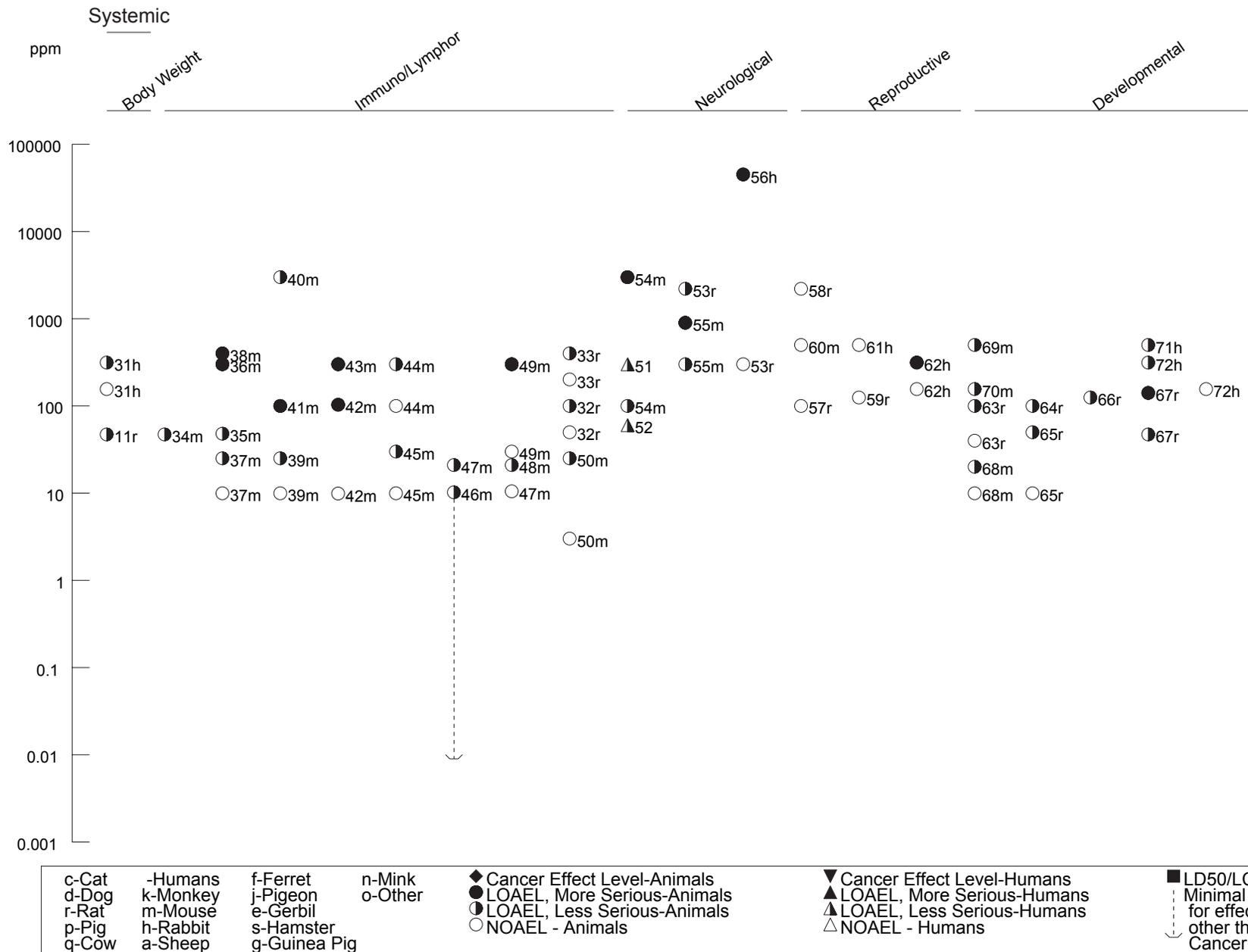


Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation (Continued)
Intermediate (15-364 days)

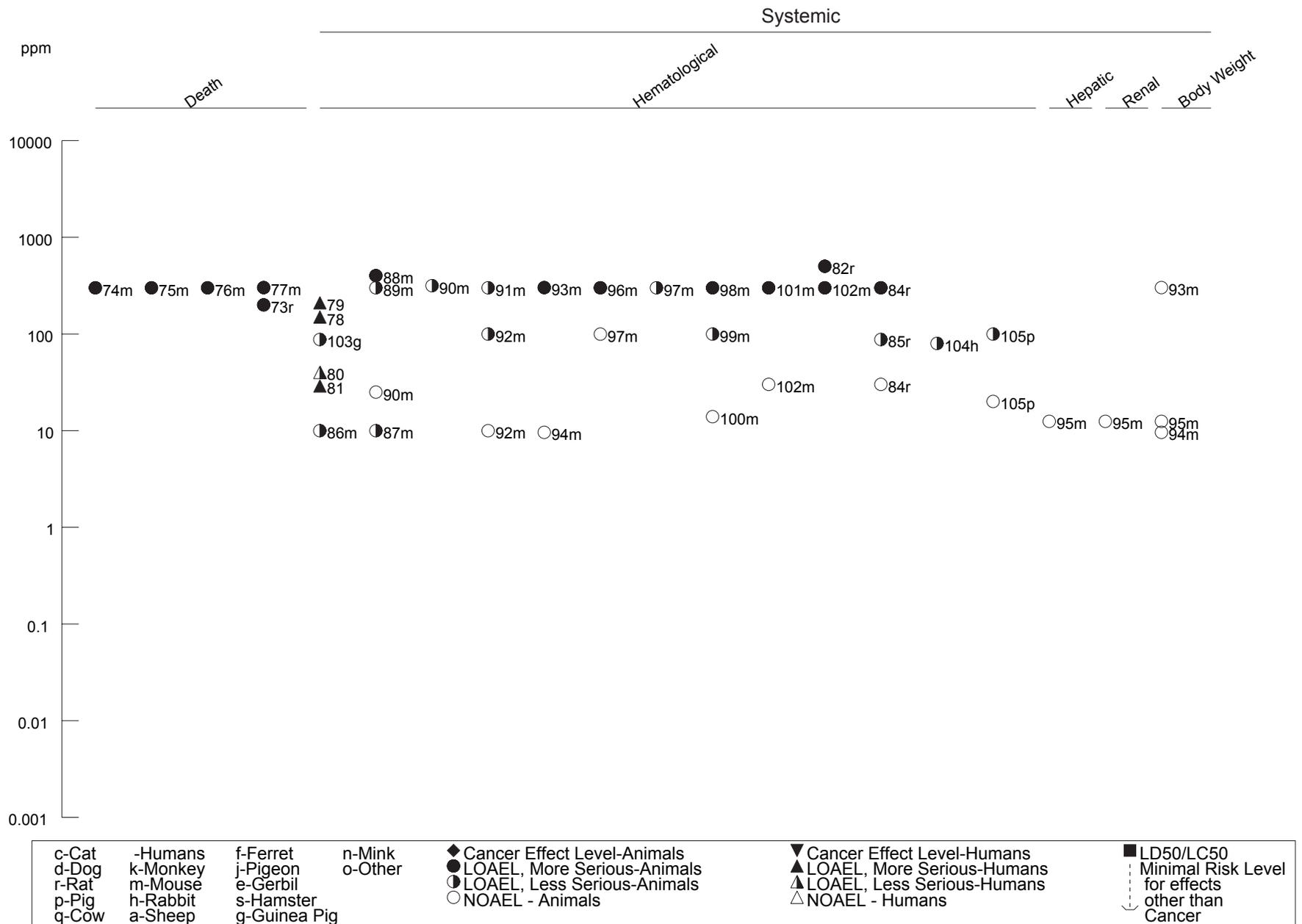
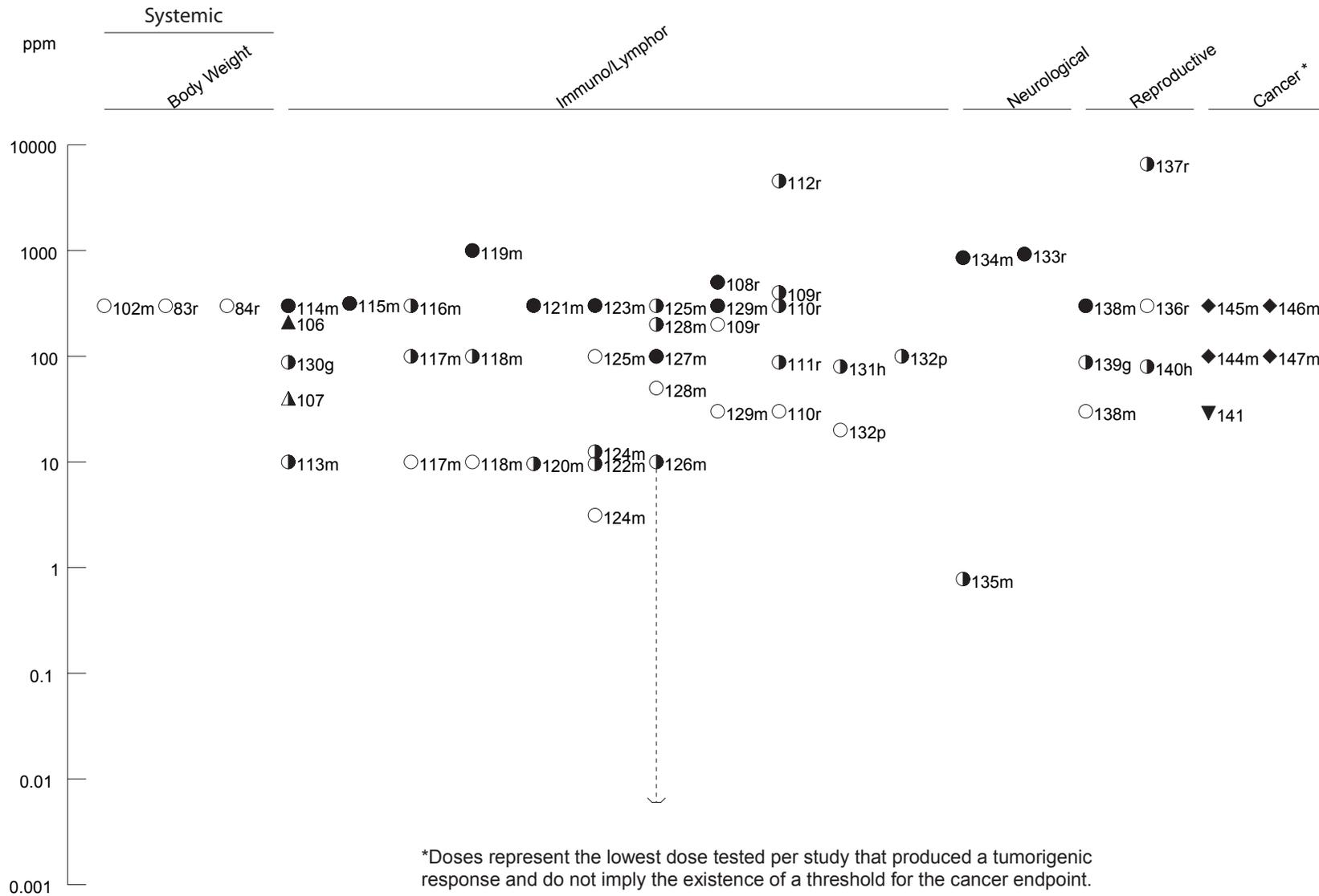


Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation (Continued)
Intermediate (15-364 days)



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

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation (Continued)
Intermediate (15-364 days)



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

Chronic (≥365 days)

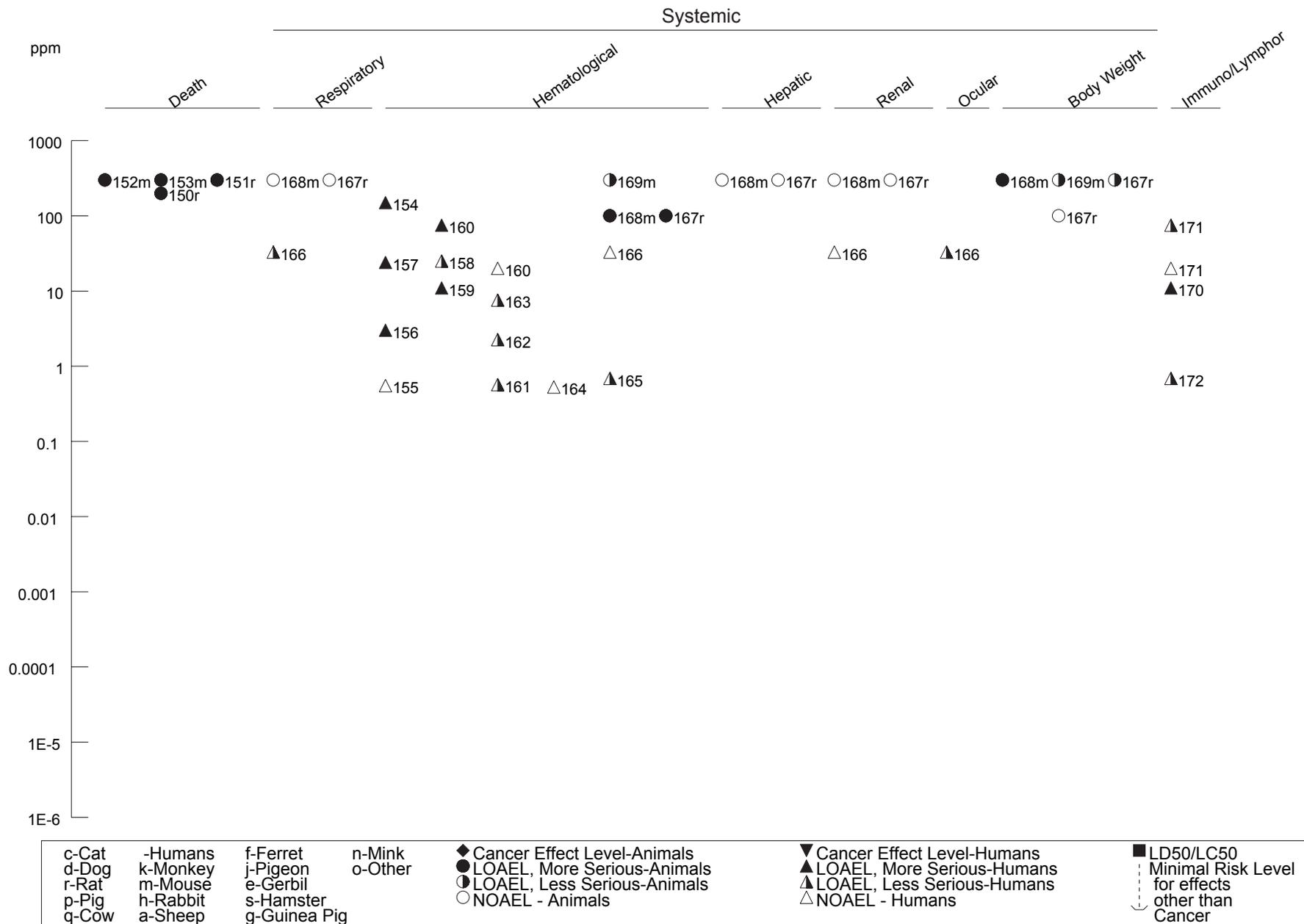
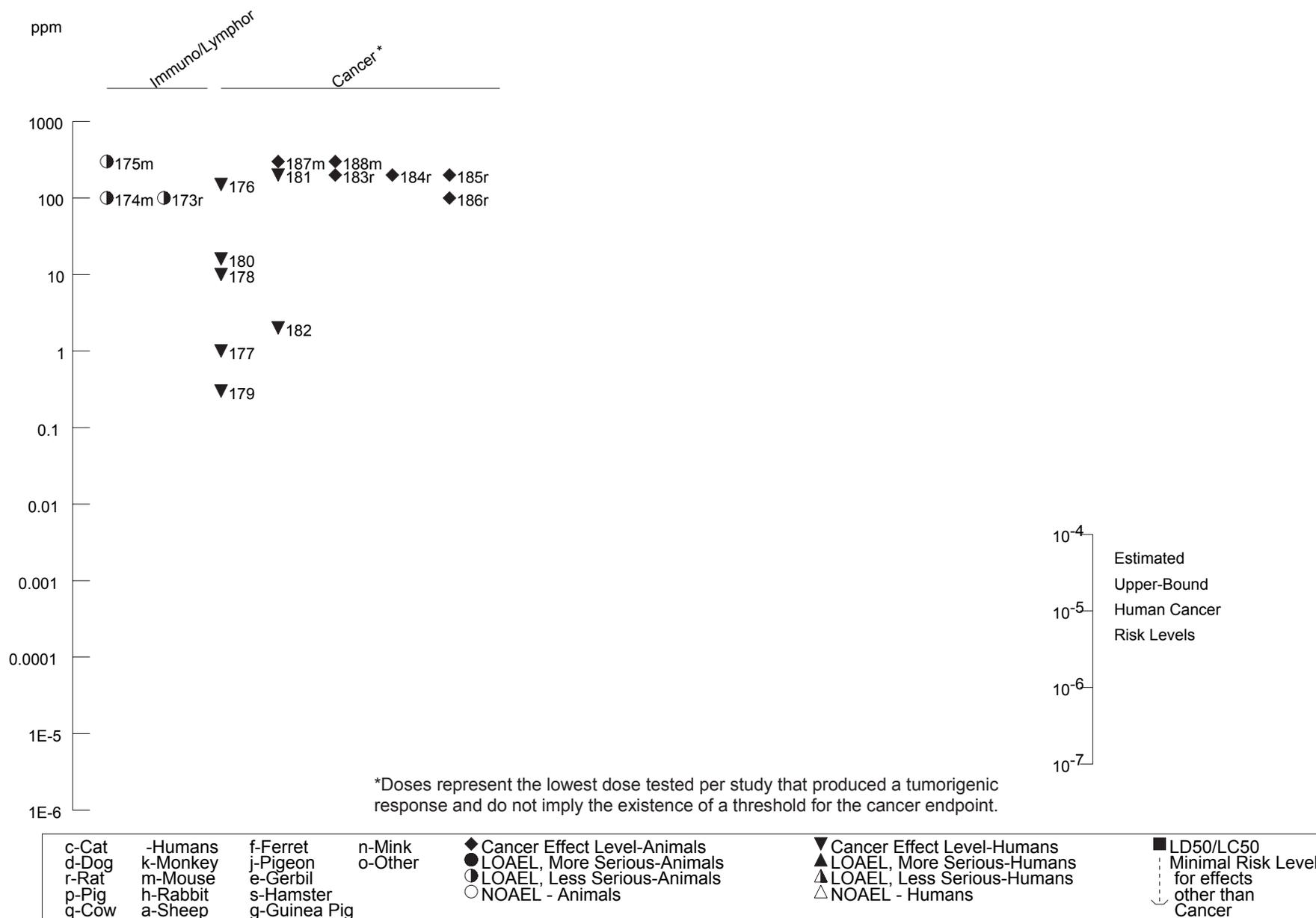


Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation (Continued)

Chronic (≥365 days)



3. HEALTH EFFECTS

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation exposure to benzene, although ventricular fibrillation has been proposed as the cause of death in some human poisonings (Avis and Hutton 1993; Winek and Collom 1971).

One animal study was found that investigated the effects of acute inhalation exposure to high concentrations of benzene vapor on the heart muscle of cats and monkeys (Nahum and Hoff 1934). Information from the electrocardiograms indicated that exposure to benzene vapor caused extra systoles and ventricular tachycardia of the prefibrillation type. Animals that had their adrenals and stellate ganglia removed did not exhibit extra systoles or ventricular tachycardia. These findings suggest that the arrhythmias were caused by catecholamine release and sympathetic discharge. This study is limited in that exact levels of exposure are not available. An additional study investigated the influence of benzene inhalation on ventricular arrhythmia in the rat (Magos et al. 1990). Rats exposed to 3,526–8,224 ppm of benzene in a closed chamber for 15 minutes exhibited an increased number of ectopic ventricular beats.

Gastrointestinal Effects. Very few data are available describing gastrointestinal effects in humans after inhalation exposure to benzene. In a case study involving the death of an 18-year-old boy who intentionally inhaled benzene, the autopsy revealed congestive gastritis (Winek and Collom 1971). No other details or data were given.

Hematological Effects. Data regarding effects on the human hematological system following acute inhalation exposure to benzene are scant, but indicate leukopenia, anemia, and thrombocytopenia after more than 2 days of occupational exposure to more than 60 ppm benzene (Midzenski et al. 1992). Epidemiological studies on persons exposed to various levels of benzene in the workplace for intermediate and chronic periods of time also indicate hematological effects. Deficiencies in most of these studies include uncertainty in estimates of historical exposure levels, concomitant exposure to other chemicals, and lack of appropriate control groups. However, sufficient data are available to show that the hematopoietic system is a critical target for benzene toxicity. Studies that were conducted well and that show effects linked to specific exposure levels are presented in Table 3-1 and Figure 3-1. Effects on leukocytes, lymphocytes, and bone marrow are also discussed in Section 3.2.1.3.

Inhalation exposure to benzene levels in excess of regulated workplace limits (8-hour TWA of 1 ppm) for several months to several years can result in deficits in the relative numbers of circulating blood cells, which may be severe enough to be considered clinical pancytopenia. Continued exposure to benzene can

3. HEALTH EFFECTS

also result in aplastic anemia or leukemia (Aksoy et al. 1974; EPA 1995; Hayes et al. 1997; IARC 1982, 1987; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1987c, 1996a, 1996b).

Pancytopenia is the reduction in the number of all three major types of blood cells: erythrocytes (red blood cells), thrombocytes (platelets), and leukocytes (white blood cells). In adults, all three major types of blood cells are produced in the red bone marrow of the vertebrae, sternum, ribs, and pelvis. The red bone marrow contains immature cells, known as multipotent myeloid stem cells, that later differentiate into the various mature blood cells. Pancytopenia results from a reduction in the ability of the red bone marrow to produce adequate numbers of these mature blood cells.

Aplastic anemia is a more severe effect of benzene and occurs when the bone marrow ceases to function and the stem cells never reach maturity. Depression in bone marrow function occurs in two stages—hyperplasia (increased synthesis of blood cell elements), followed by hypoplasia (decreased synthesis). As the disease progresses, bone marrow function decreases and the bone marrow becomes necrotic and filled with fatty tissue. This myeloblastic dysplasia without acute leukemia has been seen in persons exposed to benzene (Erf and Rhoads 1939). Aplastic anemia can progress to a type of leukemia known as acute myelogenous leukemia (Aksoy 1980), which is discussed in Section 3.2.1.7.

Early biomarkers of exposure to relatively low levels of benzene include depressed numbers of one or more of the circulating blood cell types. For example, statistically significantly decreased total red blood cells (RBCs), white blood cells (WBCs), absolute lymphocyte count, platelets, and hematocrit were reported for a group of 44 healthy subjects exposed to benzene in the workplace (median 8-hour time-weighted average [TWA] of 31 ppm; minimal exposure to other solvents) for an average of 6.3 years in China (Rothman et al. 1996a, 1996b). Age- and gender-matched workers with no history of occupational exposure to benzene served as controls. Among the 22 workers whose mean 5-day benzene exposure levels did not exceed 31 ppm (median 8-hour TWA of 13.6 ppm), significantly depressed absolute lymphocyte count, RBCs, and platelets were noted. Only absolute lymphocyte count was significantly decreased in a subgroup of 11 workers with no 8-hour TWA exceeding 31 ppm (median 8-hour TWA of 7.6 ppm).

Qu et al. (2002, 2003a, 2003b) compared hematology values in a group of 130 chronically exposed workers in China with those obtained from 51 age- and gender-matched subjects without occupational exposure to benzene. Statistically significant trends for depressed RBCs, WBCs, and neutrophils were observed in the benzene-exposed workers (average measured 4-week benzene exposure levels ranged

3. HEALTH EFFECTS

from 0.08 to 54.5 ppm just prior to blood testing). A subgroup of 73 of these workers, whose average 4-week benzene exposure level was 2.26 ppm, exhibited significantly depressed RBCs and neutrophils. This study provided exposure-response data, but apparent discrepancies in reported low-concentration results render the study of limited value for MRL derivation.

One recent cross-sectional study (Lan et al. 2004a, 2004b), performed on 250 workers exposed to benzene in shoe manufacturing industries in Tianjin, China, and 140 age- and gender-matched workers in clothing manufacturing facilities that did not use benzene, was of sufficient quality to serve as the basis for deriving a chronic-duration inhalation MRL for benzene (see footnote to Table 3-1 and Appendix A). The benzene-exposed workers had been employed for an average of 6.1 ± 2.9 years. Controls consisted of 140 age- and gender-matched workers in clothing manufacturing facilities in which measurable benzene concentrations were not found (detection limit 0.04 ppm). Benzene exposure was monitored by individual organic vapor monitors (full shift) 5 or more times during 16 months prior to phlebotomy. Benzene-exposed workers were categorized into four groups (controls, <1 , $1- <10$, and ≥ 10 ppm) according to mean benzene exposure levels measured during 1 month prior to phlebotomy. Complete blood count (CBC) and differential were analyzed mechanically. Coefficients of variation for all cell counts were $<10\%$.

Mean 1-month benzene exposure levels in the four groups (controls, <1 , $1- <10$, and ≥ 10 ppm) were <0.04 , 0.57 ± 0.24 , 2.85 ± 2.11 , and 28.73 ± 20.74 ppm, respectively. Hematological values were adjusted to account for potential confounding factors (i.e., age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index). All types of WBCs and platelets were significantly decreased in the lowest exposure group (<1 ppm), ranging in magnitude from approximately 8 to 15% lower than controls. Although similar statistical analyses for the mid- and high-exposure groups were not included in the study report, decreases in all types of WBCs and platelets were noted at these exposure levels as well; the decreases in the highest exposure group ranged in magnitude from 15 to 36%. Lymphocyte subset analysis revealed significantly decreased CD4⁺-T cells, CD4⁺/CD8⁺ ratio, and B cells. Hemoglobin concentrations were significantly decreased only within the highest (≥ 10 ppm) exposure group. Tests for a linear trend using benzene air level as a continuous variable were significant for platelets and all WBC measures except monocytes and CD8⁺-T cells. Upon restricting the linear trend analyses to workers exposed to <10 ppm benzene, excluding controls, inverse associations remained for total WBCs, granulocytes, lymphocytes, B cells, and platelets. In order to evaluate the effect of past benzene exposures on the hematological effects observed in this study, the authors compared findings for a group of workers who had been exposed to <1 ppm benzene over the previous year ($n=60$) and a subset

3. HEALTH EFFECTS

who also had <40 ppm-years lifetime cumulative benzene exposure (n=50). The authors stated that the same cell types were significantly reduced in these groups, but did not provide further information of the magnitude (i.e., percent change) of the hematological effects observed. These data suggest that the 1-month benzene exposure results could be used as an indicator of longer-term low-level benzene hematotoxicity. To demonstrate that the observed effects were attributable to benzene, significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells were noted in a subgroup (n=30; mean 1-month exposure level of 0.29 ± 0.15 ppm) of the <1 ppm group for which exposure to other solvents was negligible. Lan et al. (2004a, 2004b) also presented information on the effect of benzene on colony forming progenitor cells (data were only presented for the mid- and high-exposure groups). Benzene exposure was associated with a concentration-dependent decrease in colony formation and progenitor cells were suggested to be more sensitive than circulating cells.

As described above, several epidemiology studies compared hematological variables for benzene-exposed workers to gender- and age-matched controls and noted hematological effects at relatively low concentrations (well below 1 ppm in some exposure groups) (Lan et al. 2004a, 2004b; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b). An alternative approach was used by Collins et al. (1991, 1997) and Tsai et al. (1983, 2004). This approach utilized a defined range of clinically normal hematological values and compared the prevalence of abnormal results between benzene-exposed workers and unexposed controls. Collins et al. (1991) found no significant correlations between benzene exposure and the prevalence of abnormal hematological values among 200 workers exposed to benzene at estimated concentrations ranging from 0.01 to 1.4 ppm, relative to the prevalence of abnormal hematological values obtained from 268 unexposed workers in the same plant. In a more recent evaluation, Collins et al. (1997) found no significant correlation between exposure to benzene at an 8-hour TWA of 0.55 ppm and prevalence of clinically-defined lymphopenia (or other measures of hematotoxicity including mean corpuscular volume [MCV], and counts of WBCs, RBCs, hemoglobin, and platelets) among a group of 387 workers exposed for ≥ 5 years. Tsai et al. (1983) measured benzene levels in a Texas refinery at one time point (mean benzene concentration of 0.53 ppm) and found that hemoglobin, hematocrit, RBCs, WBCs, and thrombocytes of workers exposed for up to 21 years were within the range of normal values. Tsai et al. (2004) found no clinically adverse hematotoxic effects among a large group of 1,200 petrochemical employees with mean 8-hour TWA benzene exposure levels of 0.6 ppm from 1977 to 1988 and 0.14 ppm from 1988 to 2002. The normal range for certain hematological parameters is necessarily broad due to large interindividual differences in clinical status. Restricting the comparison of benzene-exposed and nonexposed populations to only those values

3. HEALTH EFFECTS

considered clinically abnormal or adverse may reduce the sensitivity of the study to detect meaningful changes at the population level.

Many reports of hematological effects in benzene-exposed workers involve estimated exposure levels well in excess of 1 ppm. A series of studies conducted on Turkish workers exposed to benzene-containing adhesives in various occupations showed increased severity of effects with increased levels or duration of exposure. The initial hematological study examined 217 male workers who were exposed for between 4 months and 17 years to benzene-containing solvents (Aksoy et al. 1971). The concentration of benzene in the work area ranged from 15 to 30 ppm outside work hours and reached a maximum of 210 ppm when benzene-containing adhesives were being used. Fifty-one of the workers showed clinical hematological abnormalities such as leukopenia, thrombocytopenia, eosinophilia, and pancytopenia. An additional cohort was identified that included 32 shoe manufacturers who had worked with benzene for 4 months to 15 years at concentrations of 15–30 ppm outside work hours and 210–640 ppm during the use of benzene, and who showed pancytopenia (Aksoy et al. 1972). Examination of these people revealed disruptions in bone marrow function, including cases of hypoplastic, acellular, hyperplastic, or normoblastic bone marrow. The continuing assessment further identified that ineffective erythropoiesis or increased hemolysis may have been responsible for the reticulocytosis, hyperbilirubinemia, erythroblastemia, increase in quantitative osmotic fragility, and elevated serum lactate dehydrogenase levels observed in some patients.

Leukopenia, lymphocytosis, a biphasic leukocyte response, and bone marrow hypercellularity were reported for workers in a Russian industry who were exposed to a complex of hydrocarbons that included benzene, cyclohexane, and 1,3-butadiene (Doskin 1971). Benzene levels were estimated at 3.2–12.8 ppm, which are 2–8 times the Russian standard. Leukopenia was observed by Xia et al. (1995) in Chinese workers exposed to 0.69–140 ppm (mean=6 ppm) benzene for more than 1 year. Enlarged RBCs, transient anemia, reduced hemoglobin concentrations, and clinically increased MCV were reported in workers in a chemical factory who were exposed to over 25 ppm of benzene in the workplace for an average of 9 years (Fishbeck et al. 1978).

Dosemeci et al. (1996) assessed the relative risk of abnormal hematological values with increasing benzene levels in a study of workers employed in 672 rubber and rubber glue application facilities in China between 1949 and 1987. Compared to workers with estimated exposures <5 ppm, relative risks of abnormal hematological values (indicating benzene poisoning) by estimated exposure intensity at 1.5 years prior to clinically-diagnosed benzene poisoning were 2.2 (95% confidence interval [CI] 1.7–

3. HEALTH EFFECTS

2.9), 4.7 (95% CI 3.4–6.5), and 7.2 (95% CI 5.3–9.8) for exposures in the ranges of 5–19, 20–39, and >40 ppm, respectively.

Examination of individual hematological records, demographic data, and chronological work histories of 459 workers employed at a rubber products manufacturing plant in Ohio between 1940 and 1975 revealed significant decreases in WBC and RBC counts and hemoglobin for the period of 1940–1948 when the exposure was 75 ppm (Kipen et al. 1989). The trend was not apparent during later years (1949–1975) when the exposure was decreased to 15–20 ppm. A more focused study of many of the same workers during their first year of employment revealed significantly lower WBC and RBC counts in employees exposed to benzene levels greater than the median (estimated at 40–54 ppm), compared to those with lower estimated exposure levels (Cody et al. 1993). Using a nested case-control approach at the same rubber products manufacturing plant to assess a possible exposure-response relationship between benzene and the risk of developing a low WBC or RBC count in workers for whom hematologic screening data were available, Ward et al. (1996) reported a strong correlation between low WBC counts and benzene exposure and a weak correlation between RBC count and benzene exposure.

More severe effects, including preleukemia or acute leukemia, were observed in 26 out of 28,500 benzene workers exposed to 210–650 ppm for 1–15 years (Aksoy et al. 1974). Clinical features of the preleukemia included one or more of the following: anemia, leukopenia, pancytopenia, bone marrow hyperplasia, pseudo-Pelger-Huet anomaly, and splenomegaly. A study was conducted 2–17 years following the last exposure of 44 pancytopenic patients exposed to benzene (150–650 ppm) in adhesives for 4 months to 15 years (Aksoy and Erdem 1978). Of these patients, complete remission was seen in 23, death due to complications of pancytopenia in 14, death due to myeloid metaplasia in 1, and leukemia in 6. When benzene concentrations in factories decreased in later years, less severe effects were seen. At 40 tire manufacturing plants, 231 workers were exposed for 28 months to 40 years (mean 8.8 years) to benzene-containing solvents and thinners (Aksoy et al. 1987). The decrease in benzene content of materials used in these workshops and the corresponding reduction in air concentration (most samples <1 ppm) paralleled the decrease in the number of hematological abnormalities reported for benzene-exposed workers.

Another study revealed effects, ranging from mild to severe, of benzene exposure in factory workers in China (Yin et al. 1987c). Of the 528,729 workers, 95% were exposed to mixtures of benzene, toluene, and xylene, while 5% (26,319 workers) were exposed to benzene alone at 0.02–264 ppm in air in 95% of the work stations. Over half of the work stations had levels of benzene in the air of less than 13 ppm;

3. HEALTH EFFECTS

about 1% had levels of 13–264 ppm. Benzene toxicity, as indicated by leukopenia, aplastic anemia, and leukemia, was seen in 0.94% of the workers exposed to benzene and 0.44% of the workers exposed to the mixtures. Similar toxicity was found in employees of 28 of the 141 shoe factories studied (124 cases in 2,740 employees) (Yin et al. 1987c). A positive correlation was observed for prevalence of adverse benzene effects and benzene concentration in data from these 28 shoe factories. The authors determined that the affected people were exposed to benzene concentrations >29 ppm. In one workshop, there were 4 cases of aplastic anemia in 211 workers. These workers were exposed to benzene at a mean concentration of 324 ppm during an 8-month period of employment. The prevalence of aplastic anemia in the shoe-making industry was about 5.8 times that in the general population. The main limitation of this study is the lack of information on the duration of exposure.

People who were exposed to high levels of benzene vapors in the printing industry also showed severe hematological effects. One study evaluated 332 workers who were exposed to 11–1,060 ppm of benzene for 6 months to 5 years. Detailed blood studies performed on 102 of these workers revealed benzene poisoning in 22 workers characterized by pancytopenia or other clinical signs (Goldwater 1941; Greenburg et al. 1939). Another study reported 6 cases of pancytopenia and bone marrow dysplasia in printers exposed to 24–1,060 ppm of benzene (Erf and Rhoads 1939).

Animal studies have been designed to characterize exposure-response relationships of benzene hematotoxicity; the results provide support to the human data. Animal responses to benzene exposure are variable and may depend on factors such as species, strain, duration of exposure, and whether exposure is intermittent or continuous. Wide variations have also been observed in normal hematological parameters, complicating statistical evaluation. However, the studies show that benzene exerts toxic effects at all phases of the hematological system, from stem cell depression in the bone marrow, to pancytopenia, to histopathological changes in the bone marrow. Effects on leukocytes, lymphocytes, and bone marrow are also discussed in Section 3.2.1.3.

Repeated acute-, intermediate-, and chronic-duration inhalation exposure of laboratory animals (mainly mice, but also rats, rabbits, and guinea pigs) to benzene vapor concentrations ranging from 10 to >300 ppm demonstrate significant decreases in blood values that include RBCs, total WBCs, lymphocytes, granulocytes, hematocrit, and hemoglobin (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite et al. 1982; Farris et al. 1997a, 1997b; Gill et al. 1980; Green et al. 1981a, 1981b; Li et al. 1986; Rozen and Snyder 1985; Rozen et al. 1984; Snyder et al. 1982; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956). Pancytopenia was noted in the study of Ward et al. (1985).

3. HEALTH EFFECTS

Short-duration exposure of mice to benzene has been shown to cause both concentration- and duration-related reductions in bone marrow cellularity (number of nucleated cells) and the number of colony-forming granulopoietic stem cells (CFU-C), and an increased frequency of micronucleated polychromatic erythrocytes (MN-PCE) (Toft et al. 1982). Mice that were exposed continuously at benzene concentrations ≥ 21 ppm in air for 4–10 days showed significant changes in all three parameters. Intermittent exposure (8 hours/day, 5 days/week for 2 weeks) to 21 ppm significantly reduced the number of CFU-C and elevated the frequency of MN-PCE, but did not affect bone marrow cellularity. Intermittent exposure at concentrations ≥ 50 ppm caused significant changes in all three parameters and decreased the ability of the spleen to form mature cells (as measured by numbers of colony-forming units of stem cells). When mice were intermittently exposed for 2 weeks, decreased cellularity and CFU-C per tibia were observed at 95 ppm after 6–8 hours/day exposure, whereas at 201 ppm benzene, decreased cellularity (but no effect on CFU-C) was noted after 2 hours/day exposure. A decrease in cellularity and CFU-C was observed after 4–8 hours/day exposure to 201 ppm benzene.

Results of numerous additional studies of laboratory animals (mainly mice, which are particularly sensitive to benzene hematotoxicity, but also rats and pigs) support findings of benzene-induced effects on bone marrow cellularity (hyper- and/or hypocellularity) and colony-forming stem cells, as well as granulocytic hyperplasia, following repeated acute-, intermediate-, or chronic-duration inhalation exposure to benzene vapors at concentrations ranging from 10 to 500 ppm (Baarson and Snyder 1991; Baarson et al. 1984; Chertkov et al. 1992; Corti and Snyder 1996; Cronkite et al. 1982, 1985, 1989; Dempster and Snyder 1991; Dow 1992; Farris et al. 1993, 1997b; Neun et al. 1992, 1994; Plappert et al. 1994a, 1994b; Snyder et al. 1978a, 1980, 1982; Vacha et al. 1990). For example, Dempster and Snyder (1991) observed a 50% reduction in CFU-E (erythroid progenitor cells) in bone marrow of DBA/2 mice exposed to 10 ppm benzene for 6 hours/day for 5 days. Farris et al. (1997b) reported benzene-induced decreased numbers of total bone marrow forming cells, progenitor cells, and differentiating hematopoietic cells in mice exposed to benzene vapor concentrations ≥ 200 ppm, 6 hours/day, 5 days/week for up to 8 weeks. Replication of primitive progenitor cells in the bone marrow was increased during the exposure period, presumably as compensation for cytotoxicity. Granulocytic hyperplasia was detected in the bone marrow of mice exposed to 300 ppm benzene in air for 6 hours/day, 5 days/week for 16 weeks, and held 18 months after the last exposure (Farris et al. 1993). Prolonged exposure to lower levels had a greater hematotoxic effect than exposure to higher levels for a shorter period of time. Recovery from hematotoxicity has been demonstrated following the cessation of exposure (Cronkite et al. 1982, 1985, 1989) and may be most closely associated with rate of exposure, since longer-term exposure of mice to

3. HEALTH EFFECTS

316 ppm of benzene caused irreparable hematologic injury, while the hematotoxic effects caused by shorter-term exposure to 10 times higher benzene concentrations were reversible (Cronkite et al. 1989).

One study revealed damaged erythrocytes in the peripheral blood of mice (Luke et al. 1988b). Cytotoxic damage in the bone marrow was dependent on strain and exposure duration. Peripheral blood smears were analyzed weekly from three strains of mice (DBA/2, B6C3F₁, and C57BL/6) exposed to 300 ppm benzene for 13 weeks (6 hours/day) for either 5 days/week (Regimen 1) or 3 days/week (Regimen 2). In all three strains, an initial severe depression in rate of erythropoiesis was observed. The return to normal was dependent on strain (Luke et al. 1988b) and regimen (Cronkite et al. 1989; Luke et al. 1988b). An increase in frequency of micronucleated normochromatic erythrocytes (MN-NCE) was observed to be dependent on strain (C57BL/6=B6C3F₁>DBA/2) and regimen (Regimen 1 > Regimen 2), whereas the increase in frequency of MN-PCE was dependent on strain (DBA/2>C57BL/6=B6C3F₁) but, for the most part, was not dependent on exposure regimen.

Damaged erythroblast-forming cells were also noted in bone marrow (Seidel et al. 1989). A substantial decrease in erythroid colony-forming units and smaller decreases in erythroid burst-forming units occurred in BDF₁ mice intermittently exposed to 100 ppm of benzene for 8 weeks (6 hours/day, 5 days/week). Although the effects in the 100 ppm group were not apparent at 8 weeks (the end of experiment), they did occur, and it took over 3 weeks for the erythroid burst-forming units and erythroid colony-forming units to return to their initial values. This reduction in the number of erythroid precursors was reflected by a slight reduction in the number of erythrocytes.

Benzene-induced hematotoxicity was also demonstrated in the spleen of rats and mice following intermediate- or chronic-duration repeated inhalation exposure (Snyder et al. 1978a, 1984; Ward et al. 1985). Snyder et al. (1978a, 1984) reported benzene-induced increased extramedullary hematopoiesis in the spleen. Ward et al. (1985) noted that the finding of hemosiderin in the spleen of benzene-exposed rats could be due to erythrocyte hemolysis.

Musculoskeletal Effects. A case of myelofibrosis was diagnosed in a 46-year-old man in October 1992 (Tondel et al. 1995). The patient worked from 1962 to 1979 as a gasoline station attendant. The patient was referred to the Department of Hematology, University Hospital in Linköping, Sweden, where a bone marrow biopsy was performed. The patient described symptoms of increasing muscle pain for 1 year, fatigue for 3 weeks, night sweats, and weight loss. A bone marrow biopsy showed myelofibrosis. The TWA concentration for gasoline station attendants was estimated to be <0.2 ppm. The occupational

3. HEALTH EFFECTS

standard for benzene in Sweden was 0.5 ppm (TWA) and the Swedish short-term exposure limit was 3 ppm. Ruiz et al. (1994) reported musculoskeletal effects in employees from a steel plant of Cubatao, S. Paulo, Brazil, who presented with neutropenia due to benzene exposure. Patients either were employed at the steel plant (mean time of 7 years and 4 months), or were employees of a building construction company working at repairs in the steel plant (mean time of 5 years and 5 months). Sixty percent of the workers had nonspecific clinical complaints such as myalgia.

Hepatic Effects. No specific reports of adverse hepatic effects of inhalation exposure to benzene in humans were found, although Aksoy et al. (1972) reported enlarged livers in workers chronically exposed to benzene at airborne concentrations ranging from 150 to 650 ppm.

CFY rats (20/group) were exposed to pure air, benzene (125 ppm) or benzene (400 ppm) and toluene (265 ppm) for 24 hours/day from gestational day (Gd) 7 through 14 (Tatrai et al. 1980a). The rats were then sacrificed on day 21 of pregnancy. Exposure to 125 ppm benzene caused a slight increase in relative liver weight of 4.67% compared to 4.25% in controls, which was not considered adverse. In a companion study, CFY rats were exposed to continuous benzene inhalation 24 hours/day from day 7 to day 14 of gestation at 0, 47, 141, 470, or 939 ppm atmospheric concentrations (Tatrai et al. 1980b). At 141 ppm benzene, there was a significant increase in relative maternal liver weight.

No treatment-related non-neoplastic histopathological effects on hepatic tissue were found in male Sprague-Dawley rats exposed to 0, 100, or 300 ppm benzene 5 days/week, 6 hours/day for life (Snyder et al. 1978a, 1984) or in AKR/J or C57B1/6J mice similarly exposed to 300 ppm for life (Snyder et al. 1978a, 1980).

Renal Effects. Very little data are available describing renal effects in humans after inhalation exposure to benzene. In a case study involving the death of an 18-year-old boy who intentionally inhaled benzene, the autopsy revealed acute kidney congestion (Winek and Collom 1971). No other details or data were given.

No treatment-related histopathological effects on kidney tissue were found in male Sprague-Dawley rats were exposed to 0, 100, or 300 ppm benzene 5 days/week, 6 hours/day for life (Snyder et al. 1978a, 1984) or in AKR/J or C57B1/6J mice similarly exposed to 300 ppm (Snyder et al. 1978a, 1980).

3. HEALTH EFFECTS

Dermal Effects. Dermal effects in humans have been reported after acute exposure to benzene vapors (Avis and Hutton 1993). After a fatal occupational exposure to benzene vapors on a chemical cargo ship for only minutes, autopsy reports on three victims revealed hemorrhagic respiratory tissues, and second degree burns on the face, trunk, and limbs (Avis and Hutton 1993). Skin irritation has been noted at occupational exposures of >60 ppm for up to 3 weeks (Midzenski et al. 1992). These effects are due to direct contact of the skin with the vapor, and other dermal effects resulting from direct contact of the skin are discussed in Section 3.2.3.2.

Ocular Effects. Three hundred solvent workers who had inhalation exposures for >1 year to benzene at 33 and 59 ppm for men and women, respectively, complained of eye irritation (Yin et al. 1987b).

Male Charles River CD rats exposed to 0, 1, 10, 30, or 300 ppm benzene 6 hours/day, 5 days/week for 10 weeks exhibited lacrimation at concentrations >10 ppm during the first 3 weeks of treatment (Shell 1980).

These effects are due to direct contact of the eyes with the vapor, and other ocular effects resulting from direct contact of the eyes are discussed in Section 3.2.3.2.

Body Weight Effects. Relatively few studies in animals report changes in body weight after inhalation exposure to benzene. No change in body weight was noted in Sprague-Dawley rats or CD-1 mice exposed to 300 ppm benzene for 13 weeks (Ward et al. 1985). No significant decrease in body weight was observed in CD-1 mice exposed to doses up to 4,862 ppm for 6 hours/day, for 5 days, or at lower doses of 9.6 ppm for 50 days (Green et al. 1981b). Decreases in body weight (15%) were seen in DBA/2 mice after exposure to 300 ppm benzene in air for 6 hours/day, 5 days/week for 2 weeks (Chertkov et al. 1992). Decreased body weight (16–18%) has also been noted in mice exposed to doses of approximately 200 ppm of benzene for 6 hours/day for 7 or 14 days (Aoyama 1986). C57BL mice exhibited a 59% decrease in body weight gain after exposure to 300 ppm benzene, 5 hours/day, 6 days/week over their lifetime (Snyder et al. 1980). Decreased maternal body weight and weight gain have been observed in Sprague-Dawley rats exposed to 50 ppm benzene during gestation days (Gds) 6-15 (Kuna and Kapp 1981), but not in rats exposed to doses up to 300 ppm during pre-mating, mating, gestation, and lactation (Kuna et al. 1992). CFY rats (20/group) were exposed to pure air, benzene (125 ppm), or benzene (400 ppm) plus toluene (265 ppm) for 24 hours/day from Gd 7 through 14 (Tatrai et al. 1980a). The rats were then sacrificed on day 21 of pregnancy. Exposure to 125 ppm benzene caused decreased maternal weight gain (46.74% of starting weight as opposed to 68.82% of starting

3. HEALTH EFFECTS

weight in controls). Decreased maternal weight gain was also observed in a companion experiment in which the rats were exposed to doses as low as 47 ppm using the same study design (Tatrai et al. 1980b). Decreased maternal body weight was also observed in rats exposed to 2,200 ppm benzene during gestation (Green et al. 1978). Rabbits exposed to 313 ppm benzene on Gd 7–20 exhibited decreased maternal weight gain (Ungvary and Tatrai 1985). Kunming mice exposed to 12.52 ppm benzene for 2 hours/day, 6 days/week for 30 days exhibited no adverse effect on body weight (Li et al. 1992). Sprague-Dawley rats received 100 or 300 ppm benzene vapor for 6 hours/day, 5 days/week for life (Snyder et al. 1978a, 1984). Decreased weight gain, which continued throughout the study, was observed at 30 weeks at 300 ppm, but not at 100 ppm. AKR mice exposed to 100 or 300 ppm and C57BL mice exposed to 300 ppm benzene vapor for 6 hours/day, 5 days/week for life had decreased weight gain at 300 ppm (Snyder et al. 1978a, 1980).

3.2.1.3 Immunological and Lymphoreticular Effects

Immunological effects have been reported in humans with occupational exposure to benzene. There are two types of acquired immunity, humoral and cellular, and benzene damages both. First, benzene has been shown to alter humoral immunity (i.e., to produce changes in levels of antibodies in the blood). Painters who were exposed to benzene (3–7 ppm), toluene, and xylene in the workplace for 1–21 years showed increased serum immunoglobulin values for IgM and decreased values for IgG and IgA (Lange et al. 1973b). The decreased levels of immunoglobulins may represent suppression of immunoglobulin-producing cells by benzene. Other adverse reactions, characterized by a reaction between leukocytes and agglutinins, occurred in 10 of 35 of these workers (Lange et al. 1973a). These results suggest the occurrence of allergic blood dyscrasia in some persons exposed to benzene. However, since the workers were exposed to multiple solvents, the specific role of benzene is uncertain.

The second type of immunity, cellular immunity, is affected by changes in circulating leukocytes and a subcategory of leukocytes, called lymphocytes. Leukopenia was found in a series of studies of workers exposed to benzene at levels ranging from 15 to 210 ppm in various manufacturing processes in Turkey (Aksoy et al. 1971, 1987). Another study also noted signs of preleukemia that included loss of leukocytes and other blood elements, bone marrow histopathology, and enlarged spleens (Aksoy et al. 1972, 1974). Other studies in chronically exposed workers also showed losses of lymphocytes and other blood elements (Cody et al. 1993; Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Ruiz et al. 1994; Yin et al. 1987c). In these studies, benzene levels in workplace air ranged from 1 to 1,060 ppm. Hematological effects reported in these studies are described in Section 3.2.1.2. In one study, routine

3. HEALTH EFFECTS

leukocyte counts conducted every 3 months on employees of a small-scale industry in China revealed leukopenia in workers exposed to as little as 0.69–140 ppm (mean=6 ppm) for an average period of 5–6 years (Xia et al. 1995). Leukocyte alkaline phosphatase (LAP) activity was increased in benzene workers exposed to about 31 ppm for a chronic time period (Songnian et al. 1982). Increased LAP activity is an indicator of myelofibrosis and is associated with both decreased white blood cell counts and with changes in bone marrow activity. The change in LAP activity could be used in the diagnosis of benzene poisoning since it was more sensitive than the change in the leukocyte count, although it is not a biomarker that is specific for benzene exposure. A study conducted by Li et al. (1994) during 1972–1987 examined 74,828 benzene-exposed workers employed in 672 factories and 35,805 unexposed workers from 109 factories located in 12 cities in China. Estimates of gender-specific rate ratios and a comparison of the rate ratios for females to the rate ratios for males were calculated for the incidence of hematopoietic and lymphoproliferative (HLP) disorders, comparing all exposed workers in each of the occupational groups to unexposed workers. Small increases in relative risks for all HLP disorders for both genders were observed among chemical and rubber manufacturing workers, painters, and paint manufacturers. In another study, an increase in leukocyte count and alkaline phosphatase score was observed in a pipe-fitter who was chronically exposed to 0.9 ppm benzene in addition to other solvents (Froom et al. 1994).

Animal studies support the observations made in humans and show that benzene affects humoral and cellular immunity. A decrease in spleen weight was observed in mice after exposure to benzene at a concentration of 25 ppm, 6 hours/day for 5 days, the same exposure concentration at which a decrease in circulating leukocytes was observed (Wells and Nerland 1991). Benzene decreases the formation of the B-lymphocytes that produce the serum immunoglobulins or antibodies. Exposure to benzene at 10 ppm and above for 6 days decreased the ability of bone marrow cells to produce mature B-lymphocytes in C57BL/6 mice (Rozen et al. 1984). The spleen was also inhibited from forming mature T-lymphocytes at exposure levels of 31 ppm and above. Mitogen-induced blastogenesis of B- and T-lymphocytes was depressed at 10 ppm and above. Peripheral lymphocyte counts were depressed at all levels, whereas erythrocyte counts were depressed only at 100 and 300 ppm. This study is the basis for the acute-duration inhalation MRL of 0.009 ppm (see footnote to Table 3-1 and Appendix A).

A continuation of this line of studies for 6 days to 23 weeks at 300 ppm showed continued decreases in numbers of mature B- and T-lymphocytes produced in the bone marrow, spleen, and thymus (Rozen and Snyder 1985). Abnormalities of humoral and cell-mediated immune responses following benzene exposure are presumably caused by a defect in the lymphoid stem cell precursors of both T- and B-lymphocytes. Bone marrow cellularity increased 3-fold, and the number of thymic T-cells increased

3. HEALTH EFFECTS

15-fold in benzene-exposed mice between the 6th and the 30th exposure. No corresponding increase in splenic cells was noted. The marked increase in the numbers of cells in bone marrow and thymus was interpreted by the authors to indicate a compensatory proliferation in these cell lines in response to benzene exposure, which may play a role in the carcinogenic response of C57BL mice to inhaled benzene. The lack of response in the spleen suggests a lack of lymphoid restorative capacity in that organ.

Other studies have also shown similar effects on immune functions following inhalation exposure to benzene. These include decreased numbers of circulating leukocytes and decreases in bone marrow cellularity in mice exposed to 100 ppm and higher, 24 hours/day for up to 8 days (Gill et al. 1980); decreased leukocytes and increased leukocyte alkaline phosphatase in rats exposed to 100 ppm for 7 days (Li et al. 1986); decreased leukocytes and bone marrow cellularity in DBA/2 mice exposed to 300 ppm benzene for 2 weeks (Chertkov et al. 1992); decreased leukocytes in mice exposed to 300 ppm for 10 days (Ward et al. 1985); bone marrow hyperplasia, lymphocytopenia, and anemia in mice repeatedly exposed to 100 ppm or higher for a lifetime (Snyder et al. 1978a, 1980); and leukopenia and lymphopenia in rats repeatedly exposed to 100 ppm for life (Snyder et al. 1978a, 1984). Aoyama (1986) noted decreased spleen and thymus weights and decreased levels of B- and T-lymphocytes in blood and spleen in mice exposed 6 hours/day to approximately 48 ppm of benzene for 14 days. Exposures of mice to benzene vapor concentrations ≥ 100 ppm, 6 hours/day, 5 days/week resulted in significantly reduced numbers of lymphocytes (ranging from 25 to 43% lower than controls) in the spleen, thymus, and femur as early as 1 week following the initial exposure, and persisting throughout an 8-week exposure period (Farris et al. 1997a). In rats, exposure to benzene vapors at a concentration of 400 ppm (but not 200 ppm) for 6 hours/day, 5 days/week for 4 weeks resulted in reduced thymus weight and decreased splenic lymphocytes (Robinson et al. 1997).

Reduced bone marrow cellularity was observed in Swiss Webster and C57BL/6J mice after exposure to 300 ppm benzene for 2 weeks (Neun et al. 1992). Decreased granulopoietic stem cells were observed at 21 ppm in NMRI mice after exposures of 10 days to 2 weeks (Toft et al. 1982). Green and colleagues examined the effect of benzene inhalation on peripheral blood and bone marrow and spleen cells (Green et al. 1981a, 1981b) of CD-1 mice following exposures to a number of regimens. In the acute studies, individual test groups were exposed by inhalation to mean benzene concentrations of 0, 1.1, 9.9, 103, 306, 603, 1,276, 2,416, or 4,862 ppm, 6 hours/day for 5 days. Intermediate studies consisted of two different exposure regimens and concentrations: 6 hours/day, 5 days/week for 10 weeks (50 days) to a mean concentration of 0 or 9.6 ppm benzene; or 6 hours/day, 5 days/week for 26 weeks to a mean concentration

3. HEALTH EFFECTS

of 0 or 302 ppm benzene. In the acute studies, marrow and splenic cellularities were reduced at 103 ppm. Splenic granulocytes were reduced at all exposure levels except at 9.9 ppm (Green et al. 1981b). The decrease in spleen cellularity correlated with a reduction in spleen weight at all concentrations ≥ 103 ppm. Mean spleen weights were significantly depressed at 1.1 ppm and at doses above 9.9 ppm, but not at 9.9 ppm. In Green et al. (1981a), marrow concentration of GM-CFU-C was equivalent to or greater than control values at all levels; however, splenic GM-CFU-C concentration was decreased at 103 ppm. Femoral and splenic CFU-S and GM-CFU-C per organ were depressed at 103 ppm. Absolute numbers of GM-CFU-C/femur or spleen were significantly reduced at all higher concentrations. There was no change in the colony/cluster ratio observed in the 5-day experiment. In the 50-day experiments, increased spleen weight, splenic nucleated cellularity, splenic nucleated erythrocytes, and CFU-S were seen at 9.6 ppm (Green et al. 1981a, 1981b). Exposure to 302 ppm for 26 weeks resulted in reduced marrow and spleen cellularity and decreased spleen weight (Green et al. 1981a, 1981b).

Sprague-Dawley SD/Tex rats were exposed to benzene vapor at 0 or 500 ppm for 5 days/week, 6 hours/day for 3 weeks (Dow 1992). In the bone marrow differential counts, rats showed a relative decrease in lymphoid cells at the 500 ppm dose level. There were also decreases in myeloid cells of animals exposed to 500 ppm benzene. In a companion study, purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). Exposure to 500 ppm resulted in significant decreases in total white blood cells, T-cells, peripheral blood lymphocytes, and proportion of myeloid cells in bone marrow counts.

Another series of experiments revealed that exposures as low as 25 ppm for 2 weeks caused decreases in the numbers of circulating lymphocytes but did not affect the bone marrow cellularity in C57BL/6 or CBA/Ca mice (Cronkite 1986; Cronkite et al. 1985). Increasing the duration (up to 16 weeks) or concentration (up to 300–400 ppm) produced the same effect of reduced peripheral blood lymphocytes as well as decreased bone marrow cellularity that persisted for up to 8 weeks after exposure (Cronkite et al. 1982, 1985). Similar observations were made in two groups of male CBA/Ca mice exposed to a total of 6,000 ppm of benzene by inhalation using two different regimens (Cronkite et al. 1989). One group was exposed to 316 ppm for a total of 19 times, while the second group was exposed to 3,000 ppm twice. Although both groups had significantly decreased lymphocyte counts, the lymphocyte numbers were much more depressed in the group of mice exposed to 316 ppm of benzene over 19 days. This suggests that, at relatively high exposure levels, repeated or prolonged exposure is more potent than the magnitude of exposure in causing lymphopenia. In both groups, the lymphocyte numbers had not returned to normal

3. HEALTH EFFECTS

values by 214 days post exposure. The femoral bone marrow cellularity returned to normal levels 32 days after exposure in both groups of mice.

In female BDF₁ mice (C57BL/6xDBA/2F₁ hybrids) exposed to 0, 300, or 900 ppm benzene 6 hours/day for 5 days, there was a relative increase in helper lymphocytes (CD4+) at days 3–5, leading to an increase of the T4/T8 (CD4+–CD8+) ratio from 2 in controls to higher values at 8 weeks (Plappert et al. 1994a). No concentration dependency was observed. Granulocytic hyperplasia of the spleen was observed in CBA/Ca mice exposed to 300 ppm benzene for 16 weeks (Farris et al. 1993).

Leukopenia, granulocytopenia, and lymphocytopenia were observed in C57BL/6 mice exposed to 1,000 ppm benzene for 6 hours/day, 5 days/week for up to 6 weeks (Gill et al. 1980). Decreased leukocyte counts were also noted in Sprague-Dawley rats and CD-1 mice exposed to 300 ppm benzene 6 hours/day, 5 days/week for up to 13 weeks (Ward et al. 1985). In mice, the most common compound-related histopathological findings were splenic periarteriolar lymphoid sheath depletion, lymphoid depletion in the mesenteric lymph node, and plasma cell infiltration of the mandibular lymph node. Exposure to the highest concentration caused a decrease in leukocyte and lymphocyte counts in Sprague-Dawley rats. Hematological changes at 300 ppm were accounted for by decreased leukocyte counts in males on day 14 and in females on day 91. Decreases in percentage of lymphocytes in males and females started on day 14 and lasted through day 91. Rats exhibited decreased femoral marrow cellularity as the only histological change. Treatment-related changes were not observed at lower concentrations. Rabbits, rats, and guinea pigs exposed to 80–88 ppm for 32–269 days also had decreased leukocyte counts (Wolf et al. 1956). Leukocyte alkaline phosphatase values were increased and leukocyte counts were decreased in rats exposed to 4,570 ppm for 20 weeks (Songnian et al. 1982). Exposure of C57BL mice to 10 ppm benzene for 6 hours/day, 5 days/week, for 24 weeks caused depressions in the numbers of splenic nucleated red cells and lymphocytes (Baarson et al. 1984).

Li et al. (1992) observed a 26% decrease in spleen weight in male Kunming mice exposed to 12.52 ppm benzene 2 hours/day, 6 days/week for 30 days. Examination of the bone marrow showed decreases in myelocytes, premyelocytes, myeloblasts, and metamyeloblasts at the same dose level.

Benzene also affects functional immune responses, as indicated by decreased resistance to infectious agents. Pre-exposure to benzene at 30 ppm for 5–12 days increased the bacterial counts in mice on day 4 of infection with *Listeria monocytogenes* (Rosenthal and Snyder 1985). Recovery of the immune system was noted on day 7. The effects did not occur at 10 ppm. In addition, a concentration-dependent

3. HEALTH EFFECTS

statistically significant depression was noted in T- and B-lymphocyte populations from day 1 through day 7 at 30 ppm and above. B-cells were more sensitive to benzene than were T-cells on a percentage-of-control basis. These results indicate a benzene-induced delay in immune response to *L. monocytogenes*. Concentrations of 200 or 400 ppm for 4–5 weeks (5 days/week) suppressed the primary antibody response to tetanus toxin in mice, but there was no effect at 50 ppm (Stoner et al. 1981). In another intermediate-duration exposure study, no changes were noted in the numbers of splenic B-cells, T-cells, or T-cell subsets in C57BL/6 mice exposed to 100 ppm of benzene 5 days/week for 20 days (Rosenthal and Snyder 1987). However, when splenic T-cells from mice treated with 10 ppm and 100 ppm were tested *in vitro* for their capacity to respond to foreign antigens (alloantigens) in the mixed lymphocyte reaction (MLR), the MLR response was delayed. Further analysis showing that this delayed MLR response was not due to the presence of benzene-induced suppressor cells and indicated that benzene impaired the functional abilities of alloreactive T-cells. This study is the basis for the intermediate-duration inhalation MRL of 0.006 ppm (see footnote to Table 3-1 and Appendix A). A similar *in vitro* observation was made using T-cells from mice exposed to 100 ppm of benzene 5 days/week for 3 weeks. These cells had a reduced tumor cytolytic activity, suggesting a benzene-induced impairment of cell-mediated immunity. The impaired cell-mediated immune function was also apparent *in vivo*. Mice exposed to 100 ppm for a total of 100 days were challenged with 10,000 polyoma virus-induced tumor cells (PYB6), and 9 of 10 mice had reduced tumor resistance and developed tumors that were lethal (Rosenthal and Snyder 1987).

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

3.2.1.4 Neurological Effects

Following acute inhalation of benzene, humans exhibit symptoms indicative of central nervous system effects (Cronin 1924; Flury 1928; Greenburg 1926; Midzenski et al. 1992). These symptoms, reported to occur at levels ranging from 300 to 3,000 ppm, include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. Acute exposure (5–10 minutes) to higher concentrations of benzene (approximately 20,000 ppm) can result in death, which has been associated with vascular congestion in the brain (Avis and Hutton 1993; Flury et al. 1928). Lethal exposures are also associated with nonspecific neurological symptoms similar to those reported for nonlethal exposures. These symptoms are similar to the consequences of exposure to multiple organic solvents and are reversible when symptomatic workers are removed from the problem area (Kraut et al. 1988; Yin et al. 1987b). In reports

3. HEALTH EFFECTS

of cases of benzene poisoning, subjects exhibited headaches, nausea, tremor, convulsions, and unconsciousness, among other neurological effects (Cronin 1924; Greenburg 1926; Midzenski et al. 1992; Tauber 1970).

Chronic exposure to benzene has been reported to produce neurological abnormalities in humans. Of eight patients (six with aplastic anemia and two with preleukemia) with previous occupational exposure to adhesives and solutions containing 9–88% benzene, four of the six patients with aplastic anemia showed neurological abnormalities (global atrophy of lower extremities and distal neuropathy of upper extremities) (Baslo and Aksoy 1982). Air concentrations in the workplace were reported to have reached levels of ≥ 210 ppm. These findings suggest that benzene may induce toxic effects on the nervous system involving peripheral nerves and/or spinal cord. The limitations of this study are that benzene exposure levels were not monitored and that there was a possibility of an additional exposure to toluene (6.37–9.25%).

Chronic exposure to benzene and toluene was studied in 121 workers exposed to benzene for 2–9 years (Kahn and Muzyka 1973). The air concentration of benzene between 1962 and 1965 was 6–15.6 ppm (20–50 mg/m³), while the toluene vapors did not exceed the 5 mg/m³ level. Subsequently (the authors do not specify when), the air levels of both benzene and toluene have not exceeded the 5 mg/m³ level. Seventy-four of the examined workers complained of frequent headaches (usually at the end of the work day), became tired easily, had difficulties sleeping, and complained of memory loss. The limitations of this study are that workers were exposed to both benzene and toluene and that the precise dose and duration of exposure are not known.

Tondel et al. (1995) reported the case of a gasoline station attendant who had worked from 1962 to 1979. The patient described symptoms of fatigue for 3 weeks and night sweats, among other symptoms.

The neurotoxicity of benzene has not been studied extensively in animals. Female Sprague-Dawley rats exhibited lethargy after exposure to 2,200 ppm benzene, but not 300 ppm, on Gd 6–15 (Green et al. 1978). Male albino SPF rats from a Wistar-derived strain exposed to benzene for 4 hours in glass chambers (dose not specified) exhibited depression of evoked electrical activity in the brain; the authors calculated the 30% effect level (depressed activity) as 929 ppm (Frantik et al. 1994). When female H strain mice were exposed to benzene for 2 hours, the 30% effect level for depression of evoked electrical activity in the brain was 856 ppm (Frantik et al. 1994). In rabbits, symptoms that occurred 3.7 minutes following acute exposure to benzene at 45,000 ppm were relaxation and light narcosis

3. HEALTH EFFECTS

(Carpenter et al. 1944). As the time after exposure progressed, so did the symptoms to include excitation, chewing, and tremors (after 5 minutes), loss of pupillary reflex to strong light (after 6.5 minutes), loss of blinking reflex (after 11.4 minutes), pupillary contraction (after 12 minutes), and involuntary blinking (after 15.6 minutes). Behavioral tests of C57BL/6 mice showed significant increase in licking of sweetened milk after 1 week of exposure to 300 ppm; a 90% decrease in hind limb grip strength after one exposure to 1,000 or 3,000 ppm (data for 100 ppm were not reported); and tremors after one exposure to 3,000 ppm that subsided 30 minutes after the exposure (Dempster et al. 1984). In another study, designed to reflect occupational exposure, male CD-1 and C57BL/6 mice were exposed to 300 or 900 ppm of benzene 6 hours/day for 5 days followed by 2 weeks of no exposure after which the exposure regimen was repeated for an unspecified amount of time (Evans et al. 1981). The following seven categories of behavioral activities were monitored in exposed and control animals: stereotypic behavior, sleeping, resting, grooming, eating, locomotion, and fighting. Only minimal and insignificant differences were observed between the two strains of mice. Increased behavioral activity was observed after exposure to benzene in both strains of mice. Mice exposed to 300 ppm of benzene had a greater increase than those exposed to 900 ppm, probably because of narcosis-like effects induced at the higher exposure level (Evans et al. 1981). It is not known if benzene induces behavioral changes by directly acting upon the central nervous system. It is also not known whether these changes occur before or after hematological changes.

Li et al. (1992) exposed male Kunming mice to 0, 0.78, 3.13, or 12.52 ppm benzene for 2 hours/day, 6 days/week for 30 days, and then monitored brain and blood acetylcholinesterase, forelimb grip strength, locomotor activity, and rapid response. Significantly increased grip strength was observed at 0.78 ppm, whereas at the higher doses, grip strength decreased significantly. Rapid response showed a significant increase at the low dose; the two higher doses showed a significant depressed rapid response. Locomotor activity increased at the low dose, was similar to control values at the middle dose, and decreased at the high dose. However, these changes were not significantly different from the control values. A significant decrease in acetylcholinesterase activity was noted in the brain, but it was not large enough to be considered adverse; no change in acetylcholinesterase levels in the blood was observed. However, the exposure levels used by Li et al. (1992) were more than 10-fold lower than those eliciting signs of neurotoxicity in other animal studies, an indication that the actual exposure levels in the study of Li et al. (1992) may have been higher than indicated. Uncertainty regarding actual exposure levels renders this study of little value for purposes of risk assessment.

3. HEALTH EFFECTS

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

Data on the reproductive effects of occupational exposure to benzene suggest that benzene may impair fertility in women (Mukhametova and Vozovaya 1972; Vara and Kinnunen 1946). However, the findings are inconclusive due to uncertainties in exposure assessment and the limited data collected. In one study, 30 women with symptoms of benzene toxicity were examined (Vara and Kinnunen 1946). The levels of benzene in air were not specified, but are assumed to have been much greater than the 1 ppm permitted in today's working environment. Twelve of these women had menstrual disorders (profuse or scanty blood flow and dysmenorrhea). Ten of the 12 women provided information on fertility. Of these 10 women, 2 had spontaneous abortions, and no births occurred during their employment, even though no contraceptive measures had been taken. This led the investigators to suggest that benzene has a detrimental effect on fertility at high levels of exposure. However, the study failed to provide verification that the absence of birth was due to infertility. Gynecological examinations revealed that the scanty menstruations of five of the patients were due to ovarian atrophy. This study is limited in that an appropriate comparison population was not identified. Additionally, little follow-up was conducted on the 30 women with regard to their continued work history and possible symptoms of benzene toxicity.

Disturbances of the menstrual cycle were found in women workers exposed to aromatic hydrocarbons (benzene, toluene, xylene) (Michon 1965). The exposure levels of benzene and toluene were below 0.25 ppm. The observed group consisted of 500 women, 20–40 years old. One hundred controls were included in the study. The results showed that 21% of exposed women whose work involved sitting or standing had irregular menstrual cycles compared to 12% in the control group. Brief (up to 2 days), long (6–9 days), and prolonged (over 9 days) menstrual cycles were present in 26% of women who performed lifting during their work as compared to 13% in the control group. Irregular amounts of menstrual flow and pain were also observed in female workers exposed to aromatic hydrocarbons. The major limitations of this study are that the exposure occurred from a mixture of chemicals, levels of exposure were not well defined, duration of exposure was not stated, and activities of the controls were not provided.

Another study examined the reproductive function and incidence of gynecological effects in 360 women exposed to petroleum (a major source of benzene) and chlorinated hydrocarbons both dermally and by inhalation (Mukhametova and Vozovaya 1972). However, dermal exposure was considered to be

3. HEALTH EFFECTS

negligible. The concentrations of benzene in the air were not well documented. When compared to female workers with no chemical exposure, female gluers had developed functional disturbances of the menstrual cycle. Additionally, as chemical exposure time increased, there were increases in the number of premature interruptions of pregnancy, the percentage of cases in which the membranes ruptured late, and the number of cases of intrauterine asphyxia of the fetus. The study limitations (including lack of exposure history, simultaneous exposure to other substances, and lack of follow-up) make it difficult to assess the effects of benzene on reproduction.

Reproductive competence of male workers in two organic chemical factories in France was evaluated by Stucker et al. (1994). Analysis of 1,739 pregnancies that ended in spontaneous abortion or birth was presented. Paternal exposure to benzene for each pregnancy was described as exposure in the 3 months immediately before conception, and as previous job exposures. Benzene exposure was graded at two levels: <5 ppm (low) and ≥ 5 ppm (moderate). Of the 1,739 pregnancies described, 171 ended in a spontaneous abortion (rate=9.8%). According to exposure categories, 1,277 pregnancies were defined as non-exposed (mate not exposed) and 270 whose mates were exposed at some time before conception. For the 270 pregnant women, 145 of their mates were exposed during the 3 months immediately preceding conception. The frequency of spontaneous abortion was not significantly higher for the paternal group exposed at any time before conception than in the non-exposed group; nor was it higher for the group exposed during the 3 months immediately before conception.

In CFY rats exposed to either pure air or benzene (125 ppm) for 24 hours/day from Gd 7 through 14, no effect on implantation number was observed (Tatrai et al. 1980a). No changes in maternal body weight were observed in Sprague-Dawley rats exposed to 100 ppm benzene for 6 hours/day on Gd 6–15 (Coate et al. 1984). Pregnant rabbits exposed 12 hours/day to 156.5 or 313 ppm benzene on Gd 7–20 showed an increase in the number of abortions and resorptions at 312 ppm (Ungvary and Tatrai 1985). However, in other developmental toxicity studies, no effect on the number of resorptions was seen in rats at doses as high as 2,200 ppm (Green et al. 1978), in mice at 500 ppm (Murray et al. 1979), or in rabbits at doses of 500 ppm (Murray et al. 1979).

Reproductive effects have been noted in experimental animals exposed for intermediate durations, but the levels of exposure were higher (80–6,600 ppm) than those to which humans are exposed in the modern industrial environment (Ward et al. 1985; Wolf et al. 1956). In an intermediate-duration inhalation study, groups of male and female CD-1 mice were exposed to benzene vapor concentrations of 0, 1, 10, 30, or 300 ppm, 5 days/week, 6 hours/day to benzene vapor for 13 weeks (Ward et al. 1985). Histopathological

3. HEALTH EFFECTS

changes were observed in ovaries (bilateral cysts) and testes (atrophy/degeneration, decrease in spermatozoa, moderate increase in abnormal sperm forms) of mice exposed to 300 ppm benzene; the severity of gonadal lesions was greater in the males. An inhalation study was conducted exposing rats (6,600 ppm), rabbits (80 ppm), and guinea pigs (88 ppm) to benzene for 7–8 hours/day, 5 days/week for 93, 243, and 32 or 269 days, respectively (Wolf et al. 1956). Male rats showed an increase in testicular weight after 93 days at the 6,600 ppm level. The guinea pigs showed a slight increase in average testicular weight at the 88 ppm level. Rabbits showed slight histopathological testicular changes (degeneration of the germinal epithelium) when exposed to 80 ppm. Since only one or two rabbits were used in this study, it was not possible to draw any conclusions regarding benzene's ability to induce testicular damage in the rabbits. Continuous exposure of female rats to 210 ppm benzene for 10–15 days before cohabitation with males and 3 weeks after cohabitation resulted in a complete absence of litters (Gofmekler 1968). It is not known whether this was due to failure to mate, infertility, or early preimplantation losses of fertilized ova. In a fertility study, female rats exposed up to 300 ppm benzene for 10 weeks during pre-mating, mating, gestation, and lactation showed no effect on indices of fertility, reproduction, and lactation (Kuna et al. 1992).

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

3.2.1.6 Developmental Effects

The available human data on the developmental effects of benzene after inhalation exposure are inconclusive. The studies designed specifically to investigate developmental effects are limited, primarily because of concomitant exposure to other chemicals, inadequate sample size, and lack of quantification of exposure levels (Budnick et al. 1984; Goldman et al. 1985; Heath 1983; Olsen 1983). Benzene crosses the human placenta and is present in the cord blood in amounts equal to or greater than those in maternal blood (Dowty et al. 1976). In a study of subjects with known benzene poisoning in Italy, Forni et al. (1971a) reported the case of one pregnant worker exposed to benzene in the air during her entire pregnancy. Although she had severe pancytopenia and increased chromosomal aberrations, she delivered a healthy son with no evidence of chromosomal alterations. The following year, she delivered a healthy daughter. However, increased frequency of chromatid and isochromatid breaks and sister chromatid exchange was found in lymphocytes from 14 children of female workers exposed by inhalation to benzene (dose not specified) and other organic solvents during pregnancy (Funes-Cravioto et al. 1977).

3. HEALTH EFFECTS

No mention was made of whether the mothers showed signs of toxicity or whether physical abnormalities occurred among their offspring.

There are numerous inhalation studies in which animals have been exposed to benzene during pregnancy (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). None of these studies demonstrated that benzene was teratogenic even at levels that induced maternal and fetal toxicity. Fetotoxicity was evidenced by decreased body weight and by increased skeletal variants such as missing sternebrae and extra ribs, which were not considered to be malformations. Alterations in hematopoiesis have also been observed in the fetuses and offspring of pregnant mice exposed to low levels of benzene (Keller and Snyder 1986, 1988). These studies are discussed below.

Mice exposed to 500 ppm benzene for 7 hours/day on days 6–15 of pregnancy exhibited fetal growth retardation (i.e., decreased fetal body weight) and increased minor skeletal variants (i.e., delayed ossification) (Murray et al. 1979). There were no fetal malformations and no significant effect on the incidence of pregnancy, average number of live fetuses, resorptions per litter, or maternal weight gain. No malformations in fetuses and no significant effects on incidence of pregnancy, average number of live fetuses, or resorptions per litter were observed when rabbits were exposed to benzene 7 hours/day at 500 ppm during gestation for 9 days, although an increase in minor skeletal variations was observed (Murray et al. 1979).

Pregnant mice exposed 12 hours/day to 156.5 or 313 ppm benzene on Gd 6–15 had pups with significant weight retardation and retardation of skeletal development, but no malformations (Ungvary and Tatrai 1985). A parallel study in rabbits showed that inhalation of benzene at 313 ppm caused fetal weight reduction, and an increase in minor fetal anomalies (Ungvary and Tatrai 1985).

As was the case with mice and rabbits, the fetotoxicity of benzene in rats is also demonstrated by retarded fetal weight and/or minor skeletal variants (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Tatrai et al. 1980b). In an experiment conducted by Green et al. (1978), pregnant Sprague-Dawley rats were exposed to 100, 300, or 2,200 ppm benzene for 6 hours/day on Gd 6–15. Exposure to high levels of benzene (2,200 ppm) during gestation resulted in a significant decrease in fetal weight, whereas dams breathing air containing lower levels of benzene (100 or 300 ppm) during gestation bore young that were similar in weight and crown–rump length to control pups. Statistically significant numbers of fetuses with delayed ossification were found in groups exposed to concentrations of 300 and 2,200 ppm. The

3. HEALTH EFFECTS

litter incidence of missing sternebrae was significantly increased in the 100 and 2,200 ppm exposure groups. Maternal toxicity, as indicated by a decrease in maternal weight gain, was evident only at the 2,200 ppm level. The female offspring appeared to be affected to a greater extent than males with respect to delayed ossification and missing sternebrae.

Kuna and Kapp (1981) found decreased fetal weight after exposure to 50 ppm. From a group of 151 pups examined after *in utero* exposure to 500 ppm benzene, a single pup exhibited exencephaly. In the same study, of 98 pups examined for skeletal effects after *in utero* exposures of 500 ppm, a single pup had angulated ribs and 2 others had nonsequential ossification of the forefeet. These anomalies were not statistically significant and may have resulted from maternal nutritional stress.

No significant skeletal malformations occurred in pups of rats exposed during gestation to 47 ppm for 8 days, 24 hours/day (Tatrai et al. 1980b) or 100 ppm for 10 days, 6 hours/day (Coate et al. 1984). Decreased fetal weights were seen at 47 ppm (Tatrai et al. 1980b) and 100 ppm (Coate et al. 1984), and increased fetal mortality was observed at 141 ppm (Tatrai et al. 1980b). In CFY rats exposed to pure air or 125 ppm benzene on Gd 7–14, there was a 17% decrease in placental weight, a decrease in mean fetal weight, and evidence of skeletal retardation (Tatrai et al. 1980a). Continuous exposure of female rats to 6 concentrations of benzene ranging from 0.3 to 210 ppm for 10–15 days before cohabitation with males and 3 weeks after did not affect newborn weight or induce malformations, but there were differences in the weights of individual organs of the dams at all exposure levels (Gofmekler 1968). There was a slight tendency toward decreased litter sizes at 20 ppm of benzene. A complete absence of litters resulted from exposure to 210 ppm. It is not known whether this was due to failure to mate, infertility, or early preimplantation losses of fertilized ova.

Alterations in hematopoiesis have also been observed in the fetuses and offspring of pregnant mice exposed to benzene (Keller and Snyder 1986). Administration of 20 ppm benzene to pregnant Swiss Webster mice for 6 hours/day on Gd 6–15 caused reductions in the levels of the CFU-E of the fetuses, whereas 5 and 10 ppm benzene caused enhancement of these colony-forming cells. In 2-day-old neonates, CFU-E numbers in the 5 ppm group returned to control values, but the 10 ppm neonates showed a bimodal response by litter. Granulocytic colony-forming cells were enhanced in neonates exposed *in utero* to 20 ppm benzene. Some of the mice exposed to 10 ppm prenatally were re-exposed to 10 ppm as adults. Their hematopoietic progenitor cell numbers were depressed compared with controls exposed for the first time as adults. No tests were conducted on the dams after benzene exposure.

3. HEALTH EFFECTS

In a follow-up study, pregnant Swiss Webster mice were exposed 6 hours/day on Gd 6–15 to 5, 10, or 20 ppm benzene (Keller and Snyder 1988). The results indicated that 16-day fetuses, when checked for erythrocyte and leukocyte counts, hemoglobin analysis, and the proliferating pool of differentiating hematopoietic cells, had no noteworthy change at any of the exposure levels. In contrast, 2-day neonates exposed *in utero* to all concentrations of benzene exhibited a reduced number of circulating erythroid precursor cells and, at 20 ppm, had increased numbers of hepatic hematopoietic blast cells and granulopoietic precursor cells accompanied by decreased numbers of erythropoietic precursor cells. Six-week-old adult mice exposed *in utero* to 20 ppm of benzene had a similar pattern of enhanced granulopoiesis. However, this effect was not clearly evident in 6-week-old adult mice exposed *in utero* to 5 or 10 ppm.

The results of inhalation studies conducted in experimental animals have been fairly consistent across species. It has been suggested that benzene fetotoxicity in animals is a function of maternal toxicity because the joint occurrence of a decrease in fetal weight and an increase in skeletal variants usually occurs when there is a decrease in maternal weight (Tatrai et al. 1980b). However, the mechanism underlying developmental toxicity has not been fully elucidated, and there are few data on the effect of benzene on maternal food consumption and on blood levels of benzene and its metabolites in the dams and their fetuses. There are apparently none of the usual fetotoxic findings after exposure *in utero* to low concentrations of benzene (10 ppm) (Coate et al. 1984; Kuna and Kapp 1981). As stated above, there is evidence for persistent hematopoietic anomalies in animals exposed *in utero* to benzene at 20 ppm (Keller and Snyder 1988). They may also exist at lower concentrations, but adequate testing has not been performed.

The highest NOAEL value and all reliable LOAEL values for developmental effects in each species following acute exposure are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.7 Cancer

Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and benzene-containing solvents and the occurrence of acute nonlymphocytic leukemia (ANLL), particularly the myeloid cell type (acute myelogenous leukemia, AML) (EPA 1985b, 1998; Hayes et al. 1997; IARC 1982, 1987; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1996a, 1996b). Some of the studies also provide suggestive evidence of associations between benzene exposure and non-Hodgkin's lymphoma (NHL) and multiple myeloma (Hayes et al. 1997;

3. HEALTH EFFECTS

Rinsky et al. 1987). The epidemiological studies are generally limited by confounding chemical exposures and methodological problems, including inadequate or lack of exposure monitoring and low statistical power (due to small numbers of cases), but a consistent excess risk of leukemia across studies indicates that benzene is the causal factor. Many of the earlier studies are additionally limited by a lack of information on leukemia cell types other than AML, because leukemia used to be considered a single diagnostic category for epidemiological purposes, due in part to historical nomenclature, small numbers of deaths by cell type, and unavailability of cell-type-specific rates for comparison.

Two series of studies on workers exposed to benzene in Ohio (the Pliofilm study) (e.g., Rinsky et al. 1981, 1987, 2002) and China (the NCI/CAPM study) (e.g., Hayes et al. 1997; Yin et al. 1996a, 1996b) have yielded particularly strong data on the leukemogenic potential of benzene and are summarized below. The studies of the Pliofilm cohort provide the best set of data for evaluating human cancer risks from benzene exposure because, in comparison to other published studies, the Pliofilm workers had the fewest reported co-exposures to other potentially carcinogenic substances and experienced a greater range of estimated exposures to benzene (EPA 1998). The NCI/CAPM Chinese study is one of the largest of its type ever undertaken and evaluated many thousands of benzene-exposed workers, enabling detection of significantly elevated risks at unusually low levels of exposure.

A number of studies were performed on a cohort of workers exposed to benzene in three rubber hydrochloride ('Pliofilm') manufacturing plants in Ohio (Collins et al. 1997; Finkelstein 2000; Infante 1978; Infante et al. 1977; Ireland et al. 1997; Paxton et al. 1994a, 1994b; Rinsky et al. 1981, 1987, 2002; Schnatter et al. 1996a; Wong 1995). Analyses using various expansions and updates of the cohort, statistical methods, and exposure estimates have consistently shown a significant relationship between exposure to benzene and excess mortality from leukemia. In the report by Rinsky et al. (1987), a cohort of 1,165 white males employed between 1940 and 1965 and followed through 1981 experienced increased mortality from all leukemias (9 observed versus 2.7 expected; standardized mortality ratio [SMR]=3.37, 95% CI 1.54–6.41) and multiple myeloma (4 observed versus 1 expected; SMR=4.09, 95% CI 1.10–10.47). Death rates for age-matched U.S. white males during the same calendar period were used for comparison with death rates observed in the cohort. Assessment after an additional 15 years of follow-up showed declines in the SMRs for both leukemias (2.56, 95% CI 1.43–4.22) and multiple myeloma (2.12, 95% CI 0.69–4.96), suggesting that the excess risks diminished with time since exposure (Rinsky et al. 2002). Exposures in the most recent 10 years were most strongly associated with leukemia risk, and there was no significant relation between leukemia death and benzene exposures received more than 20 years previously (Finkelstein 2000). AML accounted for most of the increased leukemia

3. HEALTH EFFECTS

(SMR=5.03, 95% CI 1.84–10.97) (Schnatter et al. 1996a; Wong 1995). The risk of all leukemias and AML, but not multiple myeloma, significantly increased with increasing cumulative exposure above 200 ppm-years (Rinsky et al. 1987, 2002; Wong 1995). Analysis of 4,417 workers from one of the Pliofilm plants showed no clear evidence that the risks of all leukemias, ANLL, multiple myeloma, or other lymphohematopoietic cancers increased with increasing exposure at lower levels of cumulative exposure (1–72 ppm-years), and the number of peak exposures over 100 ppm for ≥ 40 days was a better predictor of risk for all leukemias and multiple myeloma (Collins et al. 2003; Ireland et al. 1997). No clear associations between exposure to benzene and mortality from NHL or Hodgkin's disease were reported (Collins et al. 2003; Ireland et al. 1997; Rinsky et al. 2002).

A collaborative study between the National Cancer Institute and the Chinese Academy of Preventive Medicine (NCI/CAPM) evaluated incidence rates (occurrence of disease and cause of death) for lymphohematopoietic malignancies and other hematologic disorders in a cohort of 74,828 benzene-exposed and 35,805 nonexposed workers employed in 672 factories in 12 cities in China (Hayes et al. 1996, 1997, 2001; Linet et al. 1996). The joint NCI/CAPM study is an expansion of earlier studies performed by CAPM alone (Yin et al. 1987a, 1987b, 1987c, 1989). The workers were employed from 1972–1987, followed for an average of nearly 12 years, and worked in various job categories using benzene as a solvent for paints, varnishes, glues, coatings, and other products. The derivation of the cohort from many different factories and job types suggests that the members were concurrently exposed to many other chemicals. Findings in the exposed workers included significantly increased relative risk (RR) for all hematologic neoplasms (RR=2.6, 95% CI 1.4–4.7), all leukemias (RR=2.5, 95% CI 1.2–5.1), ANLL (RR=3.0, 95% CI 1.0–8.9), and combined ANLL and precursor myelodysplastic syndromes (ANLL/MDS; RR=4.1, 95% CI 1.4–11.6) (Hayes et al. 1997). Increases were also observed for other (unspecified) leukemias (RR=2.0, 95% CI=0.7–5.4), but the results were not statistically significant. Analysis by level of average benzene exposure (<10, 10–24, and ≥ 25 ppm) and cumulative exposure (<40, 40–99, and ≥ 100 ppm-years) indicated that the risk for all hematologic neoplasms was significantly increased at <10 ppm (RR=2.2, 95% CI 1.1–4.2) and <40 ppm-years (RR=2.2, 95% CI 1.1–4.5), respectively. The risks for all leukemias, ANLL, and ANLL/MDS were increased at exposure levels of 10–24 ppm (average) and 40–99 ppm-years (cumulative). The elevated risks showed weak tendencies to increase with increasing average and cumulative levels of exposure. Analysis by duration of exposure (<5, 5–9, or ≥ 10 years) did not show increased risk with increasing exposure duration. Analysis by occupational group (coatings, rubber, chemical, shoe, other/mixed) showed that the increased risks for ANLL and ANLL/MDS were consistent across the spectrum of industries studied, implying that the associations were due to the common exposure to benzene rather than other industry-specific exposures.

3. HEALTH EFFECTS

The results of a study of shoe factory workers in Italy are similar to those of the Pliofilm and NCI/CAPM studies in showing that the risk of leukemia increases with increasing exposure to benzene (Costantini et al. 2003; Paci et al. 1989). The cohort was followed from 1950 to 1999 and consisted of 891 men and 796 women who were exposed to estimated benzene concentrations ranging from 0 to 92 ppm, and had mean cumulative exposures of 71.8 and 43.4 ppm-years, respectively (exposure durations not reported). Leukemia risk was significantly increased in both sexes in the highest of four exposure categories and most apparent in the men. For cumulative exposures of <40, 40–99, 100–199, and >200 ppm-years, the leukemia SMR values for the men were 1.4 (95% CI 0.2–5.0), 3.7 (95% CI 0.1–20.6), 3.0 (95% CI 0.4–10.9), and 7.0 (95% CI 1.9–18.0), respectively. Leukemia subtypes were not evaluated. The findings are consistent with earlier epidemiologic studies and case reports showing increased incidences of leukemia in shoe factory and rotogravure plant workers exposed to high benzene levels during its use as a solvent (Aksoy et al. 1974, 1987; IARC 1982; Vigliani and Forni 1976).

No significant increases in leukemia were found in chemical industry workers (Bloemen et al. 2004; Bond et al. 1986; Ott et al. 1978) or petroleum industry workers (Lewis et al. 1997; Raabe and Wong 1996; Rushton and Romaniuk 1997; Schnatter et al. 1993, 1996b, 1996c; Tsai et al. 1983) exposed to lower levels of benzene. Cause-specific mortality was determined in a prospective study of 2,266 chemical workers who were exposed to benzene in various Dow Chemical Company manufacturing processes between 1938 and 1970 (Bloemen et al. 2004). The workers were followed from 1940–1996 and had an average duration of exposure, intensity of exposure, and cumulative exposure of 4.8 years, 9.6 ppm, and 39.7 ppm-years, respectively. There were no significant increases in risk for any lymphohematopoietic malignancies, including all leukemias, ANLL, chronic lymphatic leukemia (CLL), NHL, and multiple myeloma. A previous investigation of this cohort found a significantly increased risk of ‘myelogenous’ leukemias (SMR=4.44, 95% CI not reported) (Bond et al. 1986; Ott et al. 1978), but this result is less reliable than those of the current study because it is based on a much smaller number of deaths.

A meta-analysis was conducted on 19 cohorts of petroleum workers in the United States and the United Kingdom that were pooled into a single database for cell-type-specific leukemia analysis (Raabe and Wong 1996). The combined cohort consisted of 208,741 workers, mainly refinery employees who contributed more than 4.6 million person-years of observation. Benzene exposures were mainly from handling gasoline and the estimated mean and cumulative exposures for the most exposed jobs were <1 ppm and <45 ppm-years, respectively. No increased risks were found for mortality from AML, chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), or CLL. Analyses limited to

3. HEALTH EFFECTS

studies of refinery workers or studies with at least 15 years of follow-up yielded similar results. The negative results of this meta-analysis are consistent with those of case-control studies of the common leukemia cell types in petroleum workers (Rushton and Romaniuk 1997; Schnatter et al. 1993, 1996b, 1996c). A case-control study of hairy cell leukemia, a rare B-lymphoid chronic leukemia, found no association between reported exposure to benzene and the risk of this cancer in workers from various occupations with generally low levels of exposure (Clavel et al. 1996).

A possible association between occupational exposure to benzene and NHL is suggested by results of the most recent analysis (Hayes et al. 1997) of the cohort from the NCI/CAPM Chinese study summarized above. The relative risk for mortality from NHL in the whole cohort was 3.0 (95% CI 0.9–10.5), an increase that was not statistically significant. However, the risk for NHL did significantly increase at the highest level and duration of benzene exposure. For exposure to average concentrations of <10, 10–24, and ≥ 25 ppm, the RR values were 2.7 (95% CI 0.7–10.6), 1.7 (95% CI 0.3–10.2), and 4.7 (95% CI 1.2–18.1), respectively (P for trend=0.04). For cumulative exposures of <40, 40–99, and ≥ 100 ppm-years, the RR values were 3.3 (95% CI 0.8–13.1), 1.1 (95% CI 0.1–11.1), and 3.5 (95% CI 0.9–13.2), respectively (P for trend=0.02). Additionally, the risk for NHL significantly increased with increasing duration of exposure among workers exposed for <5 years (RR=0.7, 95% CI 0.1–7.2), 5–9 years (RR=3.3, 95% CI 0.7–14.7), and >10 years (RR=4.2, 95% CI 1.1–15.9) (P for trend=0.01).

Although the results of the NCI/CAPM Chinese study (Hayes et al. 1997) indicated a possible association between exposure to benzene and NHL, other cohort mortality studies found no significant increases in NHL mortality. These studies include the entire Pliofilm cohort (Rinsky et al. 2002); a total of 4,417 workers from one of the three Pliofilm plants assessed for cumulative exposures of 0, <1, 1–6, and >6 ppm-years (Collins et al. 2003); a cohort of 2,266 chemical manufacturing workers exposed to a cumulative benzene level of 39.7 ppm-years (Bloemen et al. 2004); and a meta-analysis of 26 cohorts of petroleum workers from the United States and five other countries with expected low exposures to benzene (Wong and Raabe 2000). Furthermore, case-control studies provide no indications of an association between benzene exposure and risk of NHL (Schnatter et al. 1996b; Wong and Raabe 2000), and the adequacy of the data from the NCI/CAPM Chinese study (Hayes et al. 1997) has been questioned (Dosemeci et al. 1994; Wong 1999; Wong and Raabe 2000).

Other evidence suggests a possible association between occupational exposure to benzene and increased risk of multiple myeloma. Mortality from multiple myeloma was initially elevated in the Pliofilm cohort, but appeared to diminish with increasing duration of observation. Based on an evaluation of 1,165 white

3. HEALTH EFFECTS

male workers followed through 1981, Rinsky et al. (1987) found an SMR for multiple myeloma of 4.09 (95% CI 1.10–10.47), an increase that was statistically significant. Assessment of the cohort after an additional 6 and 15 years of follow-up yielded lower SMRs of 2.91 (95% CI 0.79–7.45) (Wong 1995) and 2.12 (95% CI 0.69–4.96) (Rinsky et al. 2002), respectively, risks that were no longer statistically significant. The risk of multiple myeloma did not increase with increasing level of cumulative exposure to benzene or with duration of employment, but this finding is particularly limited by the small number of deaths. No increases in mortality from multiple myeloma were found in other cohort mortality studies, including the NCI/CAPM study of Chinese workers (no observed deaths) (Yin et al. 1996a, 1996b), the Bloemen et al. (2004) study of chemical workers (SMR=0.72, 95% CI 0.15–2.10), and the Paci et al. (1989) study of shoe factory workers (no observed deaths in males; SMR=1.11 in females, 95% CI not calculated) summarized above. Additionally, mortality from multiple myeloma was not increased in a meta-analysis of 22 other cohorts of petroleum workers with potential exposure to benzene or benzene-containing petroleum products (Wong and Raabe 1997). The combined cohort consisted of 250,816 workers (from the United States, Canada, the United Kingdom, and Australia) who were observed over a period of 55 years from 1937 to 1991. The overall SMR for multiple myeloma was 0.93 (95% CI=0.81–1.07), and analyses by type of facility/industrial process (refinery, distribution, and crude oil production and pipeline workers) and duration of observation showed no differences in risk. The individual cohorts used in the meta-analysis also had no increased risk of multiple myeloma.

As summarized above, one of the early assessments of the Pliofilm cohort found an increased risk of mortality from multiple myeloma (Rinsky et al. 1987). The implications of this finding are unclear because the risk declined to non-significant levels in subsequent follow-ups (Rinsky et al. 2002; Wong 1995), and was not supported by the findings of other cohort mortality studies (Bloemen et al. 2004; Paci et al. 1989; Wong and Raabe 2000; Yin et al. 1996a, 1996b). Additionally, population-based and hospital-based case-control studies indicate that benzene exposure is not likely to be causally related to the risk of multiple myeloma (Bezabeh et al. 1996; Heineman et al. 1992; Linet et al. 1987; Schnatter et al. 1996b; Sonoda et al. 2001; Wong and Raabe 1997). A meta-analysis of case-control studies found no significant association between occupational exposure to benzene and benzene-containing products and risk of multiple myeloma from sources categorized as benzene and/or organic solvents (summary odds ratio [OR]=0.74, 95% CI 0.60–0.90), petroleum (summary OR=1.11, 95% CI 0.96–1.28), or petroleum products (summary OR=1.08, 95% CI 0.89–1.33) (Sonoda et al. 2001).

3. HEALTH EFFECTS

Studies in animals provide supporting evidence for the carcinogenicity of inhaled benzene. As summarized below, inhalation exposure to benzene induced tumors at multiple sites in rats and mice, with a tendency towards induction of lymphomas in mice.

Carcinomas of the Zymbal gland and oral cavity were clearly increased in Sprague-Dawley rats that were exposed to 200–300 ppm benzene for 4–7 hours/day, 5 days/week for up to 104 weeks (Maltoni et al. 1982a, 1982b, 1983, 1985, 1989). The Zymbal gland is a specialized sebaceous gland and a site for benzene-induced tumors. Marginal increases in nasal cavity carcinomas, mammary tumors, and hepatomas were also observed. The significance of these findings is unclear because statistical analyses were not performed and there were no clearly increased incidences of Zymbal gland carcinoma or tumors at other sites in Sprague-Dawley rats that were similarly exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for life (Snyder et al. 1978b, 1984).

Benzene was carcinogenic in mice exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for 16 weeks and observed for 18 months or life (Cronkite 1986; Cronkite et al. 1984, 1985, 1989; Farris et al. 1993). These exposures consistently induced a variety of tumors, including leukemia (mainly thymic lymphoma) and Zymbal gland and ovarian tumors in C57BL/6 mice, and myelogenous leukemias, malignant lymphomas, and Zymbal gland and lung tumors in CBA/Ca mice. Incidences of lymphocytic lymphoma with thymic involvement were significantly increased in C57BL/6 mice similarly exposed to 300 ppm benzene for life (Snyder et al. 1980). Intermittent lifetime exposure to 300 ppm benzene (6 hours/day, 5 days/week on every third week) was more tumorigenic (in the Zymbal gland and lungs) to CD-1 and C57BL/6 mice than short-term exposure to 1,200 ppm (6 hours/day, 5 days/week for 10 weeks) followed by lifetime observation, although neither of these exposures induced leukemia (Snyder et al. 1988).

The cancer effect levels (CELs) for each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human carcinogen. The Department of Health and Human Services (NTP 2005) determined that benzene is a known carcinogen based on human evidence showing a causal relationship between exposure to benzene and cancer. IARC (1987, 2004, 2007) classified benzene in Group 1 (carcinogenic to humans) based on sufficient evidence in both humans and animals. EPA (IRIS 2007) classified benzene in Category A (known human carcinogen) based on convincing evidence in humans supported by evidence from animal

3. HEALTH EFFECTS

studies. Under EPA's most recent guidelines for carcinogen risk assessment, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies (IRIS 2007). Based on the Rinsky et al. (1981, 1987) human leukemia data, EPA derived a range of inhalation unit risk values of 2.2×10^{-6} – 7.8×10^{-6} ($\mu\text{g}/\text{m}^3$)⁻¹ for benzene (IRIS 2007). For risks ranging from 1×10^{-4} to 1×10^{-7} , the corresponding air concentrations for lifetime exposure range from 13.0–45.0 $\mu\text{g}/\text{m}^3$ (4–14 ppb) to 0.013–0.045 $\mu\text{g}/\text{m}^3$ (0.004–0.014 ppb), respectively. These risk levels are presented in Figure 3-1.

3.2.2 Oral Exposure

3.2.2.1 Death

Individual case reports of death from acute oral exposure to benzene have appeared in the literature since the early 1900s. The benzene concentrations encountered by the victims were often not known. However, lethal oral doses for humans have been estimated at 10 mL (8.8 g or 125 mg/kg for a 70-kg person) (Thienes and Haley 1972). Lethality in humans has been attributed to respiratory arrest, central nervous system depression, or cardiac collapse (Greenburg 1926). Accidental ingestion and/or attempted suicide with lethal oral doses of benzene have produced the following signs and symptoms: staggering gait; vomiting; shallow and rapid pulse; somnolence; and loss of consciousness, followed by delirium, pneumonitis, collapse, and then central nervous system depression, coma, and death (Thienes and Haley 1972). Ingestion of lethal doses may also result in visual disturbances and/or feelings of excitement and euphoria, which may quite suddenly change to weariness, fatigue, sleepiness, convulsion, coma, and death (Von Oettingen 1940).

Animal lethality data indicate that benzene is of low toxicity following acute oral exposure (O'Bryan and Ross 1988). Oral LD₅₀ values for rats ranged from 930 to 5,600 mg/kg; the values varied with age and strain of the animals (Cornish and Ryan 1965; Wolf et al. 1956). Male Sprague-Dawley rats were given various doses of benzene to determine the LD₅₀ (Cornish and Ryan 1965). The LD₅₀ for nonfasted rats was found to be 930 mg/kg. In 24-hour fasted rats, the LD₅₀ was 810 mg/kg. No increase in mortality was reported in Fischer 344 rats or B6C3F₁ mice treated with 600 mg/kg/day for up to 17 weeks (Huff et al. 1989; NTP 1986).

Sprague-Dawley rats (30–35 males, 30–35 females) were exposed to benzene by ingestion (stomach tube), in olive oil, at 0, 50, or 250 mg/kg/day for 4–5 days weekly for 52 weeks and then kept under supervision until spontaneous death (Maltoni et al. 1983). Exposure to 50 mg/kg/day benzene after

3. HEALTH EFFECTS

52 weeks resulted in deaths in 9 of 30 male (same as controls) and 2 of 30 female rats. At 250 mg/kg/day exposure, 13 of 35 males and 9 of 35 females died. For rats receiving only olive oil, 9 of 30 males and 0 of 30 females died. In a companion study, Sprague-Dawley rats were exposed to 500 mg/kg/day benzene by ingestion (stomach tube), in olive oil, 4–5 days/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Mortality rates were the same as the controls.

In a chronic-duration oral study conducted by the NTP (1986), increased mortality was observed in male Fischer 344 rats exposed to 200 mg/kg/day benzene in corn oil, and in female Fischer 344 rats exposed to 50 mg/kg/day benzene. B6C3F₁ mice given 100 mg/kg/day also had increased mortality compared to control mice.

The LD₅₀ values and all reliable LOAEL values for death in each species following acute and chronic exposure are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, musculoskeletal, hepatic, renal, endocrine, ocular, metabolic, or body weight effects in humans. Human and animal data pertaining to other systemic effects are presented below.

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

Respiratory Effects. Male and female Fischer 344 rats and B6C3F₁ mice were given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). No histopathological lesions were observed in lungs, trachea, or mainstream bronchi. After chronic-duration exposure to 50, 100, or 200 mg/kg/day (male rats) or 25, 50, or 100 mg/kg/day (female rats, male and female mice), no histopathological lesions were observed in trachea, lungs, or mainstream bronchi in rats (NTP 1986). In mice, a significantly increased incidence of alveolar hyperplasia was observed at 50 and 100 mg/kg/day in females and at 100 mg/kg/day in males.

Cardiovascular Effects. No histopathological lesions were observed in cardiac tissue from male and female Fischer 344 rats or B6C3F₁ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). After chronic-duration exposure to ≤200 mg/kg/day (male

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

Key to Figure	Species ^a (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
ACUTE EXPOSURE								
Death								
1	Human	once					126 (death)	Thienes and Haley 1972
2	Rat (Sprague-Dawley)	once (G)					930 M (LD50)	Cornish and Ryan 1965
3	Rat (Wistar)	once (GO)					5600 M (LD50)	Wolf et al. 1956
Systemic								
4	Rat (Sprague-Dawley)	Gd 6-15 daily (G)	Renal	1000 F				Exxon 1986
			Dermal		50 F (alopecia of hindlimbs and trunk)			
			Bd Wt	500 F	1000 F (body weight decreased 11%)			
			Other	50 F	250 F (decreased food consumption)			
5	Rat (CF)	1-3 d	Hepatic		1402 M (increased liver weight, biochemical changes)			Pawar and Mungikar 1975
Neurological								
6	Human	once					126 (muscular incoordination, unconsciousness)	Thienes and Haley 1972

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

(continued)

Key to Figure	Species ^a (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
7	Rat (Sprague-Dawley)	1 d			88 M (slight CNS depression)	1870 M (tremors)	Cornish and Ryan 1965	
8	Rat (Sprague-Dawley)	once (G)			950 M (altered neurotransmitter concentrations)		Kanada et al. 1994	
Reproductive								
9	Rat (Sprague-Dawley)	Gd 6-15 daily (G)		1000 F			Exxon 1986	
Developmental								
10	Rat (Sprague-Dawley)	Gd 6-15 daily (G)		1000 F			Exxon 1986	
11	Mouse (ICR/SIM)	Gd 8-12 (GO)			1300 F (decreased pup weight on neonatal days 1-3)		Seidenberg et al. 1986	
INTERMEDIATE EXPOSURE								
Death								
12	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)				250 F (9/35 died)	Maltoni et al. 1983, 1985	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
Systemic								
13	Rat (Sprague- Dawley)	52 wk 4-5 d/wk 1 x/d (GO)	Bd Wt	50	250	(body weight decreased 19%)	Maltoni et al. 1983, 1985	
14	Rat (F-344/N)	<1 yr 5 d/wk (GO)	Hemato		50	M (lymphocytopenia and leukocytopenia)	NTP 1986	
					25 ^b	F (lymphocytopenia and leukocytopenia)		
			Bd Wt	100	200	M (body weight decreased 11% or more in 25 weeks)		

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
15	Rat (F-344/N)	60-120 d 5 d/wk (GO)	Resp	600			NTP 1986	
			Cardio	600				
			Gastro	600				
			Hemato		200	(dose-related leukopenia at 60 days)		
					^b 25	(dose-related leukopenia at 120 days)		
			Musc/skel	600				
			Hepatic	600				
			Renal	600				
			Endocr	600				
	Bd Wt		200	(body weight decreased 14% in males and 16% in females)				
16	Rat (Fischer- 344)	6 wk 5 d/wk (GO)	Hepatic	400 M			Taningher et al. 1995	
			Bd Wt	400 M				
17	Rat (Wistar)	6 mo 5 d/wk (GO)	Hemato	1 F		50 F (leukopenia, erythrocytopenia)	Wolf et al. 1956	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
18	Mouse (C57BL/6)	28 d (W)	Bd Wt	1000 M			Fan 1992	
19	Mouse (CD-1)	4 wk ad lib (W)	Hemato			8 M (erythrocytopenia, increased mean corpuscular volume; leukopenia)	Hsieh et al. 1988b	
20	Mouse (CD-1)	4 wk (W)	Hemato			31.5 M (decreased erythrocyte, leukocyte counts)	Hsieh et al. 1990	
			Hepatic	31.5 M				
			Renal	31.5 M				
			Bd Wt	31.5 M				
21	Mouse (B6C3F1)	<1 yr 5 d/wk (GO)	Hemato		25 (lymphocytopenia)		NTP 1986	
			Bd Wt	100				

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
22	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)	Resp	600			NTP 1986	
			Cardio	600				
			Gastro	600				
			Hemato	200 F	^b 50 M (dose-related leukopenia)			
				25	400 F (dose-related leukopenia)			
			Musc/skel	600				
			Hepatic	600				
			Renal	600				
			Endocr	600				
Bd Wt	600							
23	Mouse (B6C3F1)	30 d ad lib (W)	Hemato	12 F	195 F (decreased leukocytes)	350 F (decreased hemoglobin, hematocrit, leukocytes, MCV, and MCH)	Shell 1992	
			Hepatic	350 F				
			Renal	350 F				
			Bd Wt	350 F				
			Other		12 F (decreased fluid intake)			

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
Immuno/ Lymphoret								
24	Rat (F-344/N)	60-120 d 5 d/wk (GO)			200	(lymphopenia at 60 days, lymphoid depletion in B-cell of the spleen)		Huff et al. 1989; NTP 1986
					25 F	dose-related lymphopenia at 120 days)		
25	Rat (Wistar)	6 mo 5 d/wk (GO)		1 F	50 F	(leukopenia)		Wolf et al. 1956
26	Mouse (C57BL/6)	28 d (W)			27 M	(decreased number of splenocytes and IL-2 production)		Fan 1992
27	Mouse (CD-1)	4 wk ad lib (W)				8 M (leukopenia and lymphopenia; enhanced splenic lymphocyte proliferation)		Hsieh et al. 1988b
28	Mouse (CD-1)	4 wk (W)				31.5 M (reduction in thymus mass; suppression of both B- and T-cell mitogeneses; suppressed IL-2 secretions; leukopenia, lymphopenia)		Hsieh et al. 1990

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	LOAEL		Reference Chemical Form	Comments
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)		
29	Mouse (CD-1)	4 wk ad lib (W)				40 M (elevated corticosterone levels; T-lymphocyte suppression)	Hsieh et al. 1991
30	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)		^b 25 M 200 F	^b 50 M (dose-related lymphopenia) 400 F		Huff et al. 1989; NTP 1986
31	Mouse (B6C3F1)	30 d ad lib (W)			12 F (decreased leukocytes)		Shell 1992
Neurological							
32	Rat (F-344/N)	60-120 d 5 d/wk (GO)		600			NTP 1986
33	Mouse (CD-1)	4 wk ad lib (W)			8 M (fluctuation of neurotransmitter levels)		Hsieh et al. 1988a
34	Mouse (CD-1)	4 wk (W)			31.5 M (decreased NE, DA, 5-HT)		Hsieh et al. 1990
35	Mouse (CD-1)	4 wk ad lib (W)		8 M	40 M (increased hypothalamic NE and VMA)		Hsieh et al. 1991

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

(continued)

Key to Figure	Species ^a (Strain)	Exposure/Duration/Frequency (Route)	System	LOAEL			Reference Chemical Form	Comments
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)		
36	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)		200	400 (intermittent tremors)		NTP 1986	
37	Mouse (B6C3F1)	30 d ad lib (W)		195 F	350 F (decreased brain weight)		Shell 1992	
Reproductive								
38	Rat (F344/N)	17 wk 5 d/wk (GO)		600			NTP 1986	
39	Mouse (B6C3F1)	17 wk 5 d/wk (GO)		600			NTP 1986	
Cancer								
40	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)				50 F (CEL: Zymbal gland carcinoma in 2/30; oral cavity carcinoma)	Maltoni et al. 1983, 1985	
41	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)				50 (CEL: Zymbal gland carcinoma) 250 (CEL: nasal cavity carcinoma, fore-stomach liver angiosarcoma; Zymbal gland carcinoma)	Maltoni et al. 1989	

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments	
					Less Serious (mg/kg/day)	Serious (mg/kg/day)			
42	Mouse (RF/J)	52 wk 4-5 d/wk (GO)				500	(CEL: mammary pulmonary leukemias)	Maltoni et al. 1989	
CHRONIC EXPOSURE									
Death									
43	Rat (F-344/N)	2 yr 5 d/wk (GO)				200 M (30/50 died)		NTP 1986	
						50 ^b F (14/50 died)			
44	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				100	(41/50 males died, 35/50 females died)	NTP 1986	
Systemic									
45	Human	6.1 yr (avg) (Occup)	Hemato		0.29 ^c		(reduced WBC and platelet counts, approximately 7-18% lower than control values)	Lan et al. 2004a	Route-to-route extrapolation from the reported LOAEL of 0.57 ppm for occupational exposure was used by ATSDR to estimate equivalent oral dose.

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments	
					Less Serious (mg/kg/day)	Serious (mg/kg/day)			
46	Rat (F-344/N)	2 yr 5 d/wk (GO)	Resp	200 M ^b 100 F			Huff et al. 1989; NTP 1986		
			Cardio	200 M ^b 100 F					
			Gastro	100	200 M (hyperkeratosis and acanthosis in nonglandular forestomach)				
			Hemato		50 M (lymphocytopenia and leukocytopenia)				
					^b 25 F (lymphocytopenia and leukocytopenia)				
			Musc/skel	200 M ^b 100 F					
			Hepatic	200 M ^b 100 F					
			Renal	200 M ^b 100 F					
			Endocr	200 M ^b 100 F					
			Dermal	200 M ^b 100 F					
Ocular	200 M ^b 100 F								

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
47	Rat (F-344/N)	2 yr 5 d/wk (GO)	Bd Wt	100 M		200 M (body weights decreased 23% in 103 weeks)	Huff et al. 1989; NTP 1986	
48	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1 x/d (GO)	Hemato			500 (decreased RBCs and WBCs after 84 weeks)	Maltoni et al. 1983, 1985	
			Bd Wt	500 (decreased body weights in 92 weeks)				

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

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
49	Mouse (B6C3F1)	2 yr 5 d/wk (GO)	Resp	50 M ^b 25 F	100 M (alveolar hyperplasia) ^b 50 F		NTP 1986	
			Cardio	100				
			Gastro		25 (epithelial hyperplasia and hyperkeratosis of forestomach)			
			Hemato		25 (lymphocytopenia; increased frequency of micronucleated normochromatic peripheral erythrocytes)			
			Musc/skel	100				
			Hepatic	100				
			Renal	100				
			Endocr		25 (hyperplasia of adrenal gland and harderian gland)			
			Dermal	100				
			Bd Wt	50	100 (mean body weight decreased 10% in 47 weeks to 19% in 103 weeks in males; decreased 14-15% in week 99-103 in females)			

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

(continued)

Key to Figure	Species ^a (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
Immuno/ Lymphoret								
50	Rat (F-344/N)	2 yr 5 d/wk (GO)			50 M (lymphoid depletion of spleen and thymus)		Huff et al. 1989; NTP 1986	
					^b 25 F (lymphoid depletion of spleen and thymus)			
51	Rat (Sprague- Dawley)	92 wk 4-5 d/wk 1 x/d (GO)			500 (decreased WBCs after 84 weeks)		Maltoni et al. 1983, 1985	
52	Mouse (B6C3F1)	2 yr 5 d/wk (GO)			25 (lymphopenia, hematopoietic hyperplasia in the bone marrow, splenic hematopoiesis)		Huff et al. 1989; NTP 1986	
Neurological								
53	Rat (F-344/N)	2 yr 5 d/wk (GO)		200 M ^b 100 F			NTP 1986	
54	Mouse (B6C3F1)	2 yr 5 d/wk (GO)		100			NTP 1986	

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

(continued)

Key to Figure	Species ^a (Strain)	Exposure/Duration/Frequency (Route)	System	LOAEL		Reference Chemical Form	Comments
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)		
Reproductive							
55	Rat (F-344/N)	2 yr 5 d/wk (GO)		200 M ^b 50 F		100 F (endometrial polyps)	NTP 1986
56	Mouse (B6C3F1)	2 yr 5 d/wk (GO)		25	(preputial gland hyperplasia in males; ovarian hyperplasia and senile atrophy in females)		NTP 1986
Cancer							
57	Rat (F-344/N)	2 yr 5 d/wk (GO)				50 M (CEL: squamous cell papillomas and carcinomas of the oral cavity) ^b 25 F (CEL: Zymbal gland carcinomas)	Huff et al. 1989; NTP 1986
58	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1 x/d (GO)				500 (CEL: Zymbal gland carcinoma; oral and nasal cavity carcinoma; angiosarcoma of the liver)	Maltoni et al. 1983, 1985

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

(continued)

Key to Figure	Species (Strain)	Exposure/Duration/Frequency (Route)	System	LOAEL			Reference Chemical Form	Comments
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)		
59	Rat (Wistar)	104 wk 4-5 d/wk 1 x/d (GO)				500 (CEL: Zymbal gland and oral and nasal cavity carcinoma; angiosarcoma of the liver)	Maltoni et al. 1989	
60	Rat (Sprague-Dawley)	104 wk 5 d/wk (GO)				50 M (CEL: Zymbal gland carcinoma)	Maltoni et al. 1989	
61	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				25 (CEL: harderian gland adenoma, lymphoma in males; CEL: lymphoma in females)	Huff et al. 1989; NTP 1986	
62	Mouse (Swiss)	78 wk 4-5 d/wk 1 x/d (GO)				500 (CEL: mammary tumors, lung tumors, Zymbal gland)	Maltoni et al. 1989	

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

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c Study results used to derive a chronic-duration oral minimal risk level (MRL) of 0.0005 mg/kg/day based on route-to-route extrapolation, as described in detail in Chapter 2 and Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts to select a point of departure, which was adjusted for intermittent exposure. An equivalent oral dose was estimated based on route-to-route extrapolation to determine a point of departure for deriving a chronic-duration oral MRL for benzene, which was divided by an uncertainty factor of 30 (10 for human variability and 3 for uncertainty in route-to-route extrapolation).

ad lib = ad libitum; Bd Wt = body weight; CEL = cancer effect level; CNS = central nervous system; d = day(s); DA = dopamine; F = female; (F) = feed; (G) = gavage; Gd = gestational day; (GO) = gavage in oil; Hemato = hematological; 5-HT = 5-hydroxytryptamine; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; mo = month(s); NE = norepinephrine; NOAEL = no-observed-adverse-effect level; NS = not specified; RBC = red blood cells; VMA = vanillin mandelic acid; (W) = water; WBC = white blood cells; wk = week(s); yr = year(s); x = times

Figure 3-2 Levels of Significant Exposure to Benzene - Oral
Acute (≤14 days)

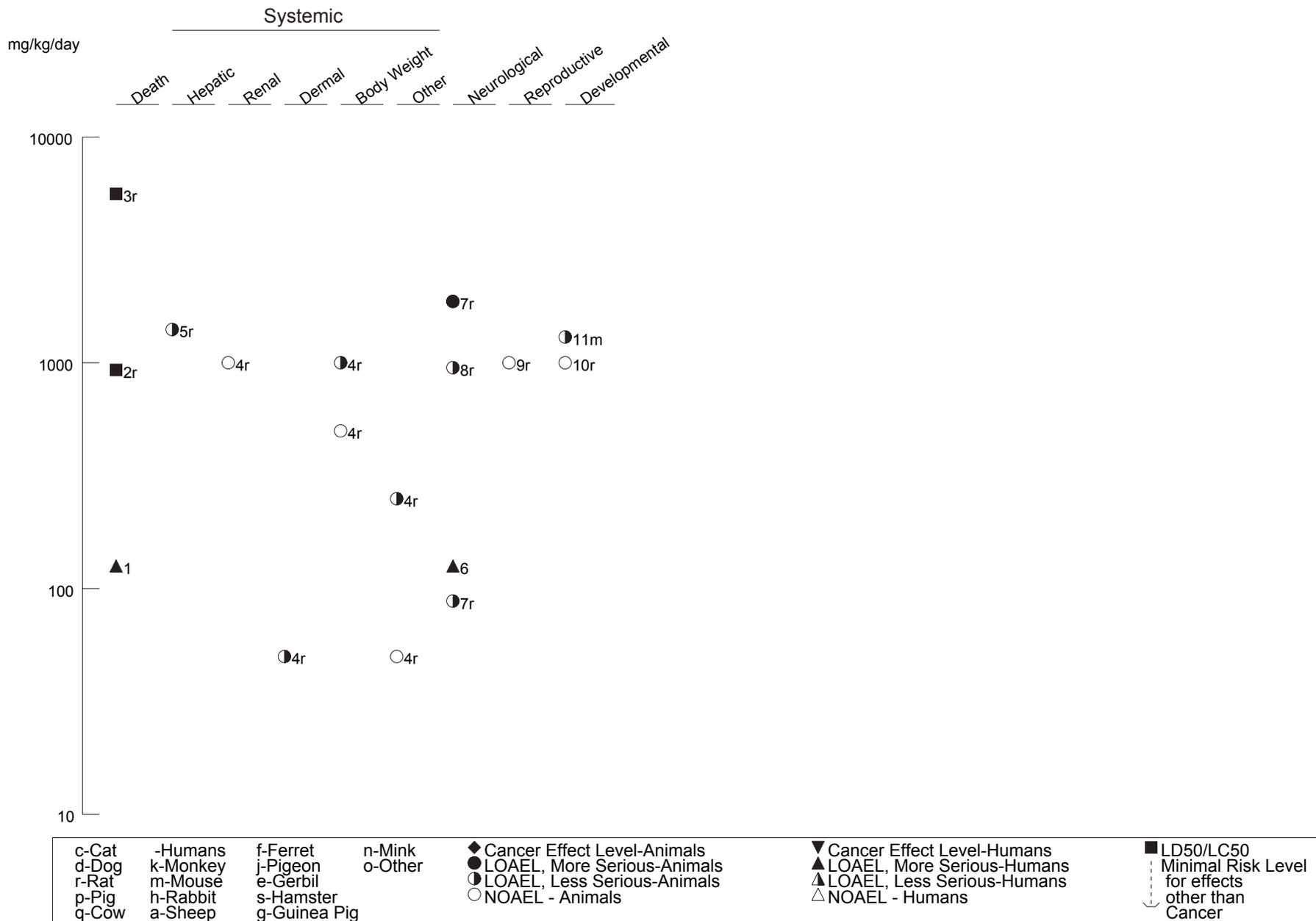


Figure 3-2 Levels of Significant Exposure to Benzene - Oral (Continued)

Intermediate (15-364 days)

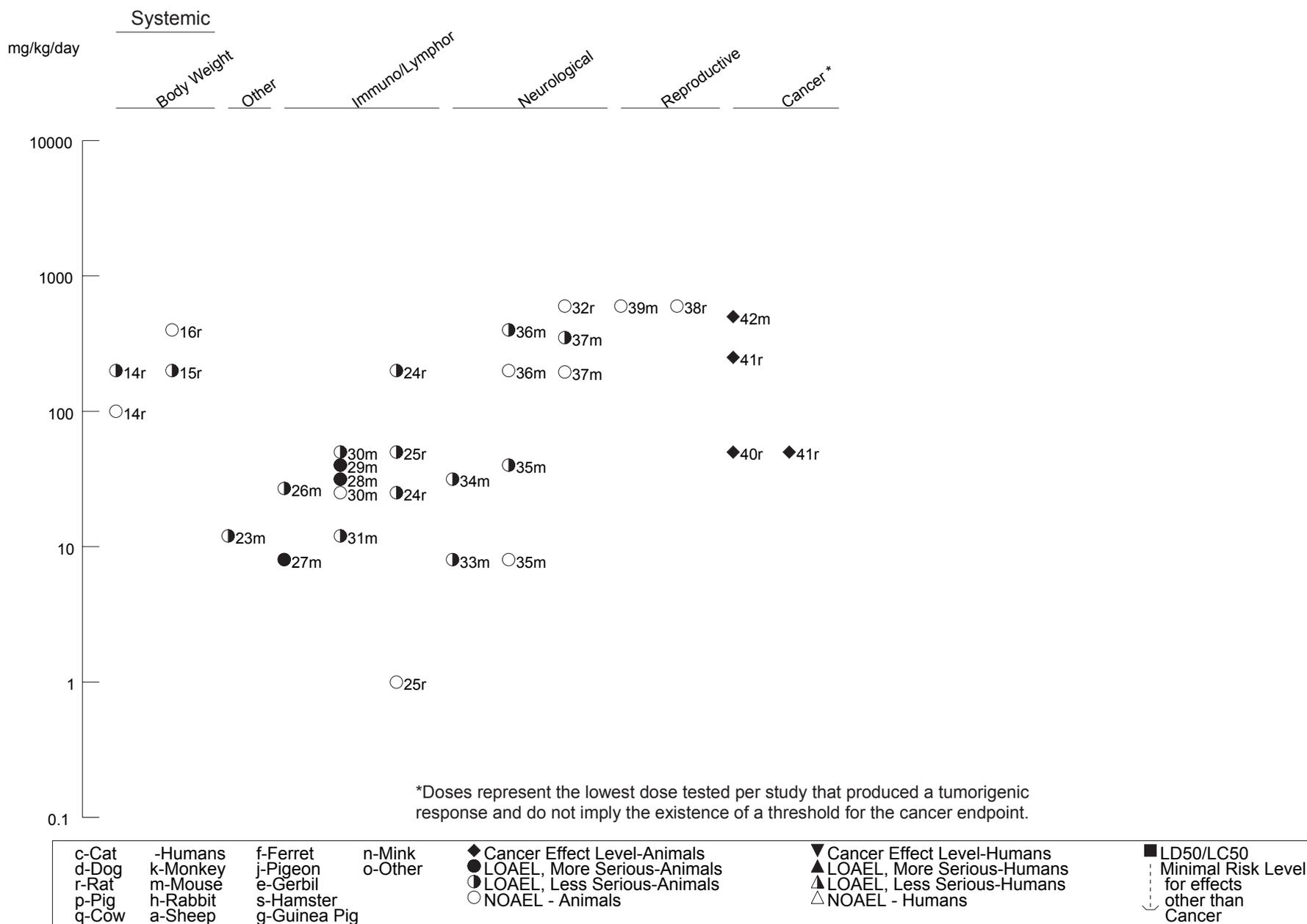
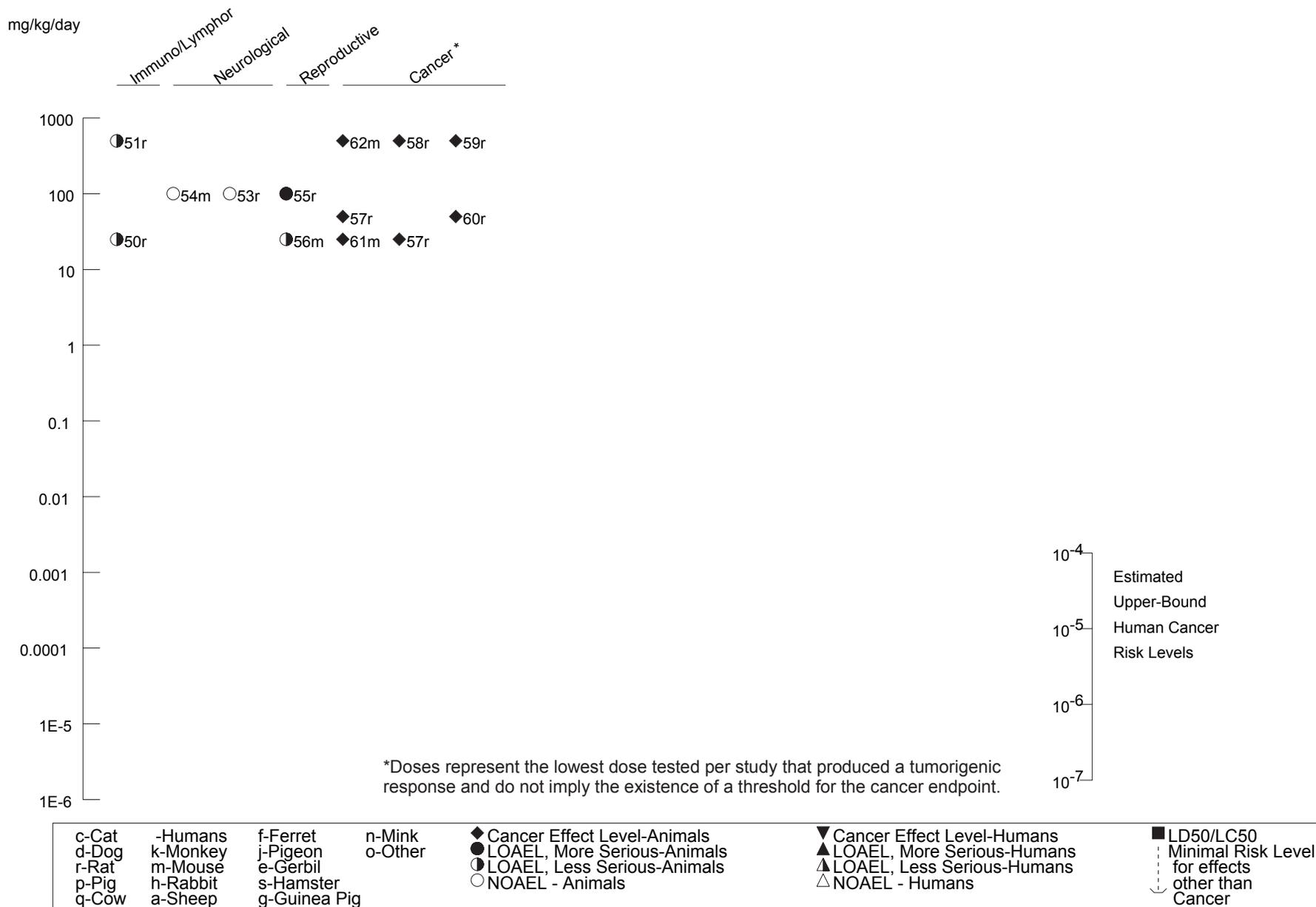


Figure 3-2 Levels of Significant Exposure to Benzene - Oral (Continued)

Chronic (≥365 days)



3. HEALTH EFFECTS

rats) or ≤ 100 mg/kg/day (female rats, male and female mice) no histopathological lesions were observed in the heart (NTP 1986).

Gastrointestinal Effects. A man swallowed an unspecified amount of benzene and survived, but developed an intense toxic gastritis and later pyloric stenosis (Greenburg 1926).

No histopathological lesions were observed in esophageal and stomach tissue or in the small intestine and colon from male and female Fischer 344 rats or B6C3F₁ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). After chronic-duration exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats, male and female mice), male rats exhibited hyperkeratosis and acanthosis in the nonglandular forestomach at 200 mg/kg/day, and mice exhibited epithelial hyperplasia and hyperkeratosis in the forestomach at 25 mg/kg/day (NTP 1986).

Hematological Effects. Prior to 1913, benzene was used as a treatment for leukemia. Benzene was given in gelatin capsules starting with 43 mg/kg/day and increasing to 71 mg/kg/day for unspecified durations (Selling 1916). Leukemia patients showed a great reduction in leukocyte count and multiple hemorrhages with advanced anemia. However, it is difficult to determine which effects were due to the leukemia and which were due to the benzene treatment.

Intermediate-duration studies in animals have revealed decreases in numbers of erythrocytes and leukocytes following exposure to benzene. Male and female Fischer 344 rats and B6C3F₁ mice were given oral doses of 0, 25, 50, 100, 200, 400, and 600 mg/kg/day benzene in corn oil for 120 days. Dose-related leukopenia and lymphopenia were observed at 200 and 600 mg/kg/day for both male and female rats killed on day 60, and at all doses in female rats killed on day 120. Dose-related leukopenia and lymphopenia were observed in male mice at 50 mg/kg/day and female mice at 400 mg/kg/day for 120 days, but not for 60 days. Mice exposed to 8 mg/kg/day in the drinking water for 4 weeks had decreased numbers of erythrocytes, increased mean corpuscular volumes, and decreased numbers of lymphocytes (Hsieh et al. 1988b, 1990). Female B6C3F₁ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased hemoglobin, hematocrit, leukocytes, MCV, and MCH were observed at 350 mg/kg/day. Decreased leukocytes also occurred at 195 mg/kg/day. Decreased leukocytes also occurred at 195 mg/kg/day. Rats gavaged with 50 mg/kg/day of benzene 5 days/week for 6 months also had decreased numbers of erythrocytes and leukocytes (Wolf et al. 1956). One chronic-duration study showed that gavage doses of 25 mg/kg/day resulted in leukopenia and/or lymphocytopenia in both rats and mice, both at the interim sacrifices at 3–18 months, and at the

3. HEALTH EFFECTS

end of 2 years (Huff et al. 1989; NTP 1986). Increased frequency of micronucleated normochromatic peripheral erythrocytes was observed in mice at 25 mg/kg/day after 2 years. Sprague-Dawley rats were exposed to 500 mg/kg benzene by ingestion (stomach tube), in olive oil, once daily, 4–5 days/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Decreased erythrocytes and leukocytes were observed after 84 weeks.

Musculoskeletal Effects. No histopathological lesions were observed in femoral tissue from male and female Fischer 344 rats or B6C3F₁ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in the sternbrae, femur, or vertebrae from Fischer 344 rats and mice exposed to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats, male and female mice) for 2 years (NTP 1986).

Hepatic Effects. Acute oral administration of 1,402 mg/kg/day benzene for 3 days induced hepatic changes in rats evidenced by increased liver weight, decreased protein in the postmitochondrial supernatant fractions (9,000 times specific gravity), and changes in hepatic drug metabolism and lipid peroxidation (Pawar and Mungikar 1975). The initiation-promotion-progression (IPP) model for the induction of malignant neoplasms in the liver was evaluated for benzene in male and female Sprague-Dawley rats (Dragan et al. 1993). Initiation was begun in 5-day-old rats with administration of a single intraperitoneal injection of diethylnitrosamine during the time when the liver is undergoing rapid growth. Promotion began at 6 months of age with phenobarbital in the feed, and continued into young adulthood. Partial hepatectomy was performed, and at the height of the regenerative proliferation phase following the hepatectomy, benzene (1 g/kg) was administered by gavage; phenobarbital treatment was maintained after the administration of benzene. A slight increase in the incidence of altered hepatic foci was observed after initiation and promotion. Few hepatic foci were observed in the livers of male Fischer 344 rats treated by gavage with 400 mg/kg/day benzene in corn oil for 5 days/week for 6 weeks (Taningher et al. 1995). No histopathological non-neoplastic lesions were observed in hepatic tissue from male and female Fischer 344 rats given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days or in male rats exposed to 50–200 mg/kg/day and female rats exposed to 25–100 mg/kg/day for 2 years (NTP 1986).

Female B6C3F₁ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). No adverse liver effects, as evidenced by gross necropsy, liver weight determination, and serum levels of hepatic enzymes, were observed. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect liver weight in CD-1

3. HEALTH EFFECTS

mice (Hsieh et al. 1990). No histopathological non-neoplastic lesions effects were observed in hepatic tissue from male and female B6C3F₁ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in male and female mice exposed to 25–100 mg/kg/day for 2 years (NTP 1986).

Renal Effects. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day benzene on Gd 6–15 and killed on Gd 20 (Exxon 1986). No adverse effects were noted in the kidneys based on gross necropsy. No adverse effects based on histological examination were observed on renal tissue or the urinary bladder from male and female Fischer 344 rats given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days or in male rats exposed to 50–200 mg/kg/day and female rats exposed to 25–100 mg/kg/day for 2 years (NTP 1986). Female B6C3F₁ mice were exposed to 0, 12, 195, or 350 mg/kg benzene in drinking water for 30 days (Shell 1992). No adverse effects were observed in the kidneys, based on kidney weights, gross examination, and blood urea nitrogen and creatinine determinations. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect kidney weight in CD-1 mice (Hsieh et al. 1990). No adverse effect based on histological examination was observed on renal tissue or the urinary bladder from male and female B6C3F₁ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in male and female mice exposed to 25–100 mg/kg/day for 2 years (NTP 1986).

Endocrine Effects. No histopathological lesions were observed in salivary, thyroid, parathyroid, pancreas, adrenal, or pituitary glands from male and female Fischer 344 rats or B6C3F₁ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). In the companion chronic-duration oral study, male Fischer 344 rats were exposed to 25, 50, 100, or 200 mg/kg/day benzene, while female rats received 25, 50, or 100 mg/kg/day benzene. Hyperplasia of the Zymbal gland was increased in low-dose males and in mid-dose females. In the adrenal gland, hyperplasia was observed in both sexes (males: 27 and 4% at 50 and 200 mg/kg, respectively; females: 34% at 25 mg/kg). In the thyroid gland, incidences of C-cell hyperplasia were 14, 26, 15, and 15% in males treated with 0, 50, 100, and 200 mg/kg, respectively. Analysis of the pituitary gland showed incidence of hyperplasia in males treated with 0, 50, 100, and 200 mg/kg at 6, 16, 20, and 10%, respectively; in females treated with 0, 25, 50, and 100 mg/kg at 11, 20, 10, and 14%, respectively. None of the increased incidences of hyperplasia in these glands were considered to be treatment-related by NTP. The non-dose-related increase of hyperplasia of the Zymbal gland could represent a progression to the neoplasms (see Section 3.2.2.7). In mice, Zymbal gland lesions showed epithelial hyperplasia in

3. HEALTH EFFECTS

males (0, 9, 30, and 26%) and in females (2, 3, 5, and 19%) exposed to 0, 25, 50, or 100 mg/kg, respectively. Hyperplasia of the adrenal cortex occurred at incidences of 4, 67, 29, and 9% in males and 10, 43, 68, and 13% in females, respectively. Hyperplasia of the harderian gland occurred at incidences of 0, 11, 22, and 15% in males and 13, 23, 22, and 21% in females, respectively (NTP 1986).

Dermal Effects. A case of accidental poisoning in which the patient survived but developed an odd skin condition consisting of swelling and edema has been reported (Greenburg 1926).

Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day benzene daily on Gd 6–15 and killed on Gd 20 (Exxon 1986). Alopecia of the hind limbs and trunk was noted in all dose groups.

No histopathological lesions were observed in the skin of male and female Fischer 344 rats and B6C3F₁ mice after chronic oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice) (NTP 1986).

Ocular Effects. No histopathological lesions were noted in the eyes of male and female Fischer 344 rats and B6C3F₁ mice after chronic-duration oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice) (NTP 1986).

Body Weight Effects. No significant change in body weight was observed in male Fischer 344 rats treated by gavage with 400 mg/kg/day benzene in corn oil for 5 days/week for 6 weeks (Taningher et al. 1995). Body weight was unaffected in male and female Fischer 344 rats given oral doses of 0, 25, 50, or 100 mg/kg/day benzene in corn oil for 120 days (NTP 1986). However, animals receiving 200, 400, or 600 mg/kg/day benzene exhibited a 14–22% decrease in body weight after 120 days. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg benzene daily on Gd 6–15 and killed on Gd 20 (Exxon 1986). Maternal body weight decreased 11% at the high dose. C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Groups of mice were killed on day 7, 14, 21, and 28 of administration, and on day 7, 14, 21, and 28 after the last administration of benzene at 200 mg/L. There was no effect of treatment on body weight. Female B6C3F₁ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). There was no significant effect on body weight at the highest treatment level. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect

3. HEALTH EFFECTS

body weight in CD-1 mice (Hsieh et al. 1990). No adverse effect was observed on body weight of male and female B6C3F₁ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986).

Sprague-Dawley rats were exposed to benzene by gavage in olive oil, at 0, 50, or 250 mg/kg/day body weight for 4–5 days/week for 52 weeks, and then kept under supervision until spontaneous death (Maltoni et al. 1983, 1985). A 19% decrease in body weight was reported in animals exposed to 250 mg/kg benzene for 52 weeks. In a companion study, Sprague-Dawley rats were exposed to 500 mg/kg benzene by ingestion (stomach tube), in olive oil, once daily, 4–5 days/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Decreased body weight was observed after 92 weeks. In a chronic-duration oral study, male Fischer 344 rats exhibited a decrease in body weight of 11% or more after 25 weeks exposure to doses of 200 mg/kg/day benzene in corn oil (NTP 1986). Females rats and male and female B6C3F₁ mice in the same study exposed to doses up to 100 mg/kg/day benzene did not show any change in body weight after 12 months of exposure, or after 2 years exposure (female rats). Male and female mice exhibited body weight effects after chronic exposure. In male mice given 100 mg/kg, mean body weights decreased from 10% after 47 weeks to 19% in 103 weeks of exposure relative to controls. In female mice given 100 mg/kg, mean body weights decreased 14–15% in weeks 99–103 of exposure (NTP 1986).

Other Systemic Effects. C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Groups of mice were killed on day 7, 14, 21, and 28 of administration, and on day 7, 14, 21, and 28 after the last administration of benzene at 200 mg/L. There was no effect of treatment on food or water consumption. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day on Gd 6–15 and killed on Gd 20 (Exxon 1986). Decreased feed consumption was noted at doses of 250 mg/kg and above, and body weight decreased 11% at the high dose. Female B6C3F₁ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased fluid consumption was observed at >12 mg/kg.

3.2.2.3 Immunological and Lymphoreticular Effects

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

3. HEALTH EFFECTS

Oral administration of benzene to CD-1 mice produced an immunotoxic effect on both the humoral and cellular immune responses (Hsieh et al. 1988b). Exposure to benzene at 8, 40, or 180 mg/kg/day for 4 weeks caused a significant dose-response reduction of total peripheral blood leukocytes and erythrocytes. Lymphocytes, but not neutrophils or other leukocytes, were decreased in number. Splenic lymphocyte proliferative response to B- and T-cell mitogens was biphasic—enhanced in the 8 mg/kg/day dosage group and depressed in the 40 and 180 mg/kg/day dosage groups. Cell-mediated immunity evaluated in mixed-lymphocyte reaction and in the ^{51}Cr -release assay showed a similar biphasic response. Antibody production was significantly suppressed in mice dosed at 40 and 180 mg/kg/day. The results indicate that administration of 40 mg/kg/day benzene has an immunosuppressive effect evident in decreased immune functions evaluated in *in vitro* assays for cell-mediated immunity and antibody production. A dose-related decrease in spleen weight was observed, which was significant only in the 180 mg/kg/day group.

C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Mice were sacrificed on days 7, 14, 21, and 28 of administration. Selected mice of the 27 mg/kg/day dose group were sacrificed on postexposure days 7, 14, and 21 in order to assess the postexposure time course of the benzene toxicity. At 27 mg/kg/day, a decreased number of splenocytes was observed on day 21 and 28 of exposure. At 154 mg/kg/day, spleen cell numbers decreased significantly as a function of time in mice treated for 14, 21, and 28 days. Benzene treatment for 3 weeks raised natural killer (NK) cell activity significantly at both doses. However, after another week of benzene treatment, NK cell activity resumed to control levels. Significant depression of interleukin-2 (IL-2) production was detected in both levels for 28 days. The NK cell activity showed normal levels on day 7, 14, and 21 after the last administration of benzene exposure for 28 days at 27 mg/kg/day. IL-2 production decreased significantly on day 7 and 14 after cessation of benzene administration, but recovered with time (43, 71, and 79% of control on day 7, 14, and 21, respectively). Spleen cell number decreased significantly on the 7th day, but recovered on day 14 and 21. Female B6C3F₁ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased leukocytes were observed at 12 mg/kg/day, and decreased spleen cell number was observed at 195 mg/kg/day.

In the NTP-sponsored intermediate-duration oral study using Fischer 344 rats and B6C3F₁ mice, dose-related leukopenia and lymphopenia were observed for both male and female Fischer 344 rats at 200 and 600 mg/kg/day killed on day 60, and at all doses in female rats killed on day 120 (NTP 1986). Decreased

3. HEALTH EFFECTS

leukocytes were observed in male and female rats exposed for 60 days to 200 and 600 mg/kg/day benzene. Lymphoid depletion in the B-cells of the spleen was observed in animals exposed to 200 mg/kg/day (3 of 5 males, 4 of 5 females) and 600 mg/kg/day (5 of 5 males, 5 of 5 females) benzene for 60 days and in animals that received 600 mg/kg/day (10 of 10 males, 10 of 10 females) benzene for 120 days. At 600 mg/kg/day benzene exposure, increased extramedullary hematopoiesis was observed in the spleen of 4 of 5 male and 3 of 5 female rats. Dose-related leukopenia and lymphopenia were observed for both male and female mice exposed for 120 days, but not for 60 days. Leukocytes and lymphocytes were significantly decreased in male mice exposed for 120 days to 50, 100, 200, 400, and 600 mg/kg/day benzene. At 120 days of exposure, leukocytes were significantly decreased in female mice at 600 mg/kg/day and lymphocytes at 400 and 600 mg/kg/day. Histological examination revealed no adverse effects in mandibular lymph node or the thymus for either rats or mice (Huff et al. 1989; NTP 1986). Rats exposed to benzene at 50 and 100 mg/kg/day for 6 months had significant leukopenia (Wolf et al. 1956).

Leukopenia and lymphopenia were observed in mice at 31.5 mg/kg/day after 4 weeks of oral exposure (Hsieh et al. 1990). Reduction in thymus mass, suppression of B- and T-cell mitogenesis, and suppressed IL-2 release were also noted. Similar results were noted at 40 mg/kg/day (Hsieh et al. 1991). Oral administration of benzene to B6C3F₁ mice and Fischer 344 rats at doses of 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice), 5 days/week for 103 weeks resulted in significant leukocytopenia and lymphocytopenia in both species (Huff et al. 1989; NTP 1986). In the thymus, lymphoid depletion was observed at 0, 10, 20, or 28% in male rats treated with 0, 50, 100, or 200 mg/kg/day, respectively. Increased incidences of lymphoid depletion of the spleen were observed in male rats treated with 0 (0%), 50 (40%), 100 (17%), and 200 (49%) mg/kg/day and in female rats treated with 0 (0%), 25 (22%), 50 (16%), and 100 (20%) mg/kg/day. In mice, an increased incidence of hematopoietic hyperplasia was observed in the bone marrow of dosed animals in both sexes. Splenic hematopoiesis was increased in dosed animals of both sexes of mice (Huff et al. 1989; NTP 1986). Maltoni et al. (1983, 1985) observed decreased leukocytes in Sprague-Dawley rats dosed with 500 mg/kg/day benzene for 84 weeks or more.

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

3. HEALTH EFFECTS

3.2.2.4 Neurological Effects

In humans, symptoms of central nervous system toxicity (including euphoria, vertigo, muscular incoordination, and unconsciousness) have been reported following one-time ingestion of benzene at 125 mg/kg (Thienes and Haley 1972).

Neurochemical profiles were conducted on rats after oral exposure to benzene (Kanada et al. 1994). Sprague-Dawley rats received a single dose of 950 mg/kg benzene by gavage and were sacrificed 2 hours after treatment. The control group received nothing. Brains were dissected into small-brain areas and stored until analysis. Acetylcholine, 3,4-dihydroxyphenylalanine (DOPA), dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine, 3-methoxy-4-hydroxyphenylglycol (MHPG), serotonin, and 5-hydroxyindoleacetic acid (5HIAA) contents in the small-brain regions were measured. Results showed that benzene decreased acetylcholine content of rat hippocampus. DOPA and norepinephrine content decreased in the rat midbrain. Dopamine, serotonin and 5HIAA content increased in the rat midbrain. Dopamine, DOPAC, norepinephrine, and 5HIAA content increased and serotonin content decreased in the rat hypothalamus after oral administration of benzene. Increased dopamine, HVA, MHPG, and serotonin content of rat medulla oblongata was observed. Decreased norepinephrine and 5HIAA content of rat medulla oblongata by benzene treatment was observed.

Oral exposure to benzene induced both synthesis and catabolism of monoamine neurotransmitters in CD-1 mice (Hsieh et al. 1988a). Mice given 8, 40, or 180 mg/kg/day of benzene for 4 weeks in drinking water exhibited changes in the levels of norepinephrine, dopamine, serotonin, and catecholamine metabolites in several brain regions but no treatment-related behavioral changes. Similar results were seen at 31.5–40 mg/kg/day (Hsieh et al. 1990, 1991). Because of the lack of association with behavioral changes, the effects on the neurotransmitters cannot be adequately assessed. Female B6C3F₁ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased brain weight was observed at 350 mg/kg/day. Sprague-Dawley rats given one oral dose of 88 mg/kg benzene exhibited slight central nervous system depression, whereas at 1,870 mg/kg/day, tremors were observed (Cornish and Ryan 1965). Histological examination of the brain revealed no treatment-related lesions after gavage treatment of male and female Fischer 344 rats and B6C3F₁ mice with doses up to 600 mg/kg/day for 120 days (NTP 1986). In the same experiment, B6C3F₁ mice exhibited tremors intermittently at doses of 400 mg/kg/day, which were more pronounced in males during the last 3 weeks of the study. No adverse effects based on histological examination of brain or spinal cord were observed

3. HEALTH EFFECTS

in male and female Fischer 344 rats and B6C3F₁ mice after chronic oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice) (NTP 1986).

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

3.2.2.5 Reproductive Effects

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

Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg benzene daily on Gd 6–15 and killed on Gd 20 (Exxon 1986). No adverse effects were noted on reproductive competency. No histological changes were reported in the prostate, testes, ovaries, mammary gland, or uterus of male and female Fischer 344 rats and B6C3F₁ mice dosed by gavage with up to 600 mg/kg/day benzene for 17 weeks (NTP 1986). In male and female Fischer 344 rats and B6C3F₁ mice after chronic oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice), endometrial stromal polyps occurred with a significant positive trend in female rats (NTP 1986). The incidence in high dose group (14/50) was significantly greater than that in the control (7/50). In mice, analysis of preputial gland lesions in male mice dosed at 0, 25, 50, or 100 mg/kg showed increased incidences of focal, diffuse or epithelial hyperplasia (5, 65, 31, and 3%, respectively). The lower incidences of hyperplasia in the higher dose groups were probably due to the progression of the preputial gland lesions to neoplasias (see Section 3.2.2.7). Various non-neoplastic and neoplastic ovarian lesions were observed in dosed female mice, including epithelial hyperplasia and senile atrophy (NTP 1986).

The NOAEL and LOAEL values for reproductive effects in rats and mice are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

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

Benzene was embryotoxic as evidenced by reduced pup body weights when mice were administered 1,300 mg/kg/day of benzene by gavage on Gd 8–12 (Seidenberg et al. 1986). No maternal toxicity was observed. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg

3. HEALTH EFFECTS

benzene daily on Gd 6–15 and killed on Gd 20 (Exxon 1986). No adverse effects were noted on morphological development.

The NOAEL value for rats and the LOAEL value for mice for developmental effects following acute oral exposure are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

Essentially no information was located regarding the oral carcinogenicity of benzene in humans. Lymphatic and hematopoietic cancers were increased in vehicle maintenance workers who occasionally siphoned gasoline by mouth (Hunting et al. 1995), but the skin and lungs were the main routes of exposure (see Section 3.2.3.7).

Benzene has been shown to be a multiple site carcinogen by the oral route in animals (Huff et al. 1989; Maltoni et al. 1983, 1985, 1989; NTP 1986). In bioassays conducted by the NTP, benzene in corn oil was administered groups of 50 F344/N rats and 50 B6C3F₁ of each sex by gavage on 5 days/week for 103 weeks (Huff et al. 1989; NTP 1986). The male rats were exposed to dose levels of 0, 50, 100, or 200 mg/kg/day, and female rats and mice of both sexes were exposed to 0, 25, 50, or 100 mg/kg/day. In the rats, benzene caused significantly increased incidences of Zymbal gland carcinomas in males at ≥ 100 mg/kg/day and females at ≥ 25 mg/kg/day, oral cavity squamous cell papillomas and carcinomas in males at ≥ 50 mg/kg/day and females at ≥ 25 mg/kg/day, and skin squamous cell papillomas and carcinomas in males at 200 mg/kg/day. In the mice, benzene mainly caused significantly increased incidences of malignant lymphomas in both sexes at ≥ 25 mg/kg/day, Zymbal gland carcinomas in males at ≥ 50 mg/kg/day and females at 100 mg/kg/day, lung alveolar/bronchiolar adenomas and carcinomas in males at ≥ 100 mg/kg/day and females at ≥ 50 mg/kg/day, Harderian gland adenomas in males at ≥ 25 mg/kg/day, preputial gland squamous cell carcinomas in males at ≥ 50 mg/kg/day, and mammary gland carcinomas in females at ≥ 50 mg/kg/day. NTP (1986) concluded that there was clear evidence of carcinogenicity of benzene in male and female F344/N rats and B6C3F₁ mice under the conditions of these studies.

Maltoni et al. (1983, 1985, 1989) assessed carcinogenicity in groups of 30–50 rats and mice of each sex that were exposed to benzene in olive oil by gavage on 4–5 days/week for up to 104 weeks and observed for life. Sprague-Dawley rats were exposed to 0, 50, or 250 mg/kg/day for 52 weeks or 0 or 500 mg/kg/day for 104 weeks; effects included increased incidences of Zymbal gland carcinomas in

3. HEALTH EFFECTS

females at ≥ 50 mg/kg/day, oral cavity carcinomas in females at 250 mg/kg/day and both sexes at 500 mg/kg/day, forestomach carcinomas in females at 500 mg/kg/day, nasal cavity and skin carcinomas in males at 500 mg/kg/day, and liver angiosarcomas in both sexes at 500 mg/kg/day. Wistar rats were exposed to 0 or 500 mg/kg/day for 104 weeks; effects included increased incidences of Zymbal gland, nasal cavity, and oral cavity carcinomas. Swiss mice were exposed to 0 or 500 mg/kg/day for 78 weeks; effects included increased incidences Zymbal gland carcinomas in males, mammary carcinomas in females, and lung adenomas in both sexes. RF/J mice were exposed to 0 or 500 mg/kg/day for 52 weeks; effects included increased incidences of mammary carcinomas in females and lung adenomas in both sexes.

As discussed in Section 3.2.1.7, there is a consensus that benzene is a human carcinogen (IARC 1987, 2004, 2007; IRIS 2007; NTP 2005). This conclusion is based on sufficient inhalation data in humans supported by animal evidence, including the oral studies summarized above. The human cancer induced by inhalation exposure to benzene is predominantly acute nonlymphocytic leukemia, whereas benzene is a multiple site carcinogen in animals by both the inhalation and oral routes. Due to the lack of oral carcinogenicity data in humans, as well as the lack of a well-demonstrated and reproducible animal model for leukemia from benzene exposure, EPA extrapolated an oral slope factor from the inhalation unit risk range (IRIS 2007). The oral slope factor ranges from 1.5×10^{-2} to 5.5×10^{-2} (mg/kg/day)⁻¹, and for cancer risks of 1×10^{-4} – 1×10^{-7} , the corresponding dose levels range from 6.7×10^{-3} – 1.8×10^{-3} to 6.7×10^{-6} – 1.8×10^{-6} mg/kg/day, respectively. These risk levels are presented in Figure 3-1.

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding deaths in animals after dermal exposure to benzene.

A cohort of 338 men was investigated as to causes of death among employees of the fleet maintenance division of Washington DC's Department of Public Works (Hunting et al. 1995). This mortality study was undertaken because of three cases of leukemia among car and mobile equipments mechanics. Preliminary evaluation showed that the garage mechanics regularly used gasoline to clean parts and wash their hands; these workers also experienced dermal and inhalation exposure to gasoline during maintenance of vehicles. The men were employed for at least 1 year between January 1, 1977, and December 31, 1989. Cause-specific SMRs were calculated. Increased risk of death was found in some categories.

3. HEALTH EFFECTS

3.2.3.2 Systemic Effects

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

Available data pertaining to hematological, dermal, and ocular effects are presented below. All reliable LOAEL values for systemic effects in humans and rabbits for acute- and chronic-duration dermal exposure are recorded in Table 3-3.

Hematological Effects. Tondel et al. (1995) reported a case of myelofibrosis that was diagnosed in a 46-year-old man who had worked from 1962 to 1979 as a gasoline station attendant. Although the exposure was primarily by inhalation, it is likely that dermal exposure also occurred.

Dermal Effects. In humans, benzene is a skin irritant. By defatting the keratin layer, it may cause erythema, vesiculation, and dry and scaly dermatitis (Sandmeyer 1981). Acute fatal exposure to benzene vapors caused second degree burns on the face, trunk, and limbs of the victims (Avis and Hutton 1993). Fifteen male workers were exposed to benzene vapors (>60 ppm) over several days during the removal of residual fuel from shipyard fuel tanks (Midzenski et al. 1992). Exposures to benzene range from 1 day to 3 weeks (mean of 5 days), 2.5–8 hours/day (mean of 5.5 hours). Workers with more than 2 days (16 hours) exposure reported mucous membrane irritation (80%), and skin irritation (13%) after exposure to the vapor.

Benzene was slightly irritating to the skin of rabbits (Wolf et al. 1956). The skin showed moderate erythema, edema, and moderate necrosis following application 1 time/day for 4 weeks.

Ocular Effects. Solvent workers who were exposed to 33 (men) or 59 (women) ppm benzene exhibited eye irritation while being exposed to the vapors (Yin et al. 1987b).

A transient increase in lacrimation was observed in male rats exposed to 10–300 ppm benzene for 6 hours/day, 5 days/week (Shell 1980). Moderate conjunctival irritation and transient corneal damage were observed in rabbits subsequent to placement of 2 drops of benzene onto the eyeball (Wolf et al. 1956).

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

Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL	LOAEL		Reference Chemical Form	Comments
				Less Serious	Serious		
ACUTE EXPOSURE							
Systemic							
Human	1-21 d 2.5-8 hr/d	Dermal		60 M ppm	(mucous membrane and skin irritation)	Midzenski et al. 1992	
Rabbit (NS)	once	Ocular		2 Unknown	(moderate conjunctival irritations; light corneal injury)	Wolf et al 1956	
INTERMEDIATE EXPOSURE							
Systemic							
Rat (CD)	10 wk 5 d/wk 6 hr/d	Ocular	1 M ppm	10 M ppm	(lacrimation)	Shell 1980	
CHRONIC EXPOSURE							
Systemic							
Human	>1 yr (occup)	Ocular		33 M ppm	(eye irritation)	Yin et al. 1987b	
				59 F ppm	(eye irritation)		

d = day(s); F = female; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; yr = year

3. HEALTH EFFECTS

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans or animals after dermal exposure to benzene.

3.2.3.4 Neurological Effects

Tondel et al. (1995) reported the case of a gasoline station attendant who had worked from 1962 to 1979 and who developed myelofibrosis. The patient described symptoms of fatigue for 3 weeks and night sweats, among other symptoms. Although the exposure was primarily by inhalation, it is probable that dermal exposure also occurred.

No studies were located regarding neurological effects in animals after dermal exposure to benzene.

3.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after dermal exposure to benzene.

3.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to benzene.

3.2.3.7 Cancer

A cohort mortality study was conducted to estimate the relative risk of hematological cancer among 335 male vehicle maintenance workers who were employed for at least 1 year between 1977 and 1989 and followed through 1991 (Hunting et al. 1995). The workers were car and mobile equipment mechanics who regularly used gasoline to clean parts and wash their hands. Exposure to gasoline also occurred via inhalation, and some of the workers occasionally siphoned gasoline by mouth. Since most of the cohort consisted of non-white (race data were missing for 9.5% of the cohort) vehicle maintenance workers who lived within the District of Columbia, death rates for age- and calendar-matched non-white males in the District of Columbia were used to calculate expected deaths for the cohort. Three deaths due to lymphatic and hematopoietic cancer were observed, yielding SMRs of 3.63 (95% CI 0.75–10.63) in the whole cohort and 4.22 (95% CI 0.87–12.34) in a subgroup of 297 workers with the highest potential for

3. HEALTH EFFECTS

exposure. Neither of these SMRs were significant, although analysis by cancer subtype showed a significantly elevated risk for leukemia and aleukemia in the high exposure subgroup (two cases, SMR=9.26, 95% CI 1.12–33.43). Aleukemia refers to leukemias in which the blood has normal or near-normal white blood cell counts, but few numbers of young leukocytes. Two additional cases of leukemia were identified among workers who were not included in the cohort (one died after the end of the follow-up period and another was still alive). Mortalities from causes other than lymphatic/hematopoietic cancer were not significantly increased.

Application of benzene to the skin of animals has not produced evidence of carcinogenicity, although most studies were inadequate for evaluation. As summarized by IARC (1982, 1987), many dermal carcinogenicity studies of chemicals other than benzene used benzene as a vehicle, and treated large numbers of control animals (mice) with benzene alone. None of these studies indicated that benzene induced skin tumors; however, all possible tumor sites usually were not examined.

3.3 GENOTOXICITY

The genotoxic effects of benzene have been studied extensively. The *in vivo* and *in vitro* data are summarized in Tables 3-4 and 3-5, respectively. In chronically-exposed humans, benzene and/or its metabolites primarily cause chromosomal aberrations (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hartwich et al. 1969; Hedli et al. 1991; Karacic et al. 1995; Kašuba et al. 2000; Major et al. 1992, 1994; Picciano 1979; Popp et al. 1992; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Sellyei and Kelemen 1971; Smith et al. 1998; Sul et al. 2002; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Türkel and Egeli 1994; Van den Berghe et al. 1979; Yardley-Jones et al. 1990; Zhang et al. 1998b, 1999).

Chromosomal aberrations in humans are frequently demonstrated in peripheral blood lymphocytes and bone marrow. Although inhalation, oral, and dermal routes are all potential pathways of exposure relevant to humans, available *in vivo* human data are usually drawn from occupational settings in which inhalation and dermal exposure routes are most prevalent. In most of these studies, chromosome abnormalities were detected in workers exposed to high concentrations of benzene, sufficient to produce blood dyscrasias. However, Qu et al. (2003a, 2003b) noted a concentration-related increase in chromosomal aberrations across a wide range of exposure concentrations, including workers with relatively low-level benzene exposure. Limitations of many of the occupational studies include lack of accurate exposure data, possible coexposure to other chemicals, and lack of appropriate control groups.

3. HEALTH EFFECTS

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

Species (test system)	End point	Result	Reference
Prokaryotic cells:			
<i>Escherichia coli</i> (host mediated DNA repair)	DNA synthesis	–	Hellmér and Bolcsfoldi 1992a
Invertebrate animal cells:			
<i>Drosophila melanogaster</i>	Sex-linked recessive lethal	–	Kale and Baum 1983
Spermatocytes	Recombination	–	
Spermatogonia	Recombination	+	
Spermatocytes	Heritable translocation	–	
Mammalian cells:			
Mouse (bone marrow)	Chromosomal aberrations	+	Giver et al. 2001; Shelby and Witt 1995; Siou et al. 1981
Mouse (bone marrow)	Chromosomal aberrations	+ ^a	Meyne and Legator 1980
Mouse (bone marrow)	Chromosomal aberrations	(+)	Tice et al. 1980; 1982
Mouse (spleen lymphocytes)	Chromosomal aberrations	+	Au et al. 1991; Rithidech et al. 1987
Mouse (lymphoid cells, myeloid cells)	Chromosomal aberrations	+	Giver et al. 2001
Rat (bone marrow)	Chromosomal aberrations	+	Fujie et al. 1990; Hoechst 1977; Philip and Jensen 1970; Styles and Richardson 1984
Rat (bone marrow)	Chromosomal aberrations	+ ^b	Anderson and Richardson 1981 ^b
Rat (bone marrow)	Chromosomal aberrations	–	Hoechst 1977
Chinese hamster (bone marrow)	Chromosomal aberrations	+	Siou et al. 1981
Rabbit (bone marrow)	Chromosomal aberrations	+	Kissling and Speck 1972; 1973
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Bogadi-Šare et al. 1997; Ding et al. 1983; Forni et al. 1971a; Kašuba et al. 2000; Picciano 1979; Sasiadek 1992; Sasiadek and Jagielski 1990; Sasiadek et al. 1989; Smith et al. 1998; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Zhang et al. 1998a, 1999
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	(+)	Yardley-Jones et al. 1990
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	–	Bogadi-Šare et al. 1997; Jablonická et al. 1987
Mouse (bone marrow)	Micronuclei	+	Shelby and Witt 1995; Shelby et al. 1993
Mouse (bone marrow PCEs)	Micronuclei	+ ^c	Ciranni et al. 1988
Mouse ^d (bone marrow PCEs)	Micronuclei	+ ^e	Suzuki et al. 1989

3. HEALTH EFFECTS

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

Species (test system)	End point	Result	Reference
Mouse (bone marrow PCEs)	Micronuclei	+	Au et al. 1990; Barale et al. 1985; Chen et al. 1994; Diaz et al. 1980; Erexson et al. 1986; Farris et al. 1996; Harper et al. 1984; Hite et al. 1980; Siou et al. 1981; Toft et al. 1982
Mouse (bone marrow PCEs)	Micronuclei	+ ^a	Meyne and Legator 1980
Mouse (bone marrow PCEs)	Micronuclei	+ ^f	Eastmond et al. 2001
Mouse (bone marrow NCEs)	Micronuclei	+	Farris et al. 1996
Mouse (bone marrow NCEs)	Micronuclei	+ ^f	Eastmond et al. 2001
Mouse (pregnant/bone marrow PCEs)	Micronuclei	(+)	Ciranni et al. 1988
Mouse (peripheral blood)	Micronuclei	+	Hayashi et al. 1992; Healy et al. 2001
Mouse (peripheral blood PCEs)	Micronuclei	+	Farris et al. 1996
Mouse (peripheral blood PCEs)	Micronuclei	+ ^{c,g}	Luke et al. 1988a
Mouse (peripheral blood NCEs)	Micronuclei	+	Barale et al. 1985; Choy et al. 1985; Farris et al. 1996; Rithidech et al. 1988
Mouse (peripheral blood NCEs)	Micronuclei	+ ^{c,f}	Luke et al. 1988a
Mouse (lung fibroblasts)	Micronuclei	+	Ranaldi et al. 1998
Mouse (fetus/liver cells)	Micronuclei	+	Ciranni et al. 1988
Rat (lymphocytes)	Micronuclei	+	Erexson et al. 1986
Chinese hamster (bone marrow)	Micronuclei	+	Siou et al. 1981
Human (lymphocytes)	Micronuclei	+	Robertson et al. 1991
Human (occupational exposure/lymphocytes)	Micronuclei	+	Liu et al. 1996
Human (occupational exposure/lymphocytes)	Micronuclei	-	Pitarque et al. 1996; Surrallés et al. 1997
Mouse (bone marrow)	Sister chromatid exchange	+	Tice et al. 1980; 1982
Mouse (pregnant/bone marrow)	Sister chromatid exchange	+	Sharma et al. 1985
Mouse (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
Mouse (fetus/liver cells)	Sister chromatid exchange	+	Sharma et al. 1985
Rat (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	+	Popp et al. 1992
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	-	Kašuba et al. 2000; Pitarque et al. 1997; Seiji et al. 1990; Yardley-Jones et al. 1988
Mouse (spleen lymphocytes)	Mutations	+	Ward et al. 1992
Mouse (lung tissue)	Mutations	+	Mullin et al. 1998
Mouse embryo (premelanocytes)	Mutations (deletions)	+	Schiestl et al. 1997
Human (bone marrow)	Mutations (gene-duplicating)	+	Rothman et al. 1995

3. HEALTH EFFECTS

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

Species (test system)	End point	Result	Reference
Human (bone marrow)	Mutations (gene-inactivating)	–	Rothman et al. 1995
Mouse (bone marrow)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lévy et al. 1996; Pathak et al. 1995; Turteltaub and Mani 2003
Mouse (white blood cells)	DNA adducts	+	Lévy et al. 1996
Mouse (liver)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Mani et al. 1999; Turteltaub and Mani 2003
Rat (bone marrow)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lévy et al. 1996; Pathak et al. 1995; Turteltaub and Mani 2003
Rat (liver)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lutz and Schlatter 1977; Mani et al. 1999; Mazzullo et al. 1989; Turteltaub and Mani 2003
Mouse (peripheral blood lymphocytes)	DNA strand breaks	+	Tuo et al. 1996
Rat (lymphocytes, bone marrow, spleen, liver)	DNA strand breaks	+	Lee et al. 2005
Human (occupational exposure/lymphocytes)	DNA strand breaks	+	Andreoli et al. 1997; Nilsson et al. 1996; Sul et al. 2002
Mouse (peripheral blood lymphocytes, bone marrow)	DNA damage	+	Chang et al. 2005
Human (occupational exposure/lymphocytes)	DNA repair efficiency	–	Hallberg et al. 1996
Rat (bone marrow)	DNA oxidative damage	+	Kolachana et al. 1993
Human (occupational exposure/lymphocytes)	DNA oxidative damage	+	Liu et al. 1996
Mouse (bone marrow)	DNA synthesis inhibition	+	Lee et al. 1988
Rabbit (bone marrow)	DNA synthesis inhibition	+	Kissling and Speck 1972
Mouse (bone marrow)	RNA synthesis inhibition	+	Kissling and Speck 1972
Rat (liver mitochondria)	RNA synthesis inhibition	+	Kalf et al. 1982

3. HEALTH EFFECTS

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

Species (test system)	End point	Result	Reference
Mouse (spermatogonia)	Sperm head abnormality	+	Topham 1980

^aThis result was observed following both oral and intraperitoneal exposure.

^bThis result was observed following both inhalation and intraperitoneal exposure.

^cMales affected to a significantly greater degree than females.

^dTwo strains of mouse were tested; Ms/Ae and CD-1. The result applies to both strains.

^eThis result was observed following both oral and intraperitoneal exposure; however, oral exposure produced the greater effect.

^fIncrease in micronuclei was exposure duration-dependent.

^gIncrease in micronuclei was exposure duration-independent.

+ = Positive result; - = negative result; (+) = weakly positive result; DNA = deoxyribonucleic acid; NCEs = normochromatic erythrocytes; PCEs = polychromatic erythrocytes; RNA = ribonucleic acid

3. HEALTH EFFECTS

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

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Prokaryotic organisms:				
<i>Salmonella typhimurium</i> (Ames test)	Gene mutation	–	–	De Flora et al. 1984
<i>S. typhimurium</i> (histidine reversion)	Gene mutation	+	–	Glatt et al. 1989
<i>S. typhimurium</i> (azaquanine reversion)	Gene mutation	+	No data	Kaden et al. 1979; Seixas et al. 1982
<i>Bacillus subtilis</i> (histidine reversion)	Gene mutation	–	–	Tannoka 1977
<i>Escherichia coli</i> (DNA polymerase 1/cell-free DNA synthetic system)	DNA synthesis inhibition	No data	–	Lee et al. 1988
<i>E. coli</i> (host mediated DNA repair)	DNA synthesis	No data	No data	Hellmér and Bolcsfoldi 1992b
Plasmid DNA Φ X-174 RF I	DNA degradation	No data	+	Li et al. 1995
Eukaryotic organisms:				
Fungi:				
<i>Aspergillus nidulans</i> (methionine suppressors)	Gene mutation	No data	–	Crebelli et al. 1986
Mammalian cells:				
Mouse (L5178Y cells/TK test)	Gene mutation	–	–	Oberly et al. 1984
Chinese hamster (ovary cell culture)	Chromosomal aberrations	–	–	Gulati et al. 1989
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	+	Eastmond et al. 1994; Morimoto 1976
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	–	Gerner-Smidt and Friedrich 1978
Human (lymphoblastoid culture)	Intrachromosomal recombination	No data	+	Aubrecht et al. 1995
Chinese hamster (ovary cell culture)	Micronuclei	–	–	Douglas et al. 1985
Human (whole blood cells)	Micronuclei	–	–	Zarani et al. 1999
Chinese hamster (ovary cell culture)	Sister chromatid exchange	–	–	Douglas et al. 1985; Gulati et al. 1989
Human (lymphocyte cell culture)	Sister chromatid exchange	+	No data	Morimoto 1983
Human (lymphocyte cell culture)	Sister chromatid exchange	No data	–	Gerner-Smidt and Friedrich 1978
Rabbit (bone marrow mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984

3. HEALTH EFFECTS

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

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Rat (liver mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984
Calf thymus DNA	DNA adducts	No data	+	Chenna et al. 1995
Human (bone marrow)	DNA adducts	No data	+	Bodell et al. 1993; Lévay and Bodell 1992
Human (leukemia cells)	DNA adducts	No data	+	Bodell et al. 1993; Lévay and Bodell 1992
Rat (hepatocytes)	DNA breaks	No data	–	Bradley 1985
Chinese hamster (ovary cell culture)	DNA breaks	+	+	Douglas et al. 1985
Chinese hamster (ovary cell culture)	DNA breaks	+	+ ^a	Lakhanisky and Hendricks 1985
Chinese hamster (V79 cell culture)	DNA breaks	–	–	Swenberg et al. 1976
Mouse (L5178Y cell culture)	DNA breaks	No data	–	Pellack-Walker and Blumer 1986
Human (leukemia cells)	DNA oxidative damage	No data	+	Kolachana et al. 1993
Human (lymphocyte cell culture)	DNA repair	No data	–	Hallberg et al. 1996
Rat liver epithelial cells	DNA hyperphosphorylation	No data	+	Dees and Travis 1994
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	(+)	Glauert et al. 1985
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	–	Probst and Hill 1985; Williams et al. 1985
Human (HeLa S3 cells)	Unscheduled DNA synthesis	–	–	Barrett 1985
Mouse (bone marrow cell culture)	DNA synthesis inhibition	No data	+	Lee et al. 1988
Mouse (bone marrow cell culture)	DNA synthesis inhibition	+	(+)	Lee et al. 1989
Calf (thymus DNA polymerase α /cell-free DNA synthetic system)	DNA synthesis inhibition	No data	+	Lee et al. 1988
Human (HeLa cells)	DNA synthesis inhibition	–	–	Painter and Howard 1982
Mouse (spleen lymphocytes)	RNA synthesis inhibition	No data	+	Post et al. 1985

3. HEALTH EFFECTS

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

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Rat (liver mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982
Cat, rabbit (bone marrow mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982

^aBenzene's effect on DNA breaks was reduced when metabolic activators were used.

– = negative results; + = positive results; (+) = weakly positive result; DNA = deoxyribonucleic acid; RNA = ribonucleic acid

3. HEALTH EFFECTS

Chromosomal aberrations observed in workers chronically exposed to benzene include hypo- and hyperdiploidy, deletions, breaks, and gaps. For example, analysis of peripheral lymphocytes of workers exposed to benzene vapors at a mean concentration of 30 ppm revealed significant increases in monosomy of chromosomes 5, 7, and 8 (but not 1), and tri- and/or tetrasomy of chromosomes 1, 5, 7, and 8 (Zhang et al. 1998b, 1999). In another series of epidemiological studies in workers chronically exposed to benzene, nonrandom effects were apparent in chromosomes 1, 2, 4, and 9; nonrandom breaks in chromosomes 2, 4, and 9 were twice as prevalent in benzene-exposed workers versus controls; and chromosomes 1 and 2 were nearly twice as prone to gaps (Sasiadek and Jagielski 1990; Sasiadek et al. 1989). Twenty-one people with hematological signs of chronic benzene poisoning exhibited significantly more chromosomal abnormalities than controls (Ding et al. 1983). A significant increase in dicentric chromosomes and unstable aberrations was noted in 36 female workers exposed to benzene in a shoe factory for up to 32 years (Kašuba et al. 2000). Significant increases in hyperploidy of chromosomes 8 and 21 and translocations between chromosomes 8 and 21 were observed in workers exposed to benzene vapors at a mean TWA of 31 ppm (Smith et al. 1998).

DNA repair efficiency was evaluated in blood lymphocytes collected from exposed or unexposed workers in a petrochemical plant (Hallberg et al. 1996). Plasmids (pCMV_{CAT}) were irradiated with UV light (254 nm) to induce thymidine dimers and were then transfected into blood lymphocytes from workers. Transfected plasmids that were repaired in the lymphocytes would express the chloramphenicol acetyltransferase reporter gene (CAT) product whereas unrepaired plasmids would not. Lymphocytes from exposed or unexposed workers did not show significant differences in their ability to repair light-damaged DNA; however, the authors suggest that the sample population was too small to detect any differences given the large individual variations in repair capacity (Hallberg et al. 1996).

Oxidative DNA damage was assessed in workers (n=87) exposed to benzene by conducting measurements of 8-hydroxy-2-deoxyguanosine (8-OHdG) in peripheral blood lymphocytes (Liu et al. 1996). 8-OHdG is formed by hydroxy radical (OH·) addition at the C-8 position of deoxyguanosine (Kasai and Nishimura 1986) and appears to be a biomarker of oxidative DNA damage (Liu et al. 1996). The exposure to benzene was classified as low, medium, or high (mean benzene levels of 2.46, 103, or 424 mg/m³, respectively [corresponding to 0.78, 32.2, or 133 ppm]). Levels of 8-OHdG in exposed workers rose in a concentration-related manner although the increases were significant only in the medium and high exposure groups. Toluene, also detected in the workplace air, did not alter levels of 8-OHdG. Formation of micronuclei, a measure of DNA damage, increased in a concentration-related manner to levels that were significantly higher than those in controls. The levels of urinary *trans,trans*-

3. HEALTH EFFECTS

muonic acid, a biomarker of benzene exposure, were well correlated with levels of 8-OHdG which, in turn, were well correlated with levels of lymphocyte micronuclei. The findings of Liu et al. (1996) are supported by the results of a study by Nilsson et al. (1996) in which concentration-related increased urinary levels of 8-OHdG were reported in male workers (n=30) at a gasoline station. Breathing zone benzene concentrations ranged from 0.003 to 0.6 ppm (mean 0.13 ppm). Significant concentration-related increases in DNA single-strand breaks were also noted in these workers. Collectively, the results of Liu et al. (1996) and Nilsson et al. (1996) provide suggestive evidence that benzene metabolites may induce reactive oxygen species, which could result in oxidative DNA damage and formation of hydroxylated bases such as 8-OHdG (Liu et al. 1996).

Rothman et al. (1995) used the glycophorin A (GPA) gene loss mutation assay to assess the nature of DNA damage in workers heavily exposed to benzene. The GPA assay measures the frequency of variant cells that have lost the expression of the M form of the GPA gene in the peripheral blood of heterozygous (MN) subjects. The variant cells possess the NN phenotype (double-copy expression of the N allele and no expression of M) or the NØ phenotype (single-copy expression of the N allele and no expression of M). The NN variants are thought to arise from mitotic recombination, chromosome loss, and reduplication, or gene conversion. The NØ variants appear to be associated with point mutations, deletions, or gene inactivation. Rothman et al. (1995) demonstrated a significant increase in the frequency of NN cells in benzene-exposed workers (compared with unexposed control subjects) in the absence of a significant effect on the frequency of NØ cells. These results suggest that benzene induces gene-duplicating, but not gene-inactivating, mutations at the GPA locus in benzene-exposed humans.

Sister chromatid exchange was not found to be a significant effect of benzene exposure in humans (Kašuba et al. 2000; Seiji et al. 1990; Yardley-Jones et al. 1988); however, the selection of control subjects in the studies by Seiji et al. (1990) and Yardley-Jones et al. (1988) was poor. Refer to Table 3-4 for a further summary of these results.

In vivo animal studies provide convincing evidence of benzene's genotoxicity (Table 3-4). Furthermore, the finding that male mice are more sensitive than females to benzene-induced chromosomal damage is consistent among reports (Armstrong and Galloway 1993; Barale et al. 1985; Choy et al. 1985; Ciranni et al. 1988; Hatakeyama et al. 1992; Meyne and Legator 1980; Siou et al. 1981). Consistently positive findings for chromosomal aberrations in bone marrow and lymphocytes in animals support the human case reports and epidemiological studies in which chromosomal damage was linked to benzene exposure. Micronucleus assays are popular methods for crudely analyzing DNA damage in animals. Positive results

3. HEALTH EFFECTS

were observed in all studies testing for increased micronuclei frequencies. One of these micronucleus assays (Luke et al. 1988a) investigated the effects of different inhalation exposure durations on polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) in peripheral blood. PCEs are newly formed erythrocytes that contain mRNA and as a result, exhibit staining when RNA staining reagents are used. NCEs are mature erythrocytes that lack mRNA and are not stained under the same conditions. The researchers found that PCEs are good indicators of recent and acute exposure, while NCEs are good indicators of accumulated long-term exposure (Luke et al. 1988a). Although no human studies were located that reported increased sister chromatid exchange in exposed individuals, increases in sister chromatid exchange were reported in mice and rats (Erexson et al. 1986; Sharma et al. 1985; Tice et al. 1980, 1982). *In vivo, trans,trans*-muconaldehyde (a metabolite of benzene) has also been shown to induce highly significant increases in sister chromatid exchanges in mice (Witz et al. 1990a). In addition to oral and inhalation routes, many researchers tested subcutaneous and intraperitoneal routes as well; the results for these alternate routes of exposure were largely positive for chromosomal aberrations in bone marrow (Anderson and Richardson 1981; Kissling and Speck 1972, 1973; Kolachana et al. 1993; Meyne and Legator 1980; Philip and Jensen 1970), micronuclei in bone marrow (Diaz et al. 1980), and sister chromatid exchange in mouse fetus liver cells (Sharma et al. 1985). Binding of benzene and/or its metabolites to DNA, RNA, and proteins has been consistently observed in rats and mice (Arfellini et al. 1985; Creek et al. 1997; Lévy et al. 1996; Mani et al. 1999; Mazullo et al. 1989; Turteltaub and Mani 2003). Arfellini et al. (1985) noted that binding to RNA and proteins was more prevalent than binding to DNA. Lévy et al. (1996) observed dose- and time-dependent formation of two DNA adducts in white blood cells and bone marrow cells of mice administered benzene for 7 days via intraperitoneal injection. Turteltaub and Mani (2003) consistently observed DNA and protein binding in mice at intraperitoneal doses as low as 5 µg/kg body weight. One study using intraperitoneal injections reported a dose-dependent increase in sperm head abnormalities in mice exposed to 0.5 or 0.6 mL benzene/kg/day (Topham 1980). The author views sperm head abnormality as a possible indication of heritable mutations. However, from this study alone, it cannot be determined if benzene causes such transmissible genetic mutations. For these and other results from animal *in vivo* studies, see Table 3-4.

Molecular mechanisms of benzene-induced genotoxicity have been studied to some extent in laboratory animals. Schiestl et al. (1997) noted benzene-induced reversion of the mouse pink-eye unstable mutation in premelanocytes of mouse embryos following intraperitoneal injection of the chemical into pregnant dams; the reversions resulted from genomic DNA deletions. Results of Chen et al. (1994) and Eastmond et al. (2001) indicate that benzene-induced micronuclei in mouse bone marrow erythrocytes are formed predominantly from chromosome breakage, but also from aneuploidy.

3. HEALTH EFFECTS

In vitro studies strongly imply that benzene's genotoxicity is derived primarily from its metabolites. Positive results were obtained for gene mutation in *Salmonella typhimurium* (Glatt et al. 1989; Kaden et al. 1979; Seixas et al. 1982) and sister chromatid exchange in human lymphocyte cell culture (Morimoto 1983) only when exogenous metabolic activators of benzene were used. Similarly, endogenous metabolic activation was required for effects to be seen on DNA synthesis in rat hepatocyte culture (Glauert et al. 1985), DNA adduct formation in rat liver mitoplasts (Rushmore et al. 1984), and RNA synthesis in rat liver mitoplasts and in rabbit and cat bone marrow mitoplasts (Kalf et al. 1982). Endogenous activation occurs naturally by enzymes already within the cells. Exogenous activation requires the addition of enzymes to cellular preparations. In one study using benzene and 13 possible metabolites, Glatt et al. (1989) found that *trans*-1,2-dihydrodiol (with metabolic activators) and the diol epoxides (with or without metabolic activators) produced histidine reversion in *S. typhimurium*. The same researchers also investigated the genotoxicity of these 13 proposed metabolites in V79 Chinese hamster cells; *anti*-diol epoxide, *syn*-diol epoxide, 1,2,3-trihydroxybenzene, 1,2,4-trihydroxybenzene, quinone, hydroquinone, catechol, phenol, and 1,2-dihydrodiol were found to produce genotoxic effects ranging from sister chromatid exchange and micronuclei increases to gene mutations (Glatt et al. 1989). Similar studies with *trans,trans*-muconaldehyde showed that this metabolite is strongly mutagenic in V79 cells and weakly mutagenic in bacteria (Glatt and Witz 1990). Chang et al. (1994) showed that muconaldehyde and its aldehyde metabolites 6-hydroxy-*trans,trans*-2,4-hexadienal and 6-oxo-*trans,trans*-hexadienoic acid were mutagenic in V79 cells.

Several studies revealed a possible connection between certain benzene metabolites and DNA damage via the formation of oxygen radicals. Lewis et al. (1988) found that both 1,2,4-benzenetriol and hydroquinone produce DNA strand breaks, 1,2,4-benzenetriol to a greater degree than hydroquinone. Consistent with these findings was the observation that 1,2,4-benzenetriol generates a greater concentration of oxygen radicals than hydroquinone. The study concluded that 1,2,4-benzenetriol damages DNA by producing oxygen radicals, while hydroquinone probably exerts its genotoxic effects by some other mechanism (Lewis et al. 1988). However, results of Li et al. (1995b) indicate that much of the benzene-mediated DNA damage may result from the generation of reactive oxygen species via a copper-redox cycling mechanism involved in the oxidation of the benzene metabolite, hydroquinone. Increased recombination, which can lead to adverse genetic changes, was observed in Chinese hamster ovary cells exposed to phenol, catechol, or benzoquinone (Winn 2003). Benzene had no effect on recombination. The observed increases in recombination were abolished by the addition of catalase to the cells, suggesting that the effect of the benzene metabolites was elicited by oxidative stress (Winn 2003).

3. HEALTH EFFECTS

Oxidative damage (manifested as DNA strand breaks) was observed in HL60 cells treated with 1,4-benzoquinone or 1,4-hydroquinone (Hiraku and Kawanishi 1996). 1,4-Benzoquinone and hydroquinone induced DNA strand breaks in Chinese hamster ovary cells while phenol, catechol, 1,2,4-benzenetriol, *trans,trans*-muconic acid, and *S*-phenyl mercapturic acid did not (Sze et al. 1996). No synergism between hydroquinone and the other metabolites was observed.

Phenol, catechol, hydroquinone, and benzene induced morphological transformation and gene mutations in Syrian hamster embryo cells (Tsutsui et al. 1997). Chromosomal aberrations, sister chromatid exchange, and unscheduled DNA synthesis were increased by the benzene metabolites while aneuploidy was observed in cells treated with benzene or catechol (Tsutsui et al. 1997). There was no significant increase ($p > 0.005$) in DNA fragmentation in human respiratory epithelial cells exposed to an atmosphere of 5 mg benzene/m³ for 8 hours, although there was evidence of an inflammatory response (Gosepath et al. 2003). Chromosomal breaks and hyperdiploidy were observed in human lymphocytes after exposure to hydroquinone *in vitro* (Eastmond et al. 1994). Aneusomy of chromosomes 7 and 8 were observed in human umbilical cord blood cells treated with hydroquinone (2, 10, or 50 μ M) for 72 hours (Smith et al. 2000). Monosomy 7 and trisomy 8 are two common clonal aberrations observed in myeloid leukemias (Smith et al. 2000). Hydroquinone (26–49 μ M) also induced monosomy of chromosomes 5, 7, and 8 in a human lymphoblast cell line (Stillman et al. 1997). Hydroquinone (10–100 μ M) and 1,2,4-benzenetriol (10–50 μ M) significantly increased monosomy 5 and 7 in human lymphocytes and long-arm deletions in chromosomes 5 and 7 (Zhang et al. 1998b). Benzene metabolites have been shown to form DNA adducts in human bone marrow and HL-60 cells (Bodell et al. 1993; Lévy and Bodell 1992). Zhang et al. (1993) showed that 1,2,4-benzenetriol increased the frequency of micronuclei formation in human lymphocytes in culture, and in HL60 cells in a dose-related manner. An increase in the level of oxidative damage to DNA was also noted in HL60 cells in culture. Extracts from human cells have been shown to have repair activity toward benzoquinone-DNA adducts *in vitro* (Chenna et al. 1995). Benzene (1–5 mM) did not elicit micronuclei formation in whole blood cells treated for 48 hours with or without metabolic activation with 10% S9 rat liver fraction for 2 hours (Zarani et al. 1999). The assay was conducted with blood collected from four subjects. Chen and Eastmond (1995) showed that benzene metabolites can adversely affect human topoisomerases, enzymes involved in DNA replication and repair. No effect of any metabolite was seen on human topoisomerase I or for topoisomerase II for hydroquinone, phenol, 2,2'-biphenol, 4,4'-biphenol, and catechol at concentrations as high as 500 μ M. 1,4-Benzoquinone and 1,2,4-benzenetriol inhibited human topoisomerase II *in vitro*, at 500 and 250 μ M without bioactivation. However, following bioactivation, phenol and 2,2'-biphenol showed inhibitory effects at doses as low as 50 μ M, whereas 4,4'-biphenol inhibited topoisomerase II at concentrations of 10 μ M. More recently,

3. HEALTH EFFECTS

Eastmond et al. (2001) demonstrated decreased activity of topoisomerase II activity in nucleated bone marrow cells of mice administered benzene by oral gavage for subchronic durations.

Available *in vitro* data suggest that benzene itself is genotoxic. Two studies reported that benzene produced DNA breaks in Chinese hamster ovary cells independent of metabolic activators (Douglas et al. 1985; Lakhnisky and Hendrickx 1985). In a study by Aubrecht et al. (1995), benzene was shown to induce intrachromosomal recombination in human lymphoblastoid cell culture. Therefore, benzene appears to have some genotoxic capabilities of its own, but its metabolites seem to be the primary genotoxins in systems in which normal metabolism is occurring. Refer to Table 3-5 for the results of these and other *in vitro* studies.

In summary, chromosome aberrations have been found consistently in bone marrow cells of persons occupationally exposed to benzene. The conclusion, based on human epidemiological studies, that benzene is a human clastogen is well supported by *in vivo* animal studies and *in vitro* cell cultures and subcellular studies. Virtually all studies that looked for effects at the chromosomal level were positive when the ability to metabolize benzene was present. These experimental results are consistent with the chromosomal damage seen in exposed humans. The leukemia observed in some benzene-exposed persons may result from the appearance of a clone of chromosomally abnormal cells in the bone marrow. With respect to genetic effects, no safe human exposure level can be determined from available epidemiological data. Significant increases in sister chromatid exchanges were produced in bone marrow cells and lymphocytes of animals. The significance of sister chromatid exchanges is unknown, but their production by a chemical is generally considered to indicate a genotoxic potential. Exposures generally occur via inhalation, and based on animal studies, effects following oral exposure may be greater than effects following inhalation exposure to comparable levels of benzene. Data presented in this section and elsewhere in this profile (Section 3.4) show that benzene metabolites are the genotoxic entities. It is possible that each metabolite causes a different genotoxic effect. Differences in metabolic capability are probably responsible for some of the variations in response to benzene seen in different test systems.

3.4 TOXICOKINETICS

The toxicokinetics of benzene has been extensively studied. Inhalation exposure is probably the major route of human exposure to benzene, although oral and dermal exposure are also important. Benzene is readily absorbed following inhalation or oral exposure. Although benzene is also readily absorbed from the skin, a significant amount of a dermal application evaporates from the skin surface. Absorbed

3. HEALTH EFFECTS

benzene is rapidly distributed throughout the body and tends to accumulate in fatty tissues. The liver serves an important function in benzene metabolism, which results in the production of several reactive metabolites. Although it is widely accepted that benzene toxicity is dependent upon metabolism, no single benzene metabolite has been found to be the major source of benzene hematopoietic and leukemogenic effects. At low exposure levels, benzene is rapidly metabolized and excreted predominantly as conjugated urinary metabolites. At higher exposure levels, metabolic pathways appear to become saturated and a large portion of an absorbed dose of benzene is excreted as parent compound in exhaled air. Benzene metabolism appears to be qualitatively similar among humans and various laboratory animal species. However, there are quantitative differences in the relative amounts of benzene metabolites.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Inhalation exposure is probably the major route of human exposure to benzene, and numerous studies of absorption of benzene after inhalation exposure in different settings have been conducted (Ashley et al. 1994; Avis and Hutton 1993; Boogaard and van Sittert 1995; Brunnemann et al. 1989; Byrd et al. 1990; Etzel and Ashley 1994; Fustinoni et al. 1995; Ghittori et al. 1995; Gordian and Guay 1995; Hajimiragha et al. 1989; Hanzlick 1995; Karacic et al. 1995; Kok and Ong 1994; Lagorio et al. 1994a; Laitinen et al. 1994; Lauwerys et al. 1994; Lindstrom et al. 1994; Mannino et al. 1995; Nomiyama and Nomiyama 1974a; Ong et al. 1994, 1995; Pekari et al. 1992; Popp et al. 1994; Rauscher et al. 1994; Rothman et al. 1995; Ruppert et al. 1995; Scherer et al. 1995; Shamy et al. 1994; Srbova et al. 1950; Yu and Weisel 1996). Existing evidence indicates that benzene is rapidly absorbed by humans following inhalation exposure. Results from a study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours showed that absorption was highest in the first few minutes of exposure, but decreased rapidly thereafter (Srbova et al. 1950). In the first 5 minutes of exposure, absorption was 70–80%, but by 1 hour, it was reduced to approximately 50% (range, 20–60%). Respiratory uptake (the amount of benzene absorbed from the lungs following inhalation of the vapors) in six volunteers including males and females exposed to 52–62 ppm benzene for 4 hours was determined to be approximately 47% (Nomiyama and Nomiyama 1974a). In a similar study, three healthy nonsmoking volunteers were exposed to benzene at levels of 1.6 or 9.4 ppm for 4 hours (Pekari et al. 1992). The amount of benzene absorbed was estimated from the difference between the concentration inhaled and the concentration exhaled. Estimates were 48% for the high dose and 52% for the low dose, supporting the evidence of Nomiyama and Nomiyama (1974a). Yu and Weisel (1996) measured the uptake of benzene by three female subjects exposed to benzene in smoke

3. HEALTH EFFECTS

generated by burning cigarettes, which resulted in airborne benzene concentrations in the range of 32–69 ppm. Average absorption for exposure periods of 30 or 120 minutes was 64% and did not appear to be influenced by exposure duration.

Studies of occupational exposure to benzene suggest that absorption occurs both by inhalation and dermally in many workplace settings. In a study conducted in 1992 in Finland, car mechanics' exposure to benzene was evaluated (Laitinen et al. 1994). Different work phases were measured at five Finnish garages. Blood samples from car mechanics (eight nonsmokers) were taken 3–9 hours after exposure to benzene. The results were approximated to the time point of 16 hours after exposure. Fourteen air samples were taken from the breathing zone and five stationary samples were collected from the middle of the garage for background concentration levels. The average background concentration (stationary samples) of gasoline vapors was $6 \pm 7 \text{ cm}^3/\text{m}^3$ ($2 \pm 2 \text{ ppm}$) and the concentration of benzene was under the detection limit of $0.2 \text{ cm}^3/\text{m}^3$ (0.1 ppm). The concentrations of benzene in the breathing zone varied from the detection limit of $0.2 \text{ cm}^3/\text{m}^3$ to $1.3 \text{ cm}^3/\text{m}^3$ ($0.1\text{--}0.4 \text{ ppm}$) for unleaded gasoline and from the detection limit to $3.7 \text{ cm}^3/\text{m}^3$ (1.2 ppm) for leaded gasoline. The highest benzene exposure level ($2.4\text{--}3.7 \text{ cm}^3/\text{m}^3$ or $0.8\text{--}1.2 \text{ ppm}$) was measured when changing the filter to the fuel pump. The mechanics worked without protective gloves, and the risk of contamination and penetration through the skin was significant. During carburetor renewal and gathering, benzene concentrations were $0.5\text{--}1.1 \text{ cm}^3/\text{m}^3$ ($0.2\text{--}0.3 \text{ ppm}$). During changing of the fuel filter to electronic fuel-injection system, benzene concentration ranged from 0.9 to $3.4 \text{ cm}^3/\text{m}^3$ ($0.3\text{--}1.1 \text{ ppm}$). The approximated benzene concentrations in blood corresponding to the time point of 16 hours after the exposure showed much higher levels of exposure than could be expected according to the corresponding air measurements (8-hour TWA). The comparison of expected benzene concentrations in blood, if no dermal exposure were present, to the levels at the time point of 16 hours after the exposure showed that the dermal route must be the source of about 68% of exposure (range 1.1–88.2%). Two of eight workers had minimal exposure through the skin (0–1.1%). The other six workers showed high dermal exposure (79.4%).

Exposure to benzene-contaminated water can also provide an opportunity for both inhalation and dermal absorption. In a series of experiments conducted in a single-family residence from June 11 to 13, 1991, exposure to benzene through contaminated residential water was monitored (Lindstrom et al. 1994). The residential water was contaminated with benzene and other hydrocarbons in 1986. Periodic testing conducted from 1986 to 1991 showed benzene concentrations ranging from 33 to 673 $\mu\text{g}/\text{L}$ (ppb). The experiment involved an individual taking a 20-minute shower with the bathroom door closed, followed by 5 minutes for drying and dressing; then the bathroom door was opened and this individual was allowed to

3. HEALTH EFFECTS

leave the house. Integrated 60- and 240-minute whole-air samples were collected from the bathroom, an adjacent bedroom, living room, and in ambient air. Glass, gas-tight syringe grab samples were simultaneously collected from the shower, bathroom, bedroom, and living room at 0, 10, 18, 20, 25, 25.5, and 30 minutes. Two members of the monitoring team were measured for 6 hours using personal Tenax gas GC monitors. For the first 30 minutes of each experiment, one member was based in the bathroom and the other in the living room. Benzene concentrations in the shower head ranged from 185 to 367 $\mu\text{g/L}$ (ppb), while drain level samples ranged from below the detectable limit (0.6 $\mu\text{g/L}$ or ppb) to 198 $\mu\text{g/L}$ (ppb). Analysis of the syringe samples suggested a pulse of benzene moving from the shower stall to the rest of the house over approximately 60 minutes. Peak levels of benzene measured 758–1,670 $\mu\text{g/m}^3$ (235–518 ppb) in the shower stall at 18–20 minutes, 366–498 $\mu\text{g/m}^3$ (113–154 ppb) in the bathroom at 10–25 minutes, 81–146 $\mu\text{g/m}^3$ (25–45 ppb) in the bedroom at 25.5–30 minutes, and 40–62 $\mu\text{g/m}^3$ (12–19 ppb) in the living room at 36–70 minutes. The individual who took the 20-minute shower had estimated inhalation doses of 79.6, 105, and 103 μg (mean=95.9 μg) for the 3 consecutive sampling days. These doses were estimated by taking the products of the concentration of benzene in water, the minute ventilation rate, the duration of exposure, and a 70% benzene absorption factor. This was 2.1–4.9 times higher than corresponding 20-minute bathroom exposures. Adding the average dose absorbed in the bathroom during the 5.5 minutes following the shower (using the overall 20–25 minutes mean syringe level of 318 $\mu\text{g/m}^3$ [99 ppb]) gave a total average shower-related inhalation dose of 113 μg . An average dermal dose of 168 μg was estimated for the 20-minute shower by multiplying the average concentration of benzene in water by the surface area of the male volunteer, an exposure factor of 75% body surface area exposed, a dermal permeability constant for benzene of 0.11 cm/hour, an exposure duration of 0.33 hours, and a unit conversion factor of 11/1,000 cm^2 . The total benzene dose resulting from the shower was estimated to be approximately 281 μg (40% via inhalation and 60% via dermal), suggesting a higher potential exposure to benzene via dermal contact from the water than through vaporization and inhalation. This exposure was 2–3.5 times higher than the mean 6-hour inhalation dose received by the sampling members. The estimated inhalation and dermal doses reported by Lindstrom et al. (1994) have not been validated by others and are therefore of questionable value for quantitative analysis.

Additional evidence of benzene absorption following inhalation exposure comes from data on cigarette smokers. Benzene levels were significantly higher in the venous blood of 14 smokers (median level of 493 ng/L) than in a control group of 13 nonsmokers (median level of 190 ng/L) (Hajimiragha et al. 1989). Cigarette smoke is known to contain benzene (Brunnemann et al. 1989; Byrd et al. 1990), and the subjects had no known exposure to other sources of benzene (Hajimiragha et al. 1989). Kok and Ong (1994)

3. HEALTH EFFECTS

report blood and urine levels of benzene as 110.9 and 116.4 ng/L, respectively for nonsmokers, and 328.8 and 405.4 ng/L, respectively for smokers. The National Association of Medical Examiners Pediatric Toxicology (PedTox) Registry reported blood benzene concentrations ranging from 0.2 to 4.9 mg/L in eight children who died in fires and were dead at the scene, indicating absorption of benzene from burning materials (Hanzlick 1995). Blood benzene levels taken from U.S. engineers (Group I) and firefighters (Group II) working at burning oil wells in Kuwait were compared to blood benzene levels from non-exposed U.S. citizens (Etzel and Ashley 1994). The median concentrations of benzene in whole blood from Groups I, II, and U.S. reference group were 0.035 µg/L (range ND–0.055 µg/L), 0.18 µg/L (range 0.063–1.1 µg/L), and 0.066 µg/L (range ND–0.54 µg/L), respectively. The median concentration in group II was generally higher than the median concentrations in group I or the reference group. Statistically significant higher concentrations of benzene ($p < 0.0001$) were found in group II smokers than in Group II nonsmokers.

Animal data confirm that benzene is rapidly absorbed through the lungs. Inhalation studies with laboratory dogs indicate that distribution of benzene throughout the animal's body is rapid, with tissue values dependent on blood supply. A linear relationship existed between the concentration of benzene in air (200–1,300 ppm) and the equilibrium concentration in blood (Schrenk et al. 1941). At these exposures, the concentrations of benzene in the blood of dogs exposed to benzene reached a steady state within 30 minutes.

In rodents, the extent of uptake increased linearly with concentration for exposures up to 200 ppm. At concentrations of >200 ppm, zero-order kinetics were observed (i.e., uptake became nonlinear, indicating saturation of the metabolic capacity). The percentage of inhaled benzene that was absorbed and retained during a 6-hour exposure period decreased from 33 to 15% in rats and from 50 to 10% in mice as the exposure concentration was increased from about 10 to 1,000 ppm (Sabourin et al. 1987). When rats and mice were exposed to approximately 300 ppm, mice had greater uptake than rats. Mice and rats had different absorption characteristics; the cumulative inhaled dose in mice was greater than that in rats (Eutermoser et al. 1986; Sabourin et al. 1987). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

3. HEALTH EFFECTS

3.4.1.2 Oral Exposure

Although definitive scientific data are not available on oral absorption of benzene in humans, case studies of accidental or intentional poisoning indicate that benzene is absorbed by the oral route (Thienes and Haley 1972).

Benzene appears to be efficiently absorbed following oral dosing in animals. Oral absorption of benzene was first demonstrated by Parke and Williams (1953a). After radiolabeled (^{14}C) benzene was administered orally to rabbits (340–500 mg/kg), the total radioactivity eliminated in exhaled air and urine accounted for approximately 90% of the administered dose, indicating that at least this much of the administered dose was absorbed. Studies in rats and mice showed that gastrointestinal absorption was greater than 97% in both species when the animals were administered benzene by gavage (in corn oil) at doses of 0.5–150 mg/kg/day (Sabourin et al. 1987). In many animal studies, benzene is administered orally in oil to insure predictable solubility and dose concentration control. This is unlike the predicted human oral exposure, which is likely to be in drinking water. There are a number of studies in which benzene has been administered to animals in the drinking water, which more closely resembles predicted human oral exposure (Lindstrom et al. 1994). Although no information was located regarding the extent of oral absorption of benzene in aqueous solutions, it is reasonable to assume that oral absorption from water solutions would be nearly 100%.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of benzene alone, or adsorbed to clay or sandy soil. Plasma concentration, half-life, tissue distribution, respiratory excretion, and urinary excretion were monitored. Peak plasma concentration of radioactivity was increased in the presence of either soil as opposed to benzene alone, while sandy soil also decreased the time to peak plasma concentration as opposed to benzene alone. Soil increased the area under the plasma radioactivity-time curve as opposed to benzene alone, a difference that was significant with clay soil. The half-life in plasma was not affected by soil.

3.4.1.3 Dermal Exposure

Studies conducted *in vivo* in humans and *in vitro* using human skin indicates that benzene can be absorbed dermally. The movement of a substance through the skin to the blood occurs by passive diffusion and has been described mathematically by Fick's law. However, this is an oversimplification of

3. HEALTH EFFECTS

the process of skin absorption; various factors (e.g., interaction of benzene with molecules within the skin) affect the transport of the solvent through the skin (Lodén 1986).

In vivo experiments on four volunteers, to whom 0.0026 mg/cm² of ¹⁴C-benzene was applied to forearm skin, indicated that approximately 0.05% of the applied dose was absorbed (Franz 1984). Absorption was rapid, with more than 80% of the total excretion of the absorbed dose occurring in the first 8 hours after application. Calculations were based on urinary excretion data and no correction was made for the amount of benzene that evaporated from the applied site before absorption occurred. In addition, the percentage of absorbed dose excreted in urine that was used in the calculation was based only on data from rhesus monkeys and may not be accurate for humans. In another study, 35–43 cm² of the forearm was exposed to approximately 0.06 g/cm² of liquid benzene for 1.25–2 hours (Hanke et al. 1961). The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be low, approximately 0.4 mg/cm²/hour. The absorption due to vapors in the same experiment was negligible. The results indicate that dermal absorption of liquid benzene is of concern, while dermal absorption from vapor exposure may not be of concern because of the low concentration of benzene in vapor form at the point of contact with the skin. No signs of acute intoxication due to liquid benzene dermally absorbed were noted. These results confirm that benzene can be absorbed through skin. However, non-benzene-derived phenol in the urine was not accounted for.

Studies of occupational exposure to benzene suggest that absorption occurs both by inhalation and dermally in many workplace settings. In a study conducted in 1992 in Finland, car mechanics' exposure to benzene was evaluated (Laitinen et al. 1994). Different work phases were measured at five Finnish garages. Blood samples from car mechanics (eight nonsmokers) were taken 3–9 hours after exposure to benzene. The results were approximated to the time point of 16 hours after exposure. Fourteen air samples were taken from the breathing zone and five stationary samples were collected from the middle of the garage for background concentration levels. The mechanics worked without protective gloves, and the risk of contamination and penetration through the skin was significant. The approximated benzene concentrations in blood corresponding to the time point of 16 hours after the exposure showed much higher levels of exposure than could be expected according to the corresponding air measurements (8 hour TWA). The comparison of expected benzene concentrations in blood, if no dermal exposure was present, to the levels at the time point of 16 hours after the exposure showed that the dermal route must be the source of about 68% of exposure (range 1.1–88.2%). Two of eight workers had minimal exposure through the skin (0–1.1%). The other six workers showed high dermal exposure (79.4%).

3. HEALTH EFFECTS

Exposure to benzene-contaminated water can also provide an opportunity for both inhalation and dermal absorption. In a series of experiments conducted in a single-family residence from June 11 to 13, 1991, exposure to benzene through contaminated residential water was monitored (Lindstrom et al. 1994). The residential water was contaminated with benzene and other hydrocarbons in 1986. Exposure was monitored for a person taking a 20-minute shower and for people in other parts of the house during and after the shower. An average dermal dose of 168 μg was estimated for a 20-minute shower using this water. The total benzene dose resulting from the shower was estimated to be approximately 281 μg (40% via inhalation and 60% via dermal), suggesting a higher potential exposure to benzene via dermal contact from the water than through vaporization and inhalation (see Section 3.4.1.1 for a more detailed discussion). This exposure was 2–3.5 times higher than the mean 6-hour inhalation dose received by the sampling team members in other parts of the house. The estimated inhalation and dermal doses reported by Lindstrom et al. (1994) have not been validated by others and are therefore of questionable value for quantitative analysis.

In vitro experiments using human skin support the fact that benzene can be absorbed dermally. An experiment on the permeability of excised human skin with regard to benzene (specific activity 99.8 mCi/mmol; total volume of applied benzene not reported) resulted in the absorption of 0.17 mg/cm² after 0.5 hours and 1.92 mg/cm² after 13.5 hours (Lodén 1986). Following application of 5, 120, 270, and 520 $\mu\text{L}/\text{cm}^2$ of benzene to human skin, total absorption was found to be 0.01, 0.24, 0.56, and 0.9 $\mu\text{L}/\text{cm}^2$, respectively. Thus, the total amount absorbed appears to increase linearly with dose. The study author indicated that evaporation of benzene did not exceed 5%. When exposure time (i.e., the time to complete evaporation) at each dose was measured and plotted as the ordinate of absorption, total absorption was found to increase linearly with exposure time. The percentage of the applied dose absorbed at each concentration was constant at about 0.2% (Franz 1984).

Using results from an *in vitro* study, it was estimated that an adult working in ambient air containing 10 ppm benzene would absorb 7.5 $\mu\text{L}/\text{hour}$ from inhalation and 1.5 $\mu\text{L}/\text{hour}$ from whole-body (2 m²) dermal exposure (Blank and McAuliffe 1985). It was also estimated that 100 cm² of smooth and bare skin in contact with gasoline containing 5% benzene would absorb 7.0 $\mu\text{L}/\text{hour}$. Diffusion through the stratum corneum was considered the most likely rate-limiting step for dermal absorption because of benzene's low water solubility (Blank and McAuliffe 1985).

3. HEALTH EFFECTS

Based on an observational study of workers in a tire factory, it was estimated that a worker exposed to benzene as a result of direct skin contact with petroleum naphtha containing 0.5% benzene could absorb 4–8 mg of benzene per day through intact skin (Susten et al. 1985). This amount absorbed was compared with an estimated 14 mg of benzene absorbed as a result of inhalation of 1 ppm for an 8-hour day. The estimate for dermal absorption is theoretical since in many facilities the concentration of benzene in rubber solvents such as petroleum naphtha is less than 0.5% and may be as low as 0.09%.

Benzene is also absorbed dermally by animals. In Rhesus monkeys, minipigs, and hairless mice, dermal absorption was <1% following a single direct (unoccluded) application of liquid benzene (Franz 1984; Maibach and Anjo 1981; Susten et al. 1985). As with humans, absorption appeared to be rapid, with the highest urinary excretion of the absorbed dose observed in the first 8 hours following exposure (Franz 1984). Multiple applications, as well as application to stripped skin, resulted in greater skin penetration (Maibach and Anjo 1981). The percentage of absorption of the applied dose of benzene in each of these animals was approximately 2–3-fold higher than that of humans.

Data indicate that soil adsorption decreases the dermal bioavailability of benzene. A study in which male rats were treated dermally with 0.004 mg/cm² ¹⁴C-benzene, with or without 1 g of clay or sandy soil, reported benzene absorption half-lives of 3.1, 3.6, and 4.4 hours for pure benzene, sandy soil, and clay soil, respectively (Skowronski et al. 1988).

Benzene in air was rapidly absorbed through the skin of hairless mice that were attached to respirators to avoid pulmonary uptake of the benzene vapors (Tsuruta 1989). The rate of absorption of benzene through the skin increased linearly with dose. The skin absorption rate for 200 ppm was 4.11 nmol/cm²/hour (0.31 µg/cm²/hour); at 1,000 ppm, the rate was 24.2 nmol/cm²/hour (1.89 µg/cm²/hour), and at 3,000 ppm, the rate was 75.5 nmol/cm²/hour (5.90 µg/cm²/hour). The skin absorption coefficient was 0.619 cm/hour.

McDougal et al. (1990) estimated permeability constants of 0.15 and 0.08 cm/hour for rat and human skin, respectively, based on the appearance of benzene in the blood of rats dermally exposed to benzene vapors at a concentration of 40,000 ppm for 4 hours. A physiologically based pharmacodynamic (PBPK) model was used to estimate the permeability of the vapor in rat and human skin. These results indicate that dermal absorption of benzene may be greater in rats than humans. Therefore, results in rats may provide a conservative estimate of dermal absorption of benzene in humans.

3. HEALTH EFFECTS

In an *in vitro* experiment using Fischer 344 rat skin, the partition coefficient for skin:air was determined for benzene at 203 ppm (Mattie et al. 1994). The partition coefficient of a chemical in skin is an indicator of the capacity of the skin to retain the chemical, and may reflect the rate at which a chemical is absorbed through the skin and enters the circulation. Results indicated a partition coefficient of 35, with an equilibration time of 4 hours. The skin:air partition coefficient is necessary for developing the dermal compartment of a PBPK model.

Based on data for skin absorption of benzene vapors in mice and occupational exposure data, Tsuruta (1989) estimated the ratio of skin absorption rate to pulmonary uptake for humans exposed to benzene to be 0.037. Dermal absorption could account for a relatively higher percentage of total benzene uptake in occupational settings where personnel, using respirators but not protective clothing, are exposed to high concentrations of benzene vapor.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Information on the distribution of benzene in humans comes primarily from case studies. The data suggest that benzene is distributed throughout the body following absorption into blood. Since benzene is lipophilic, a high distribution to fatty tissue might be expected. Following inhalation exposure to benzene, the chemical has been detected in the biological fluids and tissues of the subjects (Pekari et al. 1992; Tauber 1970; Winek and Collom 1971; Winek et al. 1967). Fluid and tissue levels of benzene have been reported in cases of both accidental and intentional lethal exposures. Levels of 0.38 mg% in blood (mg% = mg per 100 mL of blood or mg per 100 g of tissue), 1.38 mg% in the brain, and 0.26 mg% in the liver were reported in a worker who died from exposure to very high air concentrations of the chemical (Tauber 1970). An autopsy (time after death not indicated) performed on a youth who died while sniffing reagent-grade benzene revealed benzene concentrations of 2.0 mg% in blood, 3.9 mg% in brain, 1.6 mg% in liver, 1.9 mg% in kidney, 1 mg% in stomach, 1.1 mg% in bile, 2.23 mg% in abdominal fat, and 0.06 mg% in urine (Winek and Collom 1971). Benzene crosses the human placenta and is present in the cord blood in amounts equal to or greater than those in maternal blood (Dowty et al. 1976). Benzene is expected to readily bind to plasma proteins (Travis and Bowers 1989). Furthermore, benzene metabolites have been found to form covalent adducts with proteins from blood in humans (Bechtold et al. 1992b; Rappaport et al. 2002a, 2002b; Yeowell-O'Connell et al. 1998) and mice (McDonald et al. 1994). The relatively widespread distribution of benzene and its metabolites to other tissues and organs indicates that

3. HEALTH EFFECTS

protein adduct formation in the blood does not adversely affect distribution, although no confirming studies were located.

Results from animal studies indicate that absorbed benzene is distributed among several compartments. The parent compound is preferentially stored in the fat, although the relative uptake in tissues also appears to be dependent on the perfusion rate of tissues by blood.

Following a 10-minute inhalation exposure of pregnant mice to 2,000 ppm benzene, parent compound and its metabolites were found to be present in lipid-rich tissues, such as brain and fat, and in well-perfused tissues, such as liver and kidney. Benzene was also found in the placenta and fetuses immediately following inhalation of benzene (Ghantous and Danielsson 1986). During inhalation exposure of rats to 500 ppm, benzene levels reached a steady-state concentration within 4 hours in blood (11.5 µg/mL), 6 hours in fat (164.4 µg/g), and less than 2 hours in bone marrow (37.0 µg/g) (Rickert et al. 1979). Benzene was also distributed to the kidney, lung, liver, brain, and spleen. The benzene metabolites phenol, catechol, and hydroquinone were detected in blood and bone marrow following 6 hours of exposure to benzene, with levels in bone marrow exceeding the respective levels in blood. The levels of phenol in blood and bone marrow decreased much more rapidly after exposure ceased than did those of catechol or hydroquinone, suggesting the possibility of accumulation of the latter two compounds.

Benzene was rapidly distributed throughout the bodies of dogs exposed via inhalation to concentrations of 800 ppm for up to 8 hours/day for 8–22 days (Schrenk et al. 1941). Fat, bone marrow, and urine contained about 20 times the concentration of benzene in blood; benzene levels in muscles and organs were 1–3 times that in blood; and erythrocytes contained about twice the amount of benzene found in plasma. During inhalation exposure of rats to 1,000 ppm (2 hours/day, for 12 weeks), benzene was stored longer (and eliminated more slowly) in female and male rats with higher body fat content than in leaner animals (Sato et al. 1975).

Benzene was detected in the liver, lung, and blood of rats and mice examined immediately following a 6-hour exposure to benzene vapors at a concentration of 50 ppm (Sabourin et al. 1988). Sabourin and coworkers (Sabourin et al. 1987, 1988) also examined effects exposure concentration, exposure rate, and route of administration on the comparative metabolism of benzene in rats and mice. Results of these studies are summarized in Section 3.4.3 (Metabolism).

3. HEALTH EFFECTS

3.4.2.2 Oral Exposure

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

In Sprague-Dawley rats administered a single dose of 0.15, 1.5, 15, 150, or 500 mg/kg of ^{14}C -benzene by gavage, benzene was rapidly absorbed and distributed to various organs and tissues within 1 hour of administration (Low et al. 1989). One hour after rats were dosed with 0.15 or 1.5 mg/kg of benzene, tissue distribution of benzene was highest in liver and kidney, intermediate in blood, and lowest in the Zymbal gland, nasal cavity tissue, and mammary gland. At higher doses, beginning with 15 mg/kg, benzene disproportionately increased in the mammary glands and bone marrow. Bone marrow and adipose tissue proved to be depots of benzene at the higher dose levels. The highest tissue concentrations of benzene's metabolite hydroquinone 1 hour after administration of 15 mg/kg of benzene were in the liver, kidney, and blood, while the highest concentrations of the metabolite phenol were in the oral cavity, nasal cavity, and kidney. The major tissue sites of benzene's conjugated metabolites were blood, bone marrow, oral cavity, kidney, and liver for phenyl sulfate and hydroquinone glucuronide; muconic acid was also found in these sites. Additionally, the Zymbal gland and nasal cavity were depots for phenyl glucuronide, another conjugated metabolite of benzene. The Zymbal gland is a specialized sebaceous gland and a site for benzene-induced tumors. Therefore, it is reasonable to expect that lipophilic chemicals like benzene would partition readily into this gland. However, benzene did not accumulate in the Zymbal gland; within 24 hours after administration, radiolabel derived from ^{14}C -benzene in the Zymbal gland constituted less than 0.0001% of the administered dose.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of ^{14}C -benzene alone, or adsorbed to clay or sandy soil. Two hours after exposure, stomach tissue contained the highest amount of radioactivity, followed by fat in all treatment groups. No differences in tissue distribution patterns were detected for the three treatments.

3.4.2.3 Dermal Exposure

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

A study of male rats treated dermally with 0.004 mg/cm^2 of ^{14}C -benzene, with and without 1 g of clay or sandy soil, revealed soil-related differences in tissue distribution following treatment. The ^{14}C activity (expressed as a percentage of initial dose per g of tissue) 48 hours after treatment with soil-adsorbed

3. HEALTH EFFECTS

benzene was greatest in the treated skin (0.059–0.119%), followed by the kidney (0.024%) and liver (0.013–0.015%), in both soil groups. In the pure benzene group, the kidney contained the largest amount of radioactivity (0.026%), followed by the liver (0.013%) and treated skin (0.11%) (Skowronski et al. 1988). In all three groups, <0.01% of the radioactivity was found in the following tissues: duodenum, fat, bone marrow, esophagus, pancreas, lung, heart, spleen, blood, brain, thymus, thyroid, adrenal, testes, untreated skin, and remaining carcass.

3.4.3 Metabolism

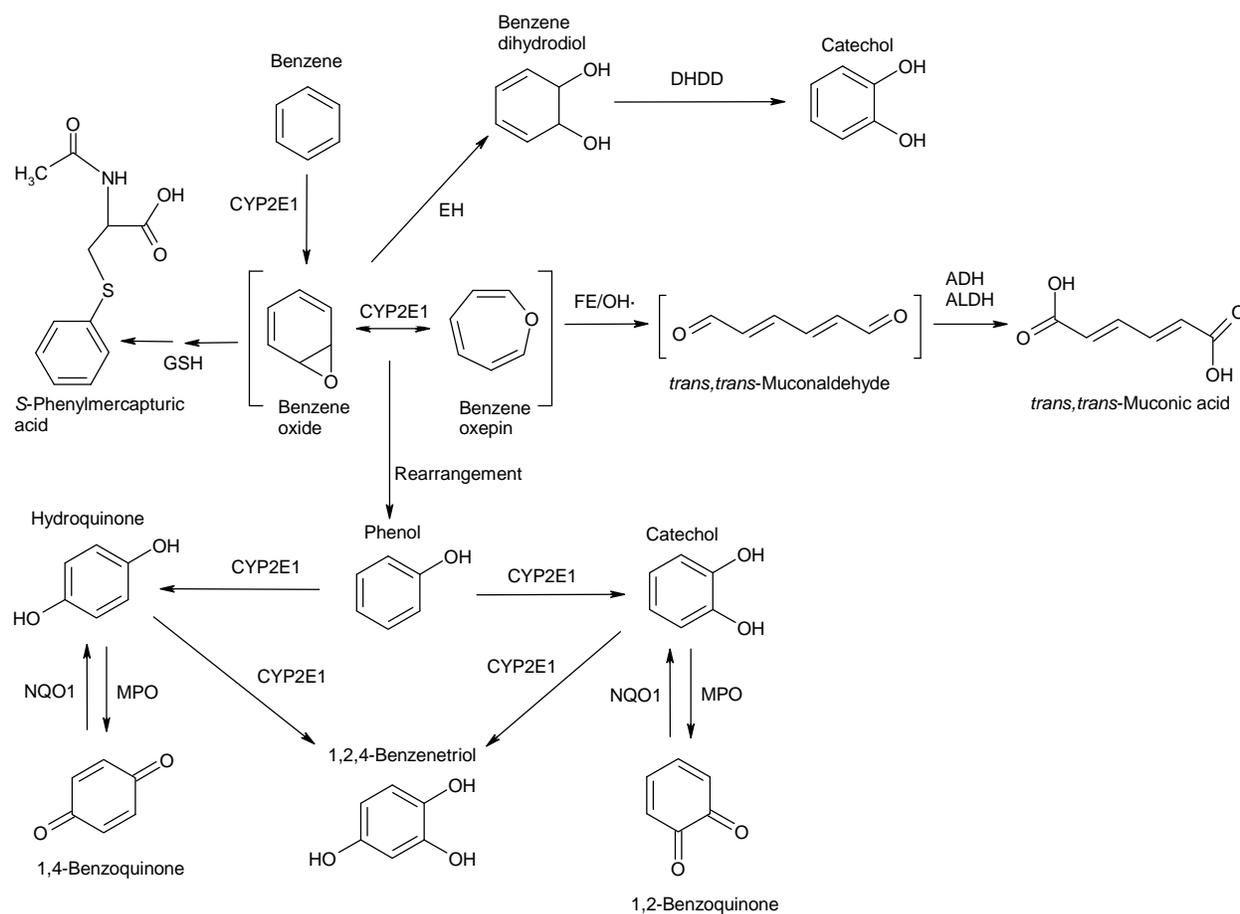
Although the metabolism of benzene has been studied extensively, the steps leading to benzene toxicity are not yet fully understood. It is generally understood that both cancer and noncancer effects are caused by one or more reactive metabolites of benzene. Available data indicate that metabolites produced in the liver are carried to the bone marrow where benzene toxicity is expressed. Benzene metabolism may occur, at least in part, in the bone marrow. Benzene metabolism has been demonstrated in isolated perfused rabbit lung preparations (Powley and Carlson 2002). As discussed in detail in Section 3.5, available evidence suggests that multiple benzene metabolites may collectively be responsible for the expression of benzene toxicity.

Data regarding metabolism of benzene in humans are derived primarily from studies using inhalation exposures. Benzene is excreted both unchanged via the lungs and as metabolites (but also as parent compound in small amounts) in the urine. The rate and percentage of excretion via the lungs are dependent on exposure dose and route. Qualitatively, the metabolism and elimination of benzene appear to be similar in humans and laboratory animals, but no directly comparable studies are available (Henderson et al. 1989; Sabourin et al. 1988).

The metabolic scheme shown in Figure 3-3 is based on results of numerous mechanistic studies of benzene metabolism (see Henderson et al. 1989; Huff et al. 1989; and Ross 1996, 2000 for comprehensive reviews of benzene metabolism). The first step is the cytochrome P-450 2E1 (CYP2E1) catalyzed oxidation of benzene to form benzene oxide (Lindstrom et al. 1997), which is in equilibrium with its oxepin (Vogel and Günther 1967). Several pathways are involved in the metabolism of benzene oxide. The predominant pathway involves nonenzymatic rearrangement to form phenol (Jerina et al. 1968), the major initial product of benzene metabolism (Parke and Williams 1953a). Phenol is oxidized in the presence of CYP2E1 to catechol or hydroquinone, which are oxidized via myeloperoxidase (MPO) to the reactive metabolites 1,2- and 1,4-benzoquinone, respectively (Nebert et al. 2002). The reverse

3. HEALTH EFFECTS

Figure 3-3. Metabolic Pathways for Benzene



ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; CYP2E1 = cytochrome P-450 2E1; DHDD = dihydrodiol dehydrogenase; EH = epoxide hydrolase; GSH = glutathione; MPO = myeloperoxidase; NQO1 = NAD(P)H:quinone oxidoreductase

Source: adapted from Nebert et al. 2002; Ross 2000

3. HEALTH EFFECTS

reaction (reduction of 1,2- and 1,4-benzoquinone to catechol and hydroquinone, respectively) is catalyzed by NAD(P)H:quinone oxidoreductase (NQO1) (Nebert et al. 2002). Both catechol and hydroquinone may be converted to the reactive metabolite 1,2,4-benzenetriol via CYP2E1 catalysis. Alternatively, benzene oxide may undergo epoxide hydrolase-catalyzed conversion to benzene dihydrodiol and subsequent dihydrodiol dehydrogenase-catalyzed conversion to catechol (Nebert et al. 2002; Snyder et al. 1993a, 1993b). Each of the phenolic metabolites of benzene (phenol, catechol, hydroquinone, and 1,2,4-benzenetriol) can undergo sulfonic or glucuronic conjugation (Nebert et al. 2002; Schrenk and Bock 1990); the conjugates of phenol and hydroquinone are major urinary metabolites of benzene (Sabourin et al. 1989a; Wells and Nerland 1991). Other pathways of benzene oxide metabolism include: (1) reaction with glutathione (GSH) to form S-phenylmercapturic acid (Nebert et al. 2002; Sabourin et al. 1988; Schafer et al. 1993; Schlosser et al. 1993; Schrenk et al. 1992; van Sittert et al. 1993), and (2) iron-catalyzed ring-opening conversion to *trans,trans*-muconic acid, presumably via the reactive *trans,trans*-muconaldehyde intermediate (Bleasdale et al. 1996; Nebert et al. 2002; Ross 2000; Witz et al. 1990b, 1990c, 1996).

Results of several studies provide strong evidence for the involvement of CYP2E1 in the oxidation of benzene. For example, no signs of benzene-induced toxicity were observed in transgenic CYP2E1 knockout mice (that do not express hepatic CYP2E1 activity) following exposure to benzene vapors (200 ppm, 6 hours/day for 5 days) that caused severe genotoxicity and cytotoxicity in wild-type mice (Valentine et al. 1996a, 1996b). Pretreatment of mice with CYP inhibitors (toluene, propylene glycol, β -diethyl amino ethyl diphenyl propyl acetate hydrogen chloride [SKF-525A]) has been demonstrated to reduce both benzene metabolite formation (Andrews et al. 1977; Gill et al. 1979; Ikeda et al. 1972; Tuo et al. 1996) and resulting genotoxicity (DNA damage as assessed by the alkaline comet assay) in mice (Tuo et al. 1996). Pretreatment with CYP inducers (3-methylcholanthrene and β -naphthoflavone) increased both benzene metabolism and benzene clastogenicity (Gad-El-Karim et al. 1986). Immunoinhibition studies in rat and rabbit hepatic microsomes provide additional support to the major role of CYP2E in benzene metabolism (Johansson and Ingelman-Sundberg 1988; Koop and Laethem 1992).

Occupationally exposed workers with a phenotype corresponding to rapid CYP2E1 metabolism were more susceptible to benzene hematotoxicity than workers not expressing this phenotype (Rothman et al. 1997). *In vitro* studies using human liver microsomes demonstrate a positive correlation between benzene metabolism and CYP2E1 activity (Nedelcheva et al. 1999; Seaton et al. 1994). Although CYP2E1 appears to be the major catalyzing agent in initial benzene metabolism, other CYPs, such as CYP2B1 and CYP2F2, may also be involved (Gut et al. 1996a, 1996b; Powley and Carlson 2000, 2001; Sheets and Carlson 2004; Sheets et al. 2004; Snyder et al. 1993a, 1993b).

3. HEALTH EFFECTS

CYPs involved in benzene metabolism are found in all tissues. However, the predominant repository is the liver, which is considered to be the primary site of benzene metabolism. By demonstrating that partial hepatectomy diminished both the rate of metabolism of benzene and its toxicity in rats exposed to benzene via subcutaneous injection, Sammett et al. (1979) provided suggestive evidence that one or more benzene metabolites formed in the liver are necessary for toxicity. *In vitro* studies have demonstrated that pulmonary microsomes of humans and laboratory animals are capable of metabolizing benzene, which appears to be catalyzed by both CYP2E1 and CYP2F2 (Powley and Carlson 1999, 2000; Sheets et al. 2004). There is some indication that CYP2E1-catalyzed benzene metabolism may also occur in bone marrow, a major target tissue of benzene toxicity. Andrews et al. (1979) demonstrated that rabbit bone marrow is capable of metabolizing benzene. Schnier et al. (1989) subsequently found that rabbit bone marrow contains CYP2E1. Irons et al. (1980) demonstrated that benzene metabolism by rat bone marrow (*in situ*) was complete and independent of metabolism by the liver, with concentrations of phenol greater than catechol and hydroquinone. Although the total metabolism by bone marrow was limited (total metabolites present were 25% of those in blood), the concentration of metabolites in the bone marrow exceeded that in the blood. Similar studies have been conducted in mice (Ganousis et al. 1992). Fibroblasts had elevated levels of glutathione-S-transferase activity relative to macrophages, whereas macrophages had higher levels of UDP-glucuronyltransferase and peroxidase activity. These data suggest that cell-specific metabolism of benzene in the marrow may contribute to the toxicity of benzene in this tissue compartment. In addition, comparison of the detoxifying activities of rat and mouse bone marrow stromal cells indicates that rats have higher levels of glutathione and quinone reductase, which are known to play critical roles in modulating hydroquinone-induced toxicity; this suggests a metabolic basis for the observed increased susceptibility of mice to benzene-induced hematotoxicity (Zhu et al. 1995). Bernauer et al. (1999, 2000) recently noted the presence of CYP2E1 in bone marrow samples of mice (several strains), rats, rabbits, and humans. However, although Irons et al. (1980) demonstrated that the isolated perfused rat femur was capable of metabolizing a very small amount of benzene (approximately 0.0002% of ¹⁴C-benzene was recovered as metabolites), neither benzene oxide nor phenol were detected in a test of benzene metabolism using microsomal preparations of bone marrow from rats (Lindstrom et al. 1999), indicating that bone marrow is not a likely source of initial metabolic oxidation for benzene. No studies were located that demonstrated the potential for human bone marrow tissue to metabolize benzene.

Mouse liver microsomes and cytosol have been shown to catalyze ring opening in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) *in vitro*, producing *trans,trans*-muconaldehyde, a six-carbon diene dialdehyde also referred to as muconic dialdehyde (Goon et al. 1993; Latriano et al.

3. HEALTH EFFECTS

1986), a known hematotoxin (Witz et al. 1985) and toxic metabolite of benzene (Henderson et al. 1989). Metabolism of benzene and *trans,trans*-muconaldehyde in the isolated perfused rat liver indicated that benzene was metabolized to muconic acid, a ring-opened metabolite of benzene (Grotz et al. 1994). *Trans,trans*-muconaldehyde was metabolized to muconic acid and three other metabolites. These studies indicate that ring-opening of benzene occurs in the liver. Other recent literature identifies the following metabolites after incubation of benzene with mouse liver microsomes: phenol, hydroquinone, *trans,trans*-muconaldehyde, 6-oxo-*trans,trans*-2,4-hexadienoic acid, 6-hydroxy-*trans,trans*-2,4-hexadienal, and 6-hydroxy-*trans,trans*-2,4-hexadienoic acid (Zhang et al. 1995a). β -Hydroxymuconaldehyde, a new metabolite, was also identified. Additional work by Zhang et al. (1995b) suggests that *cis,cis*-muconaldehyde is formed first, followed by *cis,trans*-muconaldehyde, and finally converted to *trans,trans*-muconaldehyde. Muconic dialdehyde has been shown to be metabolized *in vivo* in mice to muconic acid (Witz et al. 1990c). These data suggest that muconic dialdehyde is the precursor of muconic acid in animals exposed to benzene. Small amounts of muconic acid were found in the urine of rabbits and mice that received oral doses of ^{14}C -benzene (Gad-El-Karim et al. 1985; Parke and Williams 1953a). The percentage of this metabolite formed varied with the administered benzene dose and was quite high at low doses (17.6% of 0.5 mg/kg benzene administered to C57BL/6 mice) (Witz et al. 1990c). Other studies in animals support these results (Brondeau et al. 1992; Ducos et al. 1990; McMahon and Birnbaum 1991; Sabourin et al. 1989a; Schad et al. 1992). This pathway also appears to be active in humans (Bechtold and Henderson 1993; Ducos et al. 1990, 1992; Lee et al. 1993; Melikian et al. 1993, 1994). For instance, urine samples from male and female smokers and nonsmokers were obtained from subjects who applied for life insurance (Melikian et al. 1994). Samples from pregnant women were obtained during 7–35 weeks of pregnancy. Questionnaires were filled out on smoking history and occupation. The levels of muconic acid and cotinine (a biomarker for cigarette smoking) in the urine for the groups of pregnant and nonpregnant smokers and nonsmokers were compared with previously reported data in male smokers. Results showed the mean levels of muconic acid in the groups of male, female-nonpregnant, and female-pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher than the mean concentration of this acid in the nonsmoking groups. The differences in the mean muconic acid concentrations between smoking and nonsmoking groups were significant in male, and nonpregnant and pregnant female smokers. Mean concentrations of muconic acid levels in nonpregnant female smokers are similar to that of male smokers. Mean concentrations of muconic acid in groups of 42 male smokers and 53 female smokers were 0.22 ± 0.03 and 0.24 ± 0.02 mg/g creatinine, or 0.13 ± 0.06 and 0.13 ± 0.07 mg/mg cotinine, respectively. Mean concentrations of muconic acid in groups of 63 pregnant and 53 nonpregnant female smokers were 0.27 ± 0.04 and 0.24 ± 0.02 mg/g creatinine, or 0.24 ± 0.06 and 0.13 ± 0.07 mg/mg cotinine, respectively. Because of its relative importance in benzene toxicity,

3. HEALTH EFFECTS

additional modeling studies, including molecular orbital studies, have been conducted to further describe how *trans,trans*-muconaldehyde is transformed to muconic acid (Bock et al. 1994).

Kenyon et al. (1995) compared their urinary profile of metabolites in B6C3F₁ mice after oral dosing with phenol with the results of Sabourin et al. (1989a) who administered a comparable oral dose of benzene to B6C3F₁ mice. The analysis of Kenyon et al. (1995) indicated that phenol administration resulted in lower urinary levels of hydroquinone glucuronide, and higher levels of phenol sulfate and phenol glucuronide compared to benzene administration. Kenyon et al. (1995) hypothesized that the differences in the urinary metabolite profiles between phenol and benzene after oral dosing were due to zonal differences in the distribution of metabolizing enzymes within the liver. Conjugating enzymes are more concentrated in the periportal area of the liver, the first region to absorb the compound, whereas oxidizing enzymes are more concentrated in the pericentral region of the liver. Based on this hypothesis, during an initial pass through the liver after oral administration, phenol would have a greater opportunity to be conjugated as it was absorbed from the gastrointestinal tract into the periportal region of the liver, thus resulting in less free phenol being delivered into the pericentral region of the liver to be oxidized. With less free phenol available for oxidation, less hydroquinone would be produced, relative to conjugated phenol metabolites. In contrast, benzene must be oxidized before it can be conjugated. Therefore, metabolism of benzene would be minimal in the periportal region of the liver, with most of the benzene reaching the pericentral region to be oxidized to hydroquinone. Based on this scheme, the authors suggest that benzene administration would result in more free phenol being delivered to oxidizing enzymes in the pericentral region of the liver than administration of phenol itself (Kenyon et al. 1995).

Benzene has been found to stimulate its own metabolism, thereby increasing the rate of toxic metabolite formation. Pretreatment of mice, rats, and rabbits subcutaneously with benzene increased benzene metabolism *in vitro* without increasing CYP2E1 concentrations (Arinc et al. 1991; Gonasun et al. 1973; Saito et al. 1973). In contrast, there was no significant effect on the metabolism of benzene when Fischer 344 rats and B6C3F₁ mice, pretreated with repeated inhalation exposure to 600 ppm of benzene, were again exposed to 600 ppm benzene (Sabourin et al. 1990). The rate of benzene metabolism can be altered by pretreatment with various compounds. Benzene is a preferential substrate of CYP2E1, which also metabolizes alcohol and aniline. CYP2E1 can be induced by these substrates and is associated with the generation of hydroxyl radicals, probably via futile cycling of the cytochrome (Chepiga et al. 1991; Parke 1989; Snyder et al. 1993a, 1993b). It is possible that hydroxy radical formation by CYP2E1 may play a role in the benzene ring-opening pathway, leading to the formation of *trans,trans*-muconaldehyde. Phenol, hydroquinone, benzoquinone, and catechol have also been shown to induce CYPs in human

3. HEALTH EFFECTS

hematopoietic stem cells (Henschler and Glatt 1995). Therefore, exposure to chemicals that stimulate the activity of this enzyme system prior to exposure to benzene could increase the rate of benzene metabolism.

Both NADPH-linked and ascorbate-induced lipid peroxidation activities induced *in vitro* were lowered 5.5 and 26%, respectively, in rats following oral administration of 1,400 mg/kg/day of benzene for 3 days, followed by intraperitoneal injection of phenobarbital. These results suggest that benzene alters hepatic drug metabolism and lipid peroxidation. The decrease in lipid peroxidation could be due to the antioxidant property of the metabolites (Pawar and Mungikar 1975).

The ultimate disposition and metabolic fate of benzene depends on animal species, dose, and route of exposure. The dose of benzene affects both the total metabolism and the concentrations of individual metabolites formed. In mice, the percentage of hydroquinone glucuronide decreased as the dose increased. In both rats and mice, the percentage of muconic acid decreased as the dose increased. The shift in metabolism may affect the dose-response relationship for toxicity, and has been observed in all animal species studies thus far (Sabourin et al. 1989a, 1992; Witz et al. 1990b, 1990c). The effect of species differences in metabolism of inhaled benzene was evidenced by the fact that mice have a higher minute volume per kg body weight than rats (1.5 times higher). This caused the blood concentration of benzene to reach equilibrium more quickly in mice than in rats, but the steady-state level in blood was not influenced (Sabourin et al. 1987). Species differences in benzene metabolism following oral exposure were elucidated in rats and mice administered benzene by gavage at doses of 0.5–150 mg/kg/day (Sabourin et al. 1987). At doses below 15 mg/kg, >90% of the benzene was metabolized, while at doses above 15 mg/kg, an increasing percentage of orally administered benzene was exhaled unmetabolized. Total metabolites per unit body weight were equal in rats and mice at doses up to 50 mg/kg/day. However, total metabolites in mice did not increase at higher doses, suggesting saturation of metabolic pathways (Sabourin et al. 1987).

The integrated dose to a tissue over a 14-hour period (6-hour exposure, 8 hours following exposure) was calculated for benzene metabolites in rats and mice that were exposed to 50 ppm of radiolabeled (^3H) benzene (Sabourin et al. 1988). The major metabolic products in rats were detoxification products that were marked by phenyl conjugates. In contrast, mice had substantial quantities of the markers for toxification pathways (muconic acid, hydroquinone glucuronide, and hydroquinone sulfate) in their tissues. Muconic acid and hydroquinone glucuronide were also detected in mouse bone marrow. These results may explain why mice are more susceptible to benzene-induced toxicity than rats.

3. HEALTH EFFECTS

In a study by Orzechowski et al. (1995), hepatocytes from adult male Wistar rats and NMRI mice were incubated for 1 hour with 0.5 mM ^{14}C -benzene, and the supernatant analyzed for metabolites. Formation of sulfate conjugates of benzene, hydroquinone, and 1,2,4-benzenetriol was also studied in a separate experiment. Mouse hepatocytes produced two metabolites (1,2,4-trihydroxybenzene sulfate and hydroquinone sulfate) that were not found in rat hepatocyte incubations. These sulfate metabolites were found in incubations including benzene, or the metabolites themselves, hydroquinone and 1,2,4-benzenetriol. Mouse hepatocytes were almost three times more effective in metabolizing benzene, compared to rat hepatocytes. This difference was accounted for in the formation of hydroquinone, hydroquinone sulfate, and 1,2,4-trihydroxybenzene sulfate. These *in vitro* experiments indicate there are both quantitative and qualitative differences in rodent metabolism of benzene.

Data produced *in vitro* by mouse and rat liver microsomes also indicate species differences in benzene metabolism (Schlosser et al. 1993). Quantitation of metabolites from the microsomal metabolism of benzene indicated that after 45 minutes, mouse liver microsomes from male B6C3F₁ mice had converted 20% of the benzene to phenol, 31% to hydroquinone, and 2% to catechol. In contrast, rat liver microsomes from male Fischer 344 rats converted 23% to phenol, 8% to hydroquinone, and 0.5% to catechol. Mouse liver microsomes continued to produce hydroquinone and catechol for 90 minutes, whereas rat liver microsomes had ceased production of these metabolites by 90 minutes. Muconic acid production by mouse liver microsomes was <0.04 and <0.2% from phenol and benzene, respectively, after 90 minutes.

There are quantitative differences in the benzene metabolites produced by different species (Sabourin et al. 1988). Fischer 344 rats exposed to 50 ppm benzene had undetectable amounts of phenol, catechol, and hydroquinone in the liver, lungs, and blood. The major water-soluble metabolites were muconic acid, phenyl sulfate, prephenyl mercapturic acid, and an unknown. The unknown was present in amounts equal to the amounts of phenyl sulfate in the liver; phenyl sulfate and the unknown were the major metabolites in the liver. B6C3F₁ mice exposed to 50 ppm benzene had detectable levels of phenol and hydroquinone in the liver, lungs, and blood; catechol was detectable only in the liver and not in the lungs or blood. As in the rat, the unknown was present in amounts equal to the amounts of phenyl sulfate in the liver. Mice had more muconic acid in the liver, which indicates a greater risk for them from *trans,trans*-muconaldehyde (Sabourin et al. 1988).

3. HEALTH EFFECTS

The effect of dose rate on benzene metabolism was studied in Fischer 344 rats and B6C3F₁ mice that had either long inhalation exposures to low concentrations or short exposures to high concentrations of benzene (Sabourin et al. 1989a, 1989b). Inhalation occurred at 1 of 3 exposure regimens, all having the same integral amount of benzene: 600 ppm benzene for 0.5 hour, 150 ppm for 2 hours, or 50 ppm for 6 hours. Results indicated no dose-rate effect in rats. In mice, however, the fast exposure rate (0.5 hour times 600 ppm) produced less muconic acid in the blood, liver, and lungs. In the blood and lungs, less hydroquinone glucuronide and more prephenyl mercapturic acid were produced at the higher exposure rates. At the highest benzene exposure concentrations or fastest benzene exposure rate in mice, there was a reduction in the ratios of muconic acid and hydroquinone glucuronide to the metabolite phenylsulfate. Furthermore, with increased dose rate or increased exposure concentration, mice tended to shift a greater portion of their benzene metabolism toward detoxification pathways. Likewise, the detoxification pathways for benzene appear to be low-affinity, high-capacity pathways, whereas pathways leading to the putative toxic metabolites appear to be high-affinity, low-capacity systems (Henderson et al. 1989). Accordingly, if the exposure dose regimen, via inhalation, extends beyond the range of linear metabolism rates of benzene (200 ppm by inhalation) (Sabourin et al. 1989b), then the fraction of toxic metabolites formed relative to the amount administered will be reduced. Bois and Paxman (1992) used a PBPK model to assess effects of dose rate on the disposition of benzene metabolites. Simulations were performed for rats exposed either for 15 minutes to 32 ppm or for 8 hours to 1 ppm (equivalent 8-hour TWAs). The amount of metabolites (hydroquinone, catechol, and muconaldehyde) formed was 20% higher after the 15-minute exposure at the higher level than after the 8-hour exposure at the lower level. Differences between the model predictions (Bois and Paxman 1992) and the empirical data of Sabourin et al. (1989a, 1989b) may be related, at least in part, to the higher benzene exposure levels (50, 150, and 600 ppm) used by Sabourin and coworkers.

A number of investigators have suggested that covalent binding of benzene metabolites to cellular macromolecules is related to benzene's mechanism of toxicity, although the relationship between adduct formation and toxicity is not clear. Benzene metabolites have been found to form covalent adducts with proteins from blood in humans (Bechtold et al. 1992b). Benzene metabolites form covalent adducts with nucleic acids and proteins in rats and mice (Norpoth et al. 1988; Rappaport et al. 1996); covalently bind to proteins in mouse or rat liver, bone marrow, kidney, spleen, blood, and muscle *in vivo* (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Creek et al. 1997; Longacre et al. 1981a, 1981b; Sun et al. 1990); bind to proteins in perfused bone marrow preparations (Irons et al. 1980) and in rat and mouse liver DNA *in vivo* (Creek et al. 1997; Lutz and Schlatter 1977); and bind to DNA in rabbit and rat bone marrow mitochondria *in vitro* (Rushmore et al. 1984). Exposure-related increases in blood levels of

3. HEALTH EFFECTS

albumin adducts of benzene oxide and 1,4-benzoquinone were noted among workers occupationally exposed to benzene air concentrations ranging from 0.07 to 46.6 ppm (Rappaport et al. 2002a, 2002b). Several reactive metabolites of benzene have been proposed as agents of benzene hematotoxic and leukemogenic effects. These metabolites include benzene oxide, reactive products of the phenol pathway (catechol, hydroquinone, and 1,4-benzoquinone), and *trans,trans*-muconaldehyde. See Section 3.5.2 for a discussion of mechanisms of benzene toxicity.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Available human data indicate that following inhalation exposure to benzene, the major route for elimination of unmetabolized benzene is via exhalation. Absorbed benzene is also excreted in humans via metabolism to phenol and muconic acid followed by urinary excretion of conjugated derivatives (sulfates and glucuronides). In six male and female volunteers exposed to 52–62 ppm benzene for 4 hours, respiratory excretion (the amount of absorbed benzene excreted via the lungs) was approximately 17%; no gender-related differences were observed (Nomiyama and Nomiyama 1974a, 1974b). Results from a study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours showed that 16.4–41.6% of the retained benzene was excreted by the lungs within 5–7 hours (Srbova et al. 1950). The rate of excretion of benzene was the greatest during the first hour. The study also showed that only 0.07–0.2% of the retained benzene was excreted in the urine. Other studies suggest that benzene in the urine may be a useful biomarker of occupational exposure (Ghittori et al. 1993). Results of a study involving a single human experimental subject exposed to concentrations of benzene of 6.4 and 99 ppm for 8 hours and 1 hour, respectively, suggested that excretion of benzene in breath has three phases and could possibly have four phases. The initial phase is rapid and is followed by two (or three) slower phases (Sherwood 1988). The initial phase with a high exposure concentration (99 ppm) and a short-term exposure duration (1 hour) had a more rapid excretion rate (half-life=42 minutes) and a greater percentage of the total dose excreted (17%) than did the initial phase with a low exposure concentration (6.4 ppm) and longer exposure duration (8 hours) (half-life=1.2 hours, percentage of total dose excreted=9.3%). Subsequent phases showed an increase in the half-lives. These results also showed that urinary excretion of phenol conjugate was biphasic, with an initial rapid excretion phase, followed by a slower excretion phase. A greater proportion of the total dose was excreted in urine than in breath (Sherwood 1988). The urinary excretion of phenol in workers was measured following a 7-hour workshift exposure to 1–200 ppm benzene. A correlation of 0.881 between exposure level and urinary phenol excretion was found (Inoue et al. 1986). Urine samples were collected from randomly chosen subjects not exposed to known sources of

3. HEALTH EFFECTS

benzene, from subjects exposed to sidestream cigarette smoke, or from supermarket workers presumed exposed to benzene from polyvinyl chloride (PVC) meat packing wrap (Bartczak et al. 1994). Samples were analyzed for identification of muconic acid. Muconic acid concentrations of 8–550 ng/mL were found in all urine samples. Kok and Ong (1994) report blood and urine levels of benzene as 110.9 and 116.4 ng/L, respectively, in nonsmokers, and 328.8 and 405.4 ng/L, respectively in smokers. A significant correlation was found between benzene levels in blood and benzene levels in urine. Similar results were found for filling station attendants in Italy (Lagorio et al. 1994b).

Popp et al. (1994) reported a mean blood benzene level in car mechanics of 3.3 µg/L. Urinary muconic acid and *S*-phenylmercapturic acid levels increased during the work shift, and were well correlated with the blood levels and the benzene air levels, which reached a maximum of 13 mg/m³.

As discussed in Section 3.4.3, the mean urinary levels of muconic in groups of male, female-nonpregnant, and female-pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher than the mean concentration of this acid in the nonsmoking groups (Melikian et al. 1994). The differences in the mean muconic acid concentrations between smoking and nonsmoking groups were significant in male ($p=0.001$), and nonpregnant ($p=0.001$) and pregnant female smokers ($p=0.002$). Mean concentrations of muconic acid levels in nonpregnant female smokers are similar to that of male smokers. Mean concentrations of muconic acid in groups of 42 male smokers and 53 female smokers were 0.22 ± 0.03 and 0.24 ± 0.02 mg/g creatinine, or 0.13 ± 0.06 and 0.13 ± 0.07 mg/mg cotinine, respectively. Mean concentrations of muconic acid in groups of 63 pregnant and 53 nonpregnant female smokers were 0.27 ± 0.04 and 0.24 ± 0.02 mg/g creatinine, or 0.24 ± 0.06 and 0.13 ± 0.07 mg/mg cotinine, respectively. Mean concentrations of urinary cotinine in pregnant smokers were significantly lower than in the group of nonpregnant female smokers (1.13 ± 0.12 mg/g creatinine compared to 1.82 ± 0.14 mg/g creatinine). Benzene levels ranging from 0.01 to 0.18 µg/kg have been detected in samples of human breast milk (Fabiatti et al. 2004).

Animal data show that exhalation is the main route for excretion of unmetabolized benzene and that metabolized benzene is excreted primarily in urine. Only a small amount of an absorbed dose is eliminated in feces. A biphasic pattern of excretion of unmetabolized benzene in expired air was observed in rats exposed to 500 ppm for 6 hours, with half-times for expiration of 0.7 hour for the rapid phase and 13.1 hours for the slow phase (Rickert et al. 1979). The half-life for the slow phase of benzene elimination suggests the accumulation of benzene. The major route of excretion following a 6-hour nose-only inhalation exposure of rats and mice to various concentrations of ¹⁴C-benzene appeared to be dependent on the inhaled concentration (Sabourin et al. 1987). At similar exposures to vapor

3. HEALTH EFFECTS

concentrations of 10–1,000 ppm, the mice received 150–200% of the equivalent dose in rats on a per kg body weight basis. At all concentrations, fecal excretion accounted for <3.5% of the radioactivity for rats and <9% for mice. At lower exposure concentrations (i.e., 13–130 ppm in rats and 11–130 ppm in mice), <6% of the radioactivity was excreted in expired air. At the highest exposure concentrations (rats, 870 ppm; mice, 990 ppm), both rats and mice exhaled a significant amount of unmetabolized benzene (48 and 14%, respectively) following termination of the exposure. The majority of the benzene-associated radioactivity that was not exhaled was found in the urine and in the carcass 56 hours after the end of exposure to these high concentrations. The radioactivity in the carcass was associated with the pelt of the animals. The authors assumed that this was due to contamination of the pelt with urine, since the inhalation exposure had been nose-only. Further investigation confirmed that the radioactivity was associated with the fur of the animals. Accordingly, the percentage of the total radioactivity excreted by these animals (urine and urine-contaminated pelt) that was not exhaled or associated with feces was 47–92% for rats and 80–94% for mice. At exposures of 260 ppm in rats, 85–92% of the radioactivity was excreted as urinary metabolites, while at exposures of 130 ppm in mice, 88–94% of the radioactivity was excreted as urinary metabolites. The total urinary metabolite formation was 5–37% higher in mice than in rats at all doses. This may be explained by the greater amount of benzene inhaled by mice per kg of body weight (Sabourin et al. 1987). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

3.4.4.2 Oral Exposure

No studies were located regarding excretion in humans after oral exposure to benzene. Data on excretion of benzene or its metabolites in human breast milk after oral exposure were not found.

Radiolabeled benzene (340 mg/kg) was administered by oral intubation to rabbits; 43% of the label was recovered as exhaled unmetabolized benzene and 1.5% was recovered as carbon dioxide (Parke and Williams 1953a). Urinary excretion accounted for about 33% of the dose. The isolated urinary metabolites were mainly in the form of conjugated phenols. Phenol was the major metabolite accounting for about 23% of the dose or about 70% of the benzene metabolized and excreted in the urine. The other phenols excreted (percentage of dose) were hydroquinone (4.8%), catechol (2.2%), and trihydroxybenzene (0.3%). L-Phenyl-N-acetyl cysteine accounted for 0.5% of the dose. Muconic acid accounted for 1.3%; the rest of the radioactivity (5–10%) remained in the tissues or was excreted in the feces (Parke and Williams 1953a).

3. HEALTH EFFECTS

Mice received a single oral dose of either 10 or 200 mg/kg radiolabeled benzene (McMahon and Birnbaum 1991). Radioactivity was monitored in urine, feces, and breath. At the low dose, urinary excretion was the major route of elimination. Hydroquinone glucuronide, phenylsulfate, and muconic acid were the major metabolites at this dose, accounting for 40, 28, and 15% of the dose, respectively. At 200 mg/kg, urinary excretion decreased to account for 42–47% of the administered dose, while respiratory excretion of volatile components increased to 46–56% of the administered dose. Fecal elimination was minor and relatively constant over both doses, accounting for 0.5–3% of the dose.

The effect of dose on the excretion of radioactivity, including benzene and metabolites, following oral administration of ^{14}C -benzene (0.5–300 mg/kg) has been studied in rats and mice (Sabourin et al. 1987). At doses of <15 mg/kg for 1 day, 90% of the administered dose was excreted in the urine of both species. There was a linear relationship for the excretion of urinary metabolites up to 15 mg/kg; above that level, there was an increased amount of ^{14}C eliminated in the expired air. Mice and rats excreted equal amounts up to 50 mg/kg; above this level, metabolism apparently became saturated in mice. In rats, 50% of the 150 mg/kg dose of ^{14}C was eliminated in the expired air; in mice, 69% of the 150 mg/kg dose of ^{14}C was eliminated in expired air (Sabourin et al. 1987). The label recovered during exhalation was largely in the form of unmetabolized benzene, suggesting that saturation of the metabolic pathways had occurred. Dose also affected the metabolite profile in the urine. At low doses, a greater fraction of the benzene was converted to putative toxic metabolites than at high doses, as reflected in urinary metabolites.

Mathews et al. (1998) reported similar results following oral (gavage) administration of ^{14}C -benzene to rats, mice, and hamsters in single doses from as low as 0.2 mg/kg and up to 100 mg/kg. For example, >95% of a 0.5 mg/kg dose was recovered in the urine of rats; a small amount (3%) was recovered in expired air. At benzene doses of 10 and 100 mg/kg, elimination in the breath rose to 9 and 50%, respectively, indicating the likely saturation of benzene metabolism. Excretion in the feces was minimal at all dose levels. Similar results were noted for mice and hamsters. Both dose and species differences were noted in the composition of urinary metabolites. Phenyl sulfate was the major metabolite in rat urine at all dose levels, accounting for 64–73% of urinary radioactivity. Phenyl sulfate (24–32%) and hydroquinone glucuronide (27–29%) were the predominant urinary metabolites in mice. At a dose of 0.1 mg/kg, mice produced a considerably higher proportion of muconic acid than rats (15 versus 7%). In hamsters, hydroquinone glucuronide (24–29%) and muconic acid (19–31%) were the primary urinary metabolites. Two additional metabolites (1,2,4-trihydroxybenzene and catechol sulfate) were recovered from the urine of hamsters, but not rats or mice.

3. HEALTH EFFECTS

3.4.4.3 Dermal Exposure

Limited data on excretion of benzene after dermal exposure in humans were found. Four human male subjects were given a dermal application of 0.0024 mg/cm^2 ^{14}C benzene (Franz 1984). A mean of 0.023% (range, 0.006–0.054%) of the applied radiolabel was recovered in the urine over a 36-hour period. Urinary excretion of the radiolabel was greatest in the first two hours following skin application. More than 80% of the total excretion occurred in the first 8 hours. In another study, $35\text{--}43 \text{ cm}^2$ of the forearm were exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25–2 hours (Hanke et al. 1961). The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be low, approximately $0.4 \text{ mg/cm}^2/\text{hour}$. The absorption due to vapors in the same experiment was negligible. Although there was a large variability in the physiological values, the amount of excreted phenol was 8.0–14.7 mg during the 24-hour period after exposure. It is estimated that approximately 30% of dermally absorbed benzene is eliminated in the form of phenol in the urine.

Data on excretion of benzene or its metabolites in human breast milk after dermal exposure were not found.

Monkeys and minipigs were exposed dermally to $0.0026\text{--}0.0036 \text{ mg/cm}^2$ of ^{14}C -benzene (Franz 1984). After application, the urine samples were collected over the next 2–4 days at 5-hour intervals. The rate of excretion was highest in the first two collection periods. The total urinary excretion of radioactivity was found to be higher in monkeys than in minipigs with the same exposure. Mean excretion in monkeys was 0.065% (range, 0.033–0.135%) of the applied dose compared to 0.042% (range, 0.030–0.054%) in minipigs.

Results of a study in which male rats were dermally treated with 0.004 mg/cm^2 of ^{14}C -benzene, with or without 1g of clay or sandy soil, showed that for all treatment groups, the major routes of excretion were the urine and, to a lesser extent, the expired air (Skowronski et al. 1988). The highest amount of radioactivity in urine appeared in the first 12–24 hours after treatment (58.8, 31.3, and 25.1% of the absorbed dose, respectively, for pure benzene, sandy soil-adsorbed benzene, and clay soil-adsorbed benzene). In the group treated with pure benzene, 86.2% of the absorbed dose was excreted in the urine. Sandy soil and clay soil significantly decreased urinary excretion to 64.0 and 45.4%, respectively, of the absorbed dose during the same time period. Rats receiving pure benzene excreted 12.8% of the absorbed

3. HEALTH EFFECTS

dose in expired air within 48 hours. Only 5.9% of the radioactivity was collected in expired air 48 hours after treatment with sandy soil-adsorbed benzene, while experiments with clay soil-adsorbed benzene revealed that 10.1% of the radioactivity was located in expired air. Less than 1% of the absorbed dose was expired as $^{14}\text{CO}_2$ in all groups. The ^{14}C activity in the feces was small (<0.5% of the applied radioactivity) in all groups 48 hours after treatment. Phenol was the major urinary metabolite detected in the 0–12-hour urine samples of all treatment groups. The percentage of total urinary radioactivity associated with phenol was 37.7% for benzene alone, 44.2% for benzene adsorbed to sandy soil, and 45.5% for benzene adsorbed to clay soil. Smaller quantities of hydroquinone, catechol, and benzenetriol were also detected (Skowronski et al. 1988).

3.4.4.4 Other Routes of Exposure

The metabolic fate of benzene can be altered in fasted animals. In nonfasted rats that received an intraperitoneal injection of 88 mg of benzene, the major metabolites present in urine were total conjugated phenols (14–19% of dose), glucuronides (3–4% of dose), and free phenol (2–3% of dose). However, in rats fasted for 24 hours preceding the same exposure, glucuronide conjugation increased markedly (18–21% of dose) (Cornish and Ryan 1965). Free phenol excretion (8–10% of dose) was also increased in fasted, benzene-treated rats. There was no apparent increase in total conjugated phenol excretion in fasted rats given benzene.

When ^{14}C -benzene (0.5 and 150 mg/kg) was injected intraperitoneally into rats and mice, most of the ^{14}C -benzene and ^{14}C -metabolites were excreted in the urine and in the expired air. A smaller amount of ^{14}C -benzene was found in the feces due to biliary excretion (Sabourin et al. 1987). Monkeys were dosed intraperitoneally with 5–500 mg/kg radiolabeled benzene, and urinary metabolites were examined (Sabourin et al. 1992). The proportion of radioactivity excreted in the urine decreased with increasing dose, whereas as the dose increased, more benzene was exhaled unchanged. This indicated saturation of benzene metabolism at higher doses. Phenyl sulfate was the major urinary metabolite. Hydroquinone conjugates and muconic acid in the urine decreased as the dose increased. When C57BL/6 mice and DBA/2 mice were given benzene subcutaneously in single doses (440, 880, or 2,200 mg/kg) for 1 day, or multiple doses (880 mg/kg) 2 times daily for 3 days, no strain differences were observed in the total amount of urinary ring-hydroxylated metabolites (Longacre et al. 1981a). Although each strain excreted phenol, catechol, and hydroquinone, differences in the relative amounts of these metabolites were noted. The more sensitive DBA/2 mice excreted more phenol but less hydroquinone than the more resistant C57BL/6 mice, while both strains excreted similar amounts of catechol. DBA/2 mice excreted more

3. HEALTH EFFECTS

phenyl glucuronide but less sulfate conjugate. Both strains excreted similar amounts of phenyl mercapturic acid (Longacre et al. 1981a).

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.

3. HEALTH EFFECTS

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

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

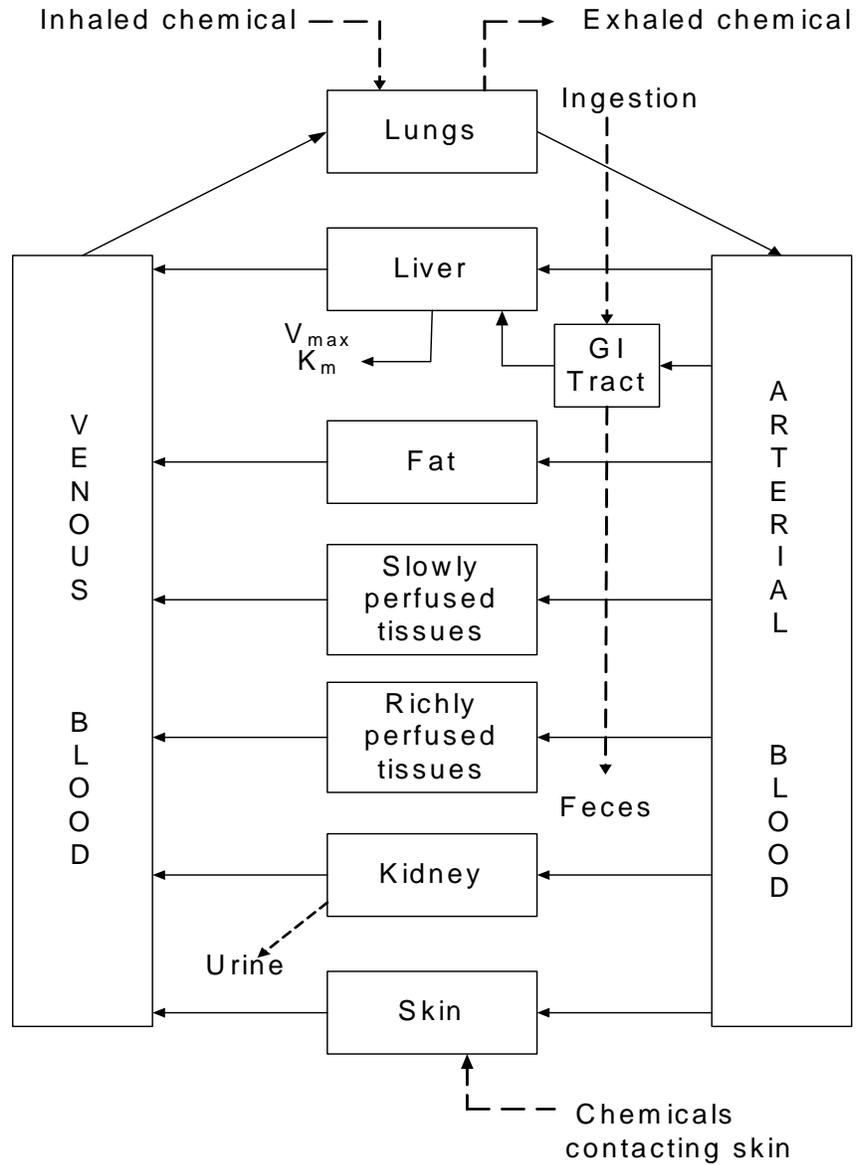
If PBPK models for benzene exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Several PBPK models have been developed that simulate the disposition of benzene in humans (Bois et al. 1996; Brown et al. 1998; Fisher et al. 1997; Medinsky et al. 1989c; Sinclair et al. 1999; Travis et al. 1990), mice (Cole et al. 2001; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990), and rats (Bois et al. 1991a; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990). A comparative summary of the models is provided in Table 3-6. All of the models have the same general structure (Figure 3-5). Most of the models simulate inhalation and oral exposures; one model provides a simulation of dermal absorption (Sinclair et al. 1999). Physiological parameters and partition coefficients for simulating benzene biokinetics of human females were reported for the Brown et al. (1998) and Fisher et al. (1997) models. Flow-limited exchange of benzene between blood and tissues is assumed in all models, with excretion of benzene in exhaled air and, in one case, to breast milk (Fisher et al. 1997). All models include simulations of blood, fat, liver, lung, and lumped compartments representing other slowly-perfused tissues (e.g., skeletal muscle) and rapidly-perfused tissues (e.g., kidneys, other viscera). Simulation of bone marrow, the primary target for benzene toxicity, is included in the models reported by Bois et al. (1991a, 1996), Sinclair et al. (1999), and Travis et al. (1990).

Simulations of metabolism in the various models vary in complexity. In the simplest representation, metabolic elimination of benzene is simulated as a single capacity-limited process, represented with

3. HEALTH EFFECTS

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

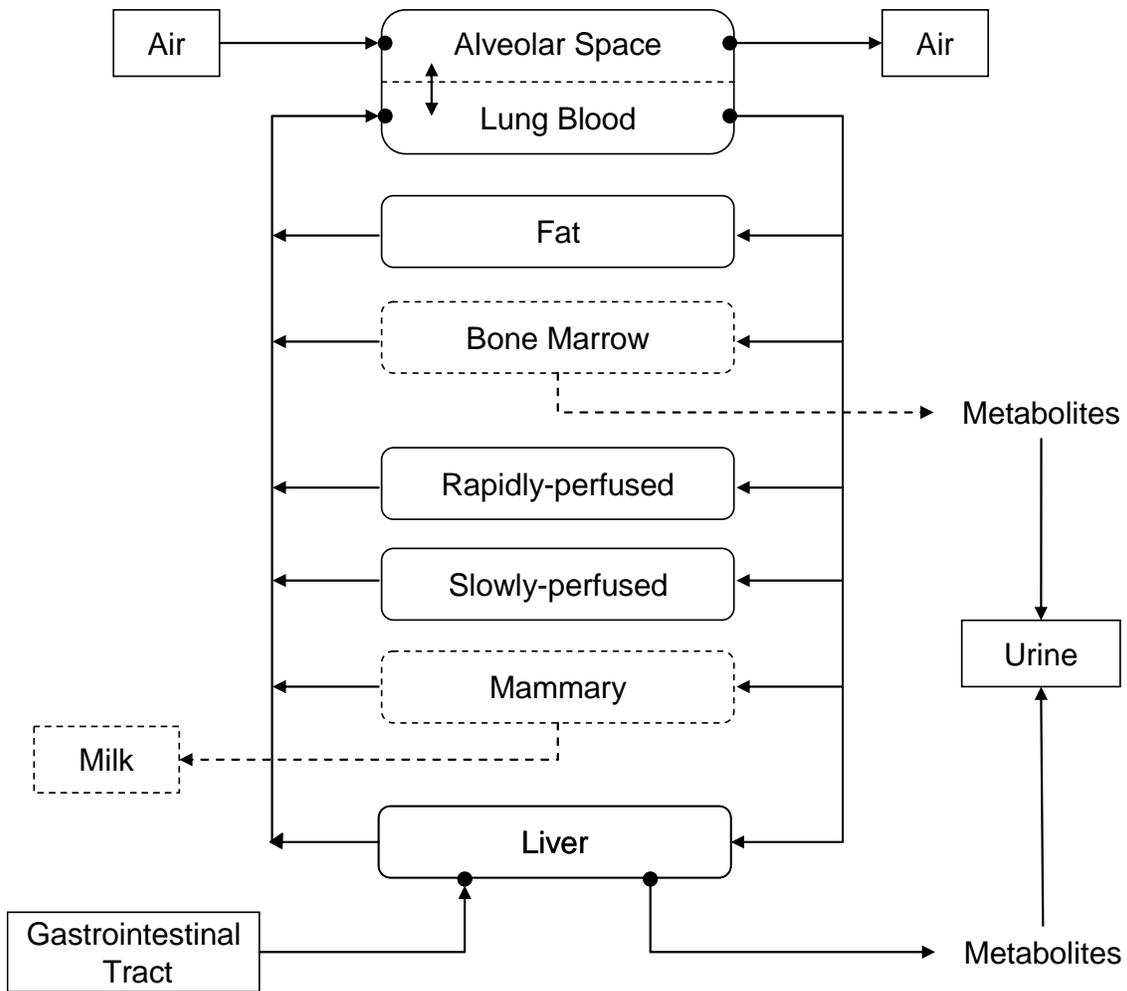


Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan and Andersen 1994

3. HEALTH EFFECTS

Figure 3-5. General Structure of Physiologically Based Pharmacokinetic Models of Benzene*



*Tissues shown with dashed lines are not simulated in all models. Flow-limited exchange of benzene between blood and tissues is assumed. Metabolism is simulated to varying degrees of complexity (see Table 3-6 for model comparison).

3. HEALTH EFFECTS

Table 3-6. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

Reference	Simulated areas					Comment
	Species ^a	Absorption pathways ^b	Tissues ^c	Metabolic pathways ^d	Excretion pathways ^e	
Bois et al. 1991a	R	IH, OR	BL, BM, FA, LI, LU, RP, SP	BM, LI: BZ→BO(c) BO→BG(c) BO→PH(f) BO→GSH(c) BG→DI(c) PH→HQ(c) PH→CA(c) PH→PHCO(c) BM, LU, LU, GI:	EH: BZ UR: PH	Simulates metabolic pathways in bone marrow, and phenol conjugation in lung and gastrointestinal tract
Bois et al. 1996	H	IH	BL, BM, FA, LU, LI, RP, SP	BM, LI: BZ→M _{tot} (c) LI: PHX _{end} →PH(z)	EH: BZ UR: M _{tot} PH	Simulates metabolic pathways in bone marrow, and endogenous production of phenolic metabolites
Brown et al. 1998	H (m,f)	IH	BL, FA, LI, LU, RP, SP	LI: BZ→M _{tot} (f)	EH: BZ	Simulates males or females
Cole et al. 2001	M	IH, OR	BL, FA, LI, LU, RP, SP	LI: BZ→BO(c) BO→PH(f) BO→PMA(f) BO→MA(f) PH→HQ(c) PH→PHCO(c) PH→CA(c) CA→THB(c) HQ→HQCO(c)	EH: BZ UR: CA MA PHCO PMA HQCO THB	All metabolism is assigned to the liver
Fisher et al. 1997	H	IH	FA, LU, LI, RP, SP, MI	LI: BZ→M _{tot} (c)	EH: BZ MI: BZ	Simulates transfer of benzene to breast milk
Medinsky et al. 1989a, 1989b, 1989c	H, M, R	IH, OR	FA, LI, LU, RP, SP	LI: BZ→BO(c) BO→PHCO(c) BO→PMA(c) BO→HQCO(c) BO→MA(c)	EH: BZ	All metabolism is assigned to the liver

3. HEALTH EFFECTS

Table 3-6. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

Reference	Simulated areas					Comment
	Species ^a	Absorption pathways ^b	Tissues ^c	Metabolic pathways ^d	Excretion pathways ^e	
Sinclair et al. 1999	H	IH, OR, DE	BL, BM, LI, LU, MU, RP	BM, LI: BZ→M _{tot} (c)	EH: BZ UR: M _{tot} PH	Simulates dermal exposure and absorption
Sun et al. 1990	M, R	IH, OR	BL, FA, LI, LU, RBC, RP, SP	LI: BZ→BO(c) BO→PHCO(c) BO→PMA(c) BO→HQCO(c) BO→MA(c) RBC: BO→HBA(c,f)	EH: BZ	Simulates formation of hemoglobin adducts in red blood cells derived from benzene oxide
Travis et al. 1990	H, M, R	IH, OR	BL, BM, FA, LI, LU, MU, RP	BM, LI: BZ→M _{tot} (c)	EH: BZ	Total metabolism of benzene in the bone marrow and liver

^aSpecies simulated: H=human; M=mouse; R=rat; m=male; f=female

^bAbsorption pathways simulated: IH=inhalation; OR=oral; DE=dermal

^cTissues simulated: BL=blood; BM=bone marrow; FA=fat; LI=liver; LU=lung; MU=muscle; RBC=red blood cells; RP=other rapidly-perfused tissues; SP=other slowly-perfused tissues

^dMetabolic pathways simulated: BZ=benzene; BD=benzene diols; BO=benzene oxide; BG=Benzen glycol; CA=catechol; HBA=hemoglobin adduct; HQ=hydroquinone; HQCO=hydroquinone conjugates; MA=muconic acid; M_{tot}=total metabolites; PH=phenol; PHCO=phenol conjugates; PMA=phenylmercapturic acid; THB=trihydroxybenzene; PHX_{end}=endogenous phenolic metabolites; (c)=capacity-limited; (f)=first-order; (z)= zero-order

^eExcretion pathways simulated: EH=exhalation; MI=breast milk; UR=urine

3. HEALTH EFFECTS

Michaelis-Menten function of benzene concentration in tissue (Bois et al. 1996; Brown et al. 1998; Fisher et al. 1997; Sinclair et al. 1999; Travis et al. 1990). In the more complex representations, the major pathways of metabolism of benzene, including conjugation reactions, are simulated as capacity-limited or first-order processes (Bois et al. 1991a; Cole et al. 2001; Medinsky et al. 1989a, 1989b, 1989c; Sun et al. 1990). In most of the models, all metabolic pathways are attributed to the liver; however, four of the models include simulations of metabolism in bone marrow (Bois et al. 1991a, 1996; Sinclair et al. 1999; Travis et al. 1990), and one model includes simulations of the formation of sulfate and glucuronide conjugates of phenol in the gastrointestinal and respiratory tracts (Bois et al. 1991a). The Sun et al. (1990) model includes a simulation of the formation of hemoglobin adducts derived from benzene oxide. In models that simulate the disposition of the metabolites, metabolites are assumed to be excreted in urine either at a rate equal to their formation (Cole et al. 2001), or in accordance with a first-order excretion rate constant (Bois et al. 1991a, 1996; Sinclair et al. 1999); the difference being, in the latter, the mass balance for formation and excretion of metabolites is simulated, allowing predictions of metabolite levels in tissues. All of the models use typical parameters and values for species-specific blood flows and tissue volumes.

Brief summaries of the models presented in Table 3-6 are provided below, with emphasis on unique features that are applicable to risk assessment.

Medinsky et al. 1989a, 1989b, 1989c

Description of the Model. The Medinsky et al. (1989a, 1989b, 1989c) model simulates absorption and disposition of benzene in the human, mouse, and rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process; absorption and excretion of benzene in the lung are assumed to be flow-limited. Exchange of benzene between blood and tissues is assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of benzene to benzene oxide as a function of the concentration of benzene in liver. Conversion of benzene oxide to phenol conjugates, phenylmercapturic acid, hydroquinone conjugates, and muconic acid are simulated as parallel, capacity-limited reactions in liver. The model simulates rates of formation of metabolites, but not the disposition (e.g., excretion) of metabolites. Metabolism parameter values (V_{max} , K_m) for the mouse and rat models were estimated by optimization of the model to observations of total metabolites formed in mice and rats exposed by inhalation or oral routes to benzene (Medinsky et al. 1989b; Sabourin

3. HEALTH EFFECTS

et al. 1987). Human metabolism parameter values were derived from allometric scaling of the values for mice (Medinsky et al. 1989c).

Risk Assessment. The model has been used to predict the amounts of benzene metabolites formed in rats and mice after inhalation or oral exposures (Medinsky et al. 1989a, 1989b). For inhalation concentrations up to 1,000 ppm, mice were predicted to metabolize at least 2–3 times more benzene than rats. For oral doses >50 mg/kg, rats were predicted to metabolize more benzene on a kg-body weight basis than mice. The model also predicts different metabolite profiles in the two species: mice were predicted to produce primarily hydroquinone glucuronide and muconic acid, metabolites linked to toxic effects, whereas rats were predicted to produce primarily phenyl sulfate, a detoxification product. These predictions agree with experimental data and provide a framework for understanding the greater sensitivity of the mouse to benzene toxicity.

Validation of the Model. The model was calibrated with data from Sabourin et al. (1987). Bois et al. (1991b) compared predictions made to observations of benzene exhaled by rats following exposures to 490 ppm benzene, reported by Rickert et al. (1979), as well as the data from which the model was calibrated (Sabourin et al. 1987). In general, the model tended to overestimate observations to which it was not specifically fitted.

Target Tissues. The model simulates amounts and concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as amounts of metabolites formed. It does not simulate concentrations of metabolites in these tissues. It does not simulate bone marrow, a target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations of mice, rats, and humans (Medinsky et al. 1989a, 1989b, 1989c).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in rodents ranging from 1 to 1,000 ppm and oral gavage doses ranging from 0.1 to 300 mg/kg (Bois et al. 1991b; Medinsky et al. 1989a, 1989b, 1989c).

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes (Medinsky et al. 1989a, 1989b, 1989c).

3. HEALTH EFFECTS

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested (single dose) benzene, including rates and amounts of major metabolites formed in mice, rats, and humans. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) the model attributes all metabolism to the liver; (3) the model does not simulate the fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites (e.g., muconaldehyde) in tissues; and (4) the model does not simulate bone marrow, a major target tissue for benzene metabolites.

Sun et al. 1990

Description of the Model. The Sun et al. (1990) model is an extension of the mouse and rat models developed by Medinsky et al. (1989a, 1989b, 1989c). The Sun et al. (1990) model includes a simulation of the formation of hemoglobin adducts derived from benzene oxide. Adduct formation is represented as the sum of capacity-limited and first-order functions of the concentration of benzene oxide in the liver. Parameter values were estimated by optimization to measurements of hemoglobin adduct formations in rats and mice exposed to single oral gavage doses of benzene (Sun et al. 1990).

Risk Assessment. The model has been applied to predicting the levels of hemoglobin adducts in mice and rats following inhalation or oral exposures to benzene. This approach could be potentially useful for predicting exposure levels that correspond to measured hemoglobin adduct levels, for use of adducts as an exposure biomarker.

Validation of the Model. The model was calibrated against measurements of hemoglobin adduct formation in mice and rats that received single oral gavage doses of benzene ranging from 0.008 to 800 mg/kg (Sun et al. 1990). The model was evaluated by comparing predictions to observations of amounts of hemoglobin adducts formed in mice and rats exposed to benzene vapor concentrations of 5, 50, or 600 ppm for 6 hours (Sabourin et al. 1989a).

Target Tissues. The model predicts hemoglobin adduct formation after oral and inhalation exposure (Sun et al. 1990).

Species Extrapolation. The model has been applied to simulations for mice and rats.

3. HEALTH EFFECTS

High-low Dose Extrapolation. The model was calibrated with observations made in mice and rats exposed to single gavage doses ranging from 0.1 to 10,000 $\mu\text{mol/kg}$ (0.008–800 mg/kg), and evaluated for predicting observations in mice and rats exposed by inhalation to 600 ppm benzene.

Interroute Extrapolation. The Sun model examined two routes of exposure, oral and inhalation. The model was found to be useful in predicting the concentrations of hemoglobin adducts in blood in rodents after oral and inhalation exposure.

Strengths and Limitations. Strengths of the model are that it extends the Medinsky et al. (1989a, 1989b, 1989c) models to simulate hemoglobin adduct formation secondary to formation of benzene oxide. A limitation of the adduct model is that it simulates production of adducts as a function of benzene oxide concentration in liver and does not consider other potential pathways of adduct formation through hydroquinone, phenol, or muconaldehyde.

Travis et al. 1990

Description of the Model. The Travis et al. (1990) model simulates the absorption and disposition of benzene in the human, mouse, and rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process. Absorption and excretion of benzene in the lung are assumed to be flow-limited, as are exchanges of benzene between blood and tissues. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolic elimination of benzene as a function of the concentration of benzene in bone marrow and liver. The model simulates rates of metabolic elimination of benzene, but not the rates of formation of specific metabolites or their disposition (e.g., excretion). For the purpose of comparing model predictions to observations, 80% of the total metabolite formed in 24 hours (and excreted in urine) was assumed to be phenol. Metabolism parameter values (V_{max} , K_m) were estimated by optimization of the model to observations of total metabolites formed (i.e., excreted in urine) in humans, mice, and rats exposed to benzene by inhalation or oral routes to benzene. The V_{max} for metabolism in bone marrow in humans was assumed to be 4% of that of liver, consistent with optimized values for rodents.

Risk Assessment. This model has been used to predict the amounts of benzene in expired air, concentrations of benzene in blood, and total amount of benzene metabolized following inhalation exposures to humans and inhalation, intraperitoneal, oral gavage, or subcutaneous exposures in mice or

3. HEALTH EFFECTS

rats (Travis et al. 1990). Cox (1996) applied the model to derive internal dose-response relationships for benzene in humans.

Validation of the Model. The model was evaluated by comparing predictions with observations made in mouse and rat inhalation studies (Rickert et al. 1979; Sabourin et al. 1987; Sato et al. 1975; Snyder et al. 1981); mouse oral gavage studies (Sabourin et al. 1987); mouse subcutaneous injection studies (Andrews et al. 1977); and rat intraperitoneal injection studies (Sato and Nakajima 1979). Predictions of benzene in expired air and/or blood concentrations were also compared to observations made in humans who inhaled concentrations ranging from 5 to 100 ppm benzene (5 ppm: Berlin et al. 1980; Sherwood 1972; 25–57 ppm: Sato et al. 1975; Sherwood 1972; Nomiyama and Nomiyama 1974a, 1974b; 99–100 ppm: Sherwood 1972; Teisinger and Fiserova-Bergerova 1955). Further evaluations of predictions of benzene in workers are reported in Sinclair et al. (1999) and Sherwood and Sinclair (1999), who compared model predictions with observations of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm.

Target Tissues. The model simulates amounts and concentrations of benzene in blood, bone marrow (a target tissue), liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues; and amounts of metabolites formed in liver and bone marrow. It does not simulate concentrations of metabolites in these tissues.

Species Extrapolation. The model has been applied to simulations for mice, rats, and humans (Sherwood and Sinclair 1999; Sinclair et al. 1999; Travis et al. 1990).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in humans ranging from 1 to 1,110 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999; Travis et al. 1990). Evaluations of predictions in rodents included observations made during inhalation exposures that ranged from 11 to 1,000 ppm and oral gavage doses that ranged from 0.5 to 300 mg/kg.

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., benzene concentration in blood, amount benzene metabolized) resulting from exposures by these routes (Travis et al. 1990).

Strengths and Limitations. Strengths of the model are that it simulates (1) disposition of inhaled and ingested (single dose) benzene in mice, rats, and humans; and (2) concentrations of benzene, and

3. HEALTH EFFECTS

rates and amount of benzene metabolized in bone marrow, a target tissue for benzene metabolites.

Limitations of the model include: (1) the model simulates metabolic elimination of benzene, but not the rates of formation of major metabolites; and (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues.

Fisher et al. 1997

Description of the Model. The Fisher et al. (1997) model extends the model reported by Travis et al. (1990) to include a simulation of lactational transfer of benzene to breast milk in humans. Other tissues simulated include blood, fat, liver, lung, other slowly-perfused tissues, and rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited, as is excretion of benzene in breast milk. The lactational transfer model includes simulations of breast milk production and loss from nursing; the latter is represented as a first-order process. Estimates of blood:air and blood:milk partition coefficients during lactation (from which the milk:blood partition coefficient could be calculated) were measured in nine lactating subjects (Fisher et al. 1997). The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites and their disposition (e.g., excretion) are not simulated. Metabolism parameter values (K_m , V_{max}) and tissue:blood partition coefficients were derived from Travis et al. (1990).

Risk Assessment. This model has been used to predict benzene concentrations in breast milk and lactational transfers to breast feeding infants (Fisher et al. 1997). Exposures to the threshold limit value (TLV) (10 ppm, 8 hours/day, 5 days/week) were predicted to yield 0.053 mg of benzene in breast milk per 24 hours. This approach has potential applicability to assessing lactational exposures to infants resulting from maternal exposures.

Validation of the Model. The lactation model was evaluated (Fisher et al. 1997) by comparing predictions for perchloroethylene (not benzene) with those predicted by a perchloroethylene model developed by Schreiber (1993). Other components of the biokinetics model were derived from the Travis et al. (1990) model, which has undergone evaluations against data obtained from studies in humans.

Target Tissues. The model simulates concentrations of benzene in blood, breast milk, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as rates of metabolic

3. HEALTH EFFECTS

elimination of benzene. It does not simulate concentrations of metabolites in these tissues and does not simulate metabolism in bone marrow, a major target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations for humans (Fisher et al. 1997).

High-low Dose Extrapolation. The lactational model has not been evaluated for simulating inhalation exposures to benzene in humans; therefore, applicability to high-low dose extrapolations cannot be assessed.

Interroute Extrapolation. The model was developed to simulate inhalation exposures. Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates the disposition of inhaled benzene in females during lactation, including transfers of benzene to breast milk and nursing infants; concentrations of benzene in blood and tissues; and rates of metabolic elimination of benzene metabolized. Limitations of the model include that the model does not simulate rates of formation of major metabolites and that the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Sinclair et al. 1999

Description of the Model. The Sinclair et al. (1999) model is an extension of the human model developed by Travis et al. (1990) to include a simulation of first-order urinary excretion of total metabolites and phenol, and dermal absorption of benzene.

Risk Assessment. The model has been applied to predicting the levels of benzene in exhaled air and phenol in urine in workers exposed to benzene (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Validation of the Model. The model was evaluated against measurements of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999).

3. HEALTH EFFECTS

Target Tissues. The model simulates amounts and concentrations of benzene in blood, bone marrow (a target tissue), liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues; rates of metabolic elimination of benzene in liver and bone marrow; and excretion of total metabolites formed and phenol.

Species Extrapolation. The model has been applied to simulations for humans (Sinclair et al. 1999).

High-low Dose Extrapolation. The model was evaluated against observations of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Interroute Extrapolation. The model simulates inhalation, oral, and dermal exposures.

Strengths and Limitations. Strengths of the model are that it extends the Travis et al. (1990) model to include simulation of dermal absorption of benzene.

Bois et al. 1991a

Description of the Model. The Bois et al. (1991a) model simulates absorption and disposition of benzene and the benzene metabolite, phenol, in the rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene and phenol are simulated as a first-order function for dose. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene and phenol between blood and tissues. Excretion of phenol is simulated as a first-order transfer to urine. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in bone marrow, liver, gastrointestinal tract, and respiratory tract (see Table 3-6). All pathways are assumed to be capacity-limited reactions, except for the spontaneous hydrolysis of benzene oxide to form phenol, which is simulated as a first-order process. The model simulates rates of formation of metabolites and first-order excretion of phenol; however, disposition (e.g., excretion) of other metabolites is not simulated. Parameter values, including metabolism parameter values, were optimized to a reference set of observations of metabolites formed in rats exposed by inhalation or to single gavage doses of benzene (see below).

3. HEALTH EFFECTS

Risk Assessment. This model has been used to predict amounts of benzene and phenol metabolites formed in rats during oral gavage exposures to benzene equivalent to those administered in NTP (1986) and to inhalation exposures equivalent to the OSHA PEL (Bois and Paxman 1992; Bois et al. 1991a). Model simulations indicate that dose rate may be an important factor in benzene toxicity. For example, when the model was applied to simulations for rats exposed either for 15 minutes to a benzene vapor concentration of 32 ppm or for 8 hours to 1 ppm (equivalent 8-hour TWAs), the amount of metabolites (hydroquinone, catechol, and muconaldehyde) formed was 20% higher after the 15-minute exposure at the higher level than after the 8-hour exposure at the lower level (Bois and Paxman 1992). These metabolites have been identified as being important in the genesis of bone marrow toxicity after benzene exposure (Eastmond et al. 1987). These types of analyses, if extended to humans, would be applicable to evaluations of the adequacy of short-term exposure limits.

Validation of the Model. The model was calibrated (Bois and Paxman 1992; Bois et al. 1991a) with observations made in rats exposed to single oral gavage doses of benzene, or to inhalation exposures of 13–870 ppm (Sabourin et al. 1987, 1989b), in rats administered single parenteral doses of phenol (Cassidy and Houston 1984), and in *in vitro* metabolism studies (Sawahata and Neal 1983). Further evaluations against data not used in the calibration were not reported.

Target Tissues. The model simulates amounts and concentrations of benzene and phenol in bone marrow, a target tissue for benzene metabolites, as well as in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues. The model also simulates amounts of specific metabolites formed and urinary excretion of the major urinary metabolite, phenol. It does not simulate concentrations of metabolites, other than phenol, in these tissues.

Species Extrapolation. The model has been applied to simulations for rats. A human model has been developed that implements a scaled-down version of the rat metabolism model (see Bois et al. 1996).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in rats ranging from 13 to 870 ppm and oral gavage doses ranging from 15 to 300 mg/kg.

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes.

3. HEALTH EFFECTS

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested benzene (and phenol), including rates and amounts of most of the major metabolites formed in rats. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) although the model simulates the fate of benzene and phenol, it does not simulate the fate of other metabolites formed and, therefore, cannot be used to predict concentrations of these metabolites in tissues; and (3) the model, as configured in Bois et al. (1991a), does not simulate benzene disposition in humans.

Bois et al. 1996

Description of the Model. The Bois et al. (1996) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates metabolic elimination of benzene as a single capacity-limited (i.e., Michaelis-Menten) reaction, occurring in bone marrow and liver. Endogenous formation of phenolic metabolites is also simulated as a zero-order process occurring in liver. The model simulates first-order excretion of total metabolites and the phenol fraction (approximately 80% of total). Parameter values (physiological and chemical) were estimated by Bayesian optimization techniques (Markov Chain Monte Carlo analysis) using reference observations of benzene concentration in blood and urinary excretion of phenol in human subjects who were exposed to benzene in air (Pekari et al. 1992).

Risk Assessment. The model has been used to predict rates and amounts of benzene metabolized in human populations (Bois et al. 1996). The population model (population geometric means and standard deviations of parameter values) was derived using Markov Chain Monte Carlo analysis with observations from three human subjects serving as the reference data for inter-individual variability (from Pekari et al. 1992). The population model predicts probability distributions of model outputs (for example, rates or amounts of benzene metabolized for a given exposure). This approach could be used to evaluate uncertainty factors in risk assessments intended to account for uncertainties in our understanding of benzene pharmacokinetics variability.

Validation of the Model. The model was calibrated with observations of benzene concentrations in blood and urinary phenol levels, made in three human subjects who were exposed to 1.7 or 10 ppm

3. HEALTH EFFECTS

benzene for 4 hours (Pekari et al. 1992). Further evaluations against data not used in the calibration have not been reported.

Target Tissues. The model simulates amounts and concentrations of benzene in bone marrow, a target of benzene toxicity, as well as blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues. Amounts of total metabolites formed and excreted are simulated; however, the model does not simulate concentrations of metabolites in these tissues.

Species Extrapolation. The model has been applied to simulations for humans (Bois et al. 1996).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in human subjects ranging from 1.7 to 10 ppm (Bois et al. 1996).

Interroute Extrapolation. The model simulates inhalation exposures. Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled benzene and rates of total metabolism in humans. Limitations include that the model has not been evaluated for multiple exposures and that the model simulates total metabolism of benzene, and not the rates of formation of the major metabolites of benzene of toxicological interest.

Brown et al. 1998

Description of the Model. The Brown et al. (1998) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites, or their disposition (e.g., excretion), are not simulated. For the purpose of comparing model predictions to observations, 80% of the total metabolites formed and excreted in urine (i.e., amount of benzene eliminated by metabolism) in 24 hours was assumed to be phenol. The K_m parameter for metabolism was derived from Travis et al. 1990; the V_{max} was estimated by optimization of the model to observations of blood concentrations of

3. HEALTH EFFECTS

benzene and benzene in exhaled breath of female and male subjects who were exposed to 25 ppm benzene for 2 hours (Sato et al. 1975). Partition coefficients for males and females were derived from vial equilibrium studies conducted on blood and/or tissues from males and females (Fisher et al. 1997; Paterson and Mackay 1989).

Risk Assessment. This model has been used to predict the benzene concentrations in blood and amounts of benzene metabolized in females and males who experience the same inhalation exposure scenarios. Females were predicted to metabolize 23–26% more benzene than similarly-exposed males. This difference was attributed, in part, to a higher blood:air partition coefficient for benzene in females.

Validation of the Model. The model was calibrated by comparing predictions of blood concentrations of benzene and benzene in exhaled breath of female and male subjects who were exposed to 25 ppm benzene for 2 hours (Brown et al. 1998; Sato et al. 1975). Further evaluations against data not used in the calibration were not been reported.

Target Tissues. The model simulates concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as rates of metabolic elimination of benzene. It does not simulate concentrations of metabolites in these tissues and does not simulate metabolism in bone marrow, a major target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations for humans.

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in humans. Evaluations of predictions included observations made during inhalation exposures to 25 ppm (Brown et al. 1998; Sato et al. 1975).

Interroute Extrapolation. The model simulates inhalation and has been applied to predicting internal dose metrics (e.g., benzene concentration in blood, amount benzene metabolized) resulting from exposures by this route (Brown et al. 1998). Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled benzene in female and male humans as well as the concentrations of benzene and rates of metabolic elimination of benzene metabolized. Limitations of the model include: (1) the model does not simulate

3. HEALTH EFFECTS

rates of formation of major benzene metabolites; (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues; and (3) the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Cole et al. 2001

Description of the Model. The Cole et al. (2001) model simulates absorption and disposition of benzene in the mouse. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in liver (see Table 3-6). Capacity-limited reactions in bone marrow and liver include benzene to benzene oxide, phenol to hydroquinone, phenol to catechol, catechol to trihydroxybenzene, and conjugation of phenol and hydroquinone. First-order reactions in liver include conversion of benzene oxide to phenol, muconic acid, and phenylmercapturic acid. The model simulates rates of formation of metabolites, tissue distribution of benzene oxide, phenol, and hydroquinone; and first-order excretion of metabolites in urine. Capacity-limited metabolism parameter values were estimated from *in vitro* studies of mouse liver (Lovern et al. 1999; Nedelcheva et al. 1999; Seaton et al. 1995); first-order parameters were estimated by optimization of model output to observations of metabolites formed in mice exposed by inhalation or to single gavage doses (Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988). Blood:tissue partition coefficients for benzene and metabolites were derived from Medinsky et al. (1989a) or estimated based on the n-octanol-water partition coefficient (Poulin and Krishnan 1995).

Risk Assessment. This model has been used to predict amounts of benzene exhaled and amounts of benzene metabolites produced in mice during inhalation exposures or following oral gavage exposures to benzene (Cole et al. 2001).

Validation of the Model. The model was calibrated with observations made in mice exposed to single oral gavage doses of benzene, or to inhalation exposures (Cole et al. 2001; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988). Further evaluations against data not used in the calibration have not been reported.

3. HEALTH EFFECTS

Target Tissues. The model simulates amounts and concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly perfused and slowly perfused tissues; rates of formation of metabolites; tissue distribution of benzene oxide, phenol, and hydroquinone; and first-order excretion of metabolites in urine. It does not simulate concentrations of metabolites in bone marrow, a target tissue for benzene metabolites.

Species Extrapolation. The model has been applied to simulations for mice (Cole et al. 2001).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in mice (50 ppm) and oral gavage doses ranging from 0.1 to 100 mg/kg (Cole et al. 2001; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988).

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes (Cole et al. 2001).

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested benzene, including rates and amounts of major metabolites. Most of the metabolism parameter values were derived empirically from *in vitro* studies, rather than by model optimization. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) the model does not simulate the metabolism of benzene in bone marrow, a major target of benzene toxicity; (3) the model, as configured in Cole et al. (2001), does not simulate benzene disposition in humans.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Benzene is readily absorbed via all natural routes of exposure (inhalation, oral, and dermal) and distributed throughout the body via the blood. Based on physical properties such as slight water solubility, high lipid solubility, and nonpolarity, benzene is expected to enter the blood via passive diffusion from gut, lungs, and skin. Benzene is expected to readily bind to plasma proteins (Travis and Bowers 1989). Being moderately lipophilic, benzene tends to accumulate in fatty tissues. However, benzene metabolism is relatively rapid and required for hematopoietic and leukemogenic effects to be expressed. Multiple reactive metabolites appear to be involved in benzene toxicity. As discussed in detail in Section 3.5.2, potential candidates include benzene oxide, phenolic metabolites (phenol,

3. HEALTH EFFECTS

catechol, hydroquinone, 1,2,4-benzenetriol, and 1,2- and 1,4-benzoquinone), and *trans,trans*-muconaldehyde. Both human and animal data demonstrate the importance of CYP2E1 in benzene metabolism (see Section 3.4.3 for a detailed discussion). Metabolism is assumed to take place primarily in the liver, with some secondary metabolism in the bone marrow, the site of characteristic benzene toxicity. Processes involved in transport of hepatic metabolites of benzene to the critical toxicity target (bone marrow) are not known, although some degree of covalent binding of reactive benzene metabolites to blood proteins is expected. At relatively low exposure levels, urinary excretion of conjugated benzene derivatives represents the major excretory pathway for benzene. Biliary excretion represents a minor excretory pathway.

3.5.2 Mechanisms of Toxicity

Numerous mechanistic studies have been conducted in an effort to elucidate mechanisms of benzene-induced hematotoxic and leukemogenic effects, widely recognized as the most critical effects of benzene exposure. Conversely, benzene-induced effects on reproduction, development, and the nervous system have not been studied in sufficient detail to assess mechanisms of toxicity for these end points. The database of information for benzene-induced hematotoxic and leukemogenic effects has been reviewed extensively (e.g., Bird et al. 2005; Irons 2000; Morgan and Alvares 2005; Ross 1996, 2000, 2005; Schnatter et al. 2005; Smith 1996a, 1996b; Snyder 2000a, 2000b, 2002; Snyder and Hedli 1996; Snyder and Kalf 1994). It is generally believed that reactive hepatic metabolites of benzene are transported to the major toxicity target (bone marrow). Additional metabolism likely occurs in bone marrow. Phenolic metabolites (phenol, hydroquinone, catechol, 1,2,4-benzenetriol, and 1,2- and 1,4-benzoquinone) appear to play a major role in benzene toxicity. Smith (1996a, 1999b) noted that the phenolic metabolites can be metabolized by bone marrow peroxidases, such as myeloperoxidase (MPO), to highly reactive semiquinone radicals and quinones that stimulate the production of reactive oxygen species. These steps lead to damage to tubulin, histone proteins, topoisomerase II, other DNA associated proteins, and DNA itself (clastogenic effects such as strand breakage, mitotic recombination, chromosome translocations, and aneuploidy). Damage to stem or early progenitor cells would be expressed as hematopoietic and leukemogenic effects.

Results of several mechanistic studies demonstrate that benzene hematotoxicity is dependent upon metabolism (see Section 3.4.3 for a detailed discussion). Inhibition of benzene metabolism reduced its toxicity (Andrews et al. 1977). Partial hepatectomy decreased both benzene metabolism and toxicity (Sammatt et al. 1979). Pretreatment with inducers of metabolism increased both benzene metabolism and

3. HEALTH EFFECTS

toxicity (Gad-El-Karim et al. 1985, 1986). Inhibition of CYPs (enzymes that catalyze oxidation pathways in benzene metabolism) reduced benzene-induced genotoxicity (Tuo et al. 1996). Mice lacking CYP2E1 or microsomal epoxide hydrolase expression were not susceptible to benzene levels known to cause myelotoxicity and cytotoxicity in wild type mice (Bauer et al. 2003; Valentine et al. 1996a, 1996b). Occupationally-exposed workers with a phenotype corresponding to rapid CYP2E1 metabolism were more susceptible to benzene toxicity than those expressing slow CYP2E1 metabolism (Rothman et al. 1997). The enzyme NAD(P)H:quinone oxidoreductase (NQO1), which maintains quinones in reduced form where they are more readily conjugated and excreted (Nebert et al. 2002), is another example of the importance of metabolism in benzene hematotoxicity. Between 22% (Caucasian) and 45% (Asian) of the population is homozygous for an NQO1 allele whereby NQO1 production is negligible. Rothman et al. (1997) found that workers homozygous for an NQO1 allele whereby NQO1 production is negligible (wild type) exhibited a 2.4-fold increased risk for benzene hematotoxicity than workers with the normal genotype. Greater than 7-fold increased risk of benzene hematotoxicity was noted in workers who expressed both rapid CYP2E1 metabolism and the NQO1 wild type (Rothman et al. 1997).

Benzene metabolism involves the production of reactive metabolites that may act directly on cellular macromolecules (proteins and DNA). No single metabolite has been implicated; effects are probably due to many metabolites, which include benzene oxide, reactive products of the phenol pathway (catechol, hydroquinone, and 1,4-benzoquinone), and *trans,trans*-muconaldehyde. Evidence that benzene oxide may play a role in benzene toxicity includes findings that benzene oxide is a product of oxidative benzene metabolism in mouse, rat, and human liver microsomes (Lovern et al. 1997), benzene oxide can be released from the liver into the blood (Lindstrom et al. 1997), benzene oxide-protein adducts have been found in the blood and bone marrow of mice exposed to benzene (McDonald et al. 1994), and benzene oxide hemoglobin and albumin adducts have been detected in the blood of workers exposed to benzene (Rappaport et al. 2002a, 2002b; Yeowell-O'Connell et al. 1998).

Urinary *trans,trans*-muconic acid has been detected in humans and animals following benzene exposure, although its purported reactive precursor (*trans,trans*-muconaldehyde; see Figure 3-3) has not been detected *in vivo*. The highly reactive *trans,trans*-muconaldehyde, which has been found in mouse hepatic microsomes (Latriano et al. 1986), can undergo reductive and oxidative metabolism (Goon et al. 1992) and has been shown to be hematotoxic (Witz et al. 1985). A small amount (<0.05%) of parenterally-administered *trans,trans*-muconaldehyde to mice reached the bone marrow (Zhang et al. 1997). Rivedal and Witz (2005) found *trans,trans*-muconaldehyde to be a strong inhibitor of gap junction intercellular communication in rat liver epithelial cells.

3. HEALTH EFFECTS

Phenolics (phenol, catechol, hydroquinone; 1,2,4-benzenetriol; 1,2- and 1,4-benzoquinone) are major metabolites of benzene that have been shown to persist in bone marrow following inhalation exposure (Rickert et al. 1979; Sabourin et al. 1988). Hydroquinone induces chromosomal damage in lymphocytes *in vitro* in a manner similar to that observed in lymphocytes of benzene-exposed workers (Eastmond et al. 1994; Stillman et al. 1997; Zhang et al. 1998b). Kolachana et al. (1993) demonstrated that both hydroquinone and 1,2,4-benzenetriol cause oxidative damage to DNA in mouse bone marrow (*in vivo*) and human myeloid cells (*in vitro*). Glutathione adducts of 1,4-benzoquinone have been shown to be hematotoxic in bone marrow of mice exposed to benzene (Bratton et al. 1997). Additional evidence that phenolic metabolites may play an important role in benzene toxicity includes the finding that MPO, an enzyme found in high concentration in bone marrow (Bainton et al. 1971), catalyzes the oxidation of polyphenols to reactive quinones, semiquinones, and oxygen radicals (Nebert et al. 2002). This can lead to strand breaks and inhibition of topoisomerases and microtubule assembly, which could result in chromosome damage (Chen and Eastmond 1995; Eastmond et al. 2001; Irons and Neptune 1980; Smith 1996a, 1996b). Eastmond et al. (2005) demonstrated that hydroquinone can be readily activated to a potent topoisomerase II inhibitor in the presence of human MPO and H₂O₂ and that partial inhibition occurs at hydroquinone concentrations as low as 50 nM. Irons and Neptune (1980) suggested that benzene-derived hydroquinone may inhibit cell replication by covalently binding to tubulin, a protein essential for spindle formation in mitosis.

Benzene-induced effects on DNA have been studied in some detail. Schwartz et al. (1985) demonstrated that benzene metabolites inhibit mitochondrial DNA polymerase. Hydroquinone has been shown to inhibit ribonucleotide reductase, a key step in DNA synthesis (Li et al. 1997, 1998). Rushmore et al. (1984) found that benzene metabolites covalently bind to mitochondrial DNA and inhibit RNA synthesis. Benzene metabolites bound to hepatic DNA have been observed in animals following inhalation exposure to radiolabeled benzene (Lutz and Schlatter 1977). However, the reported levels of DNA adduct formation appear to be low. For example, Creek et al. (1997) observed adducts to both protein and DNA in the range of nanograms per kilogram in mice given radiolabeled benzene.

Benzene-induced DNA damage may result from the oxidation of DNA by reactive oxygen species that are produced during benzene metabolism. Both 1,4-benzoquinone and hydroquinone are known to increase superoxide, nitric oxide, and hydrogen peroxide in HL-60 cells (Rao and Snyder 1995). Chen et al. (2004) observed nitric oxide-derived benzene metabolites, namely nitrobenzene, nitrophenyl, and nitrophenol isomers in the bone marrow of mice 1 hour following intraperitoneal injection of a 400 mg/kg

3. HEALTH EFFECTS

dose of benzene. These nitro metabolites were either not detected in other tissues or were present in much smaller concentrations, indicating that they were most likely produced in bone marrow. Brunmark and Cadenas (1988) described a metabolic pathway from hydroquinone leading to the production of glutathionyl-benzenetriol, which can undergo autoxidation leading to superoxide formation. Rao (1996) suggested that benzene-induced DNA damage is mediated by the release of free iron in the bone marrow (probably by polyphenolic metabolites), followed by the chelation of iron by hydroquinone or benzenetriol to yield a reactive oxygen-generating species such as superoxide, which causes oxidative damage to DNA.

The expression of benzene toxicity may involve multiple benzene metabolites. All of the known unconjugated metabolites of benzene, with the exception of phenol and 1,2,4-benzenetriol, have been shown to decrease erythropoiesis (Snyder and Hedli 1996). In mice, the combination of phenol and hydroquinone resulted in exacerbated loss of bone marrow cellularity (Eastmond et al. 1987), increased peroxidatic activation of hydroquinone (Subrahmanyam et al. 1989, 1990), and increased DNA damage (Lévay and Bodell 1992; Marrazzini et al. 1994). Combinations of either phenol and hydroquinone, or phenol and catechol, were more hematotoxic than any of the metabolites given alone (Guy et al. 1991). The combination of hydroquinone and muconaldehyde was the most potent in inhibiting erythropoiesis (Snyder et al. 1989). Catechol was found to stimulate the peroxidase-mediated activation of hydroquinone and produced a synergistic genotoxic effect in lymphocytes (Robertson et al. 1991).

3.5.3 Animal-to-Human Extrapolations

Pathways of benzene metabolism are generally similar among various rodent and nonhuman primate species. However, species differences exist regarding capacity to metabolize benzene and relative proportions of various benzene metabolites formed.

Species differences exist in absorption and retention of benzene. For example, following 6-hour exposures to low concentrations (7–10 ppm) of benzene vapors, mice retained 20% of the inhaled benzene, whereas rats and monkeys retained only 3–4% (Sabourin et al. 1987, 1992). Mice exhibit a greater overall capacity to metabolize benzene, compared to rats. An Inhalation exposure to 925 ppm resulted in an internal dose of 152 mg/kg in mice, approximately 15% of which was excreted as parent compound, and an internal dose of 116 mg/kg in rats, approximately 50% of which was excreted unchanged (Henderson et al. 1992; Sabourin et al. 1987).

3. HEALTH EFFECTS

The proportions of benzene metabolites produced depend on both species and exposure concentration. Hydroquinones and muconic acid (potential sources of benzene toxicity) were detected in much higher concentrations in the blood, liver, lung, and bone marrow of mice than rats, following a 6-hour inhalation exposure to benzene at a concentration of 50 ppm (Sabourin et al. 1988). It is generally understood that metabolic profiles of benzene in mice and humans are more similar than those of humans and rats. Sabourin et al. (1989a) noted increased production of detoxification metabolites (phenylglucuronide and prephenylmercapturic acid) and decreased production of potentially toxic metabolites (hydroquinones and muconic acid) in both mice and rats exposed to benzene at much higher concentrations (600 ppm in air or 200 mg/kg orally), which indicates that extrapolation of toxicological results from studies using high exposure concentrations to low exposure scenarios may result in an underestimation of risk.

Recent PBPK models have tried to address benzene metabolism in an effort to derive animal-to-human extrapolations (Bois et al. 1991a, 1996; Cole et al. 2001; Medinsky 1995; Medinsky et al. 1989a, 1989b, 1989c; Travis et al. 1990). Each model described a multicompartamental model that attempted to relate the generation of metabolites to end points of benzene toxicity. The generation of hydroquinone and muconaldehyde in the liver, with further metabolism in the bone marrow, has been addressed as well as the available data allow. However, the models are not sufficiently refined to allow them to accurately predict human metabolism.

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

3. HEALTH EFFECTS

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 information was located to indicate that benzene may adversely affect the endocrine system.

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

3. HEALTH EFFECTS

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 clear evidence of age-related differences in susceptibility to benzene toxicity was located. Benzene crosses the placenta and can be found in cord blood at concentrations that equal or exceed those of maternal blood (Dowty et al. 1976). Nursing infants can be exposed to benzene in the breast milk (Fabietti et al. 2004). Limited animal studies indicate that *in utero* exposure to benzene results in hematological changes similar to those observed in animals exposed only as adults (Corti and Snyder 1996; Keller and Snyder 1986, 1988). There is some indication that parental occupational exposure to benzene may play a role in childhood leukemia (Buckley et al. 1989; McKinney et al. 1991; Shaw et al.

3. HEALTH EFFECTS

1984; Shu et al. 1988). However, none of these studies indicate whether children may be at greater risk than adults for benzene toxicity. Results of Infante-Rivard et al. (2005) indicate that maternal exposure to benzene during pregnancy or from 2 years before pregnancy up to birth does not result in increased risk of childhood acute lymphoblastic leukemia (ALL), a frequent form of childhood cancer (Infante-Rivard et al. 2005). However, no information was located regarding the risk of childhood AML, the form of leukemia most frequently associated with exposure to benzene.

Children could potentially be at increased risk for significant benzene exposure via the inhalation route based on higher activity levels and ventilation rates than adults. However, no information was located to indicate that children are at increased risk for benzene toxicity. Age-related differences in benzene metabolism could potentially affect susceptibility. Results of one human study indicate that CYP2E1, a major enzyme involved in benzene metabolism, is not present in the fetus, but appears in rapidly increasing concentrations during early postnatal development (Vieira et al. 1996). This suggests that fetuses and neonates may be at decreased risk of benzene toxicity due to a reduced metabolic capacity. No information was located regarding potential age-related differences in pharmacodynamic processes such as benzene-target interactions in the hematopoietic system.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the

3. HEALTH EFFECTS

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 benzene 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 benzene 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 Benzene

Several biomarkers of exposure to benzene have been reported in the literature. Unmetabolized benzene can be detected in the expired air and urine of humans exposed to benzene vapors (Farmer et al. 2005; Fustinoni et al. 2005; Ghittori et al. 1993; Nomiyama and Nomiyama 1974a, 1974b; Sherwood 1988; Srbova et al. 1950; Waidyanatha et al. 2001). Urinary phenol measurements have routinely been used for monitoring occupational exposure to benzene (OSHA 1987), and urinary phenol levels appear to be correlated with exposure levels (Astier 1992; Inoue et al. 1986, 1988b; Karacic et al. 1987; Pagnotto et al. 1961; Pekari et al. 1992).

Urinary *trans,trans*-muconic acid has been widely studied as a biomarker of exposure to benzene (Boogaard and van Sittert 1995, 1996; Ducos et al. 1990, 1992; Inoue et al. 1989b, 2000; Lee et al. 1993; Melikian et al. 1993, 1994; Pezzagno et al. 1999; Popp et al. 1994; Qu et al. 2005; Rothman et al. 1998; Ruppert et al. 1997; Sanguinetti et al. 2001; van Sittert et al. 1993; Weaver et al. 2000). Urinary *S*-phenylmercapturic acid levels have also been correlated with occupational exposure to benzene

3. HEALTH EFFECTS

(Boogaard and van Sittert 1995, 1996; Farmer et al. 2005; Inoue et al. 2000; Jongeneelen et al. 1987; Popp et al. 1994; Qu et al. 2005). Significant exposure-response trends for urinary *trans,trans*-muconic acid and *S*-phenylmercapturic acid levels have been demonstrated in occupationally-exposed subjects at exposure levels of ≤ 1 ppm (Qu et al. 2005). The American Conference of Governmental Industrial Hygienists (ACGIH) has established 25 μg *S*-phenylmercapturic acid/g creatinine in the urine and 500 μg *trans,trans*-muconic acid/g creatinine in the urine as Biological Exposure Indices (BEIs) for benzene exposure in the workplace (ACGIH 2006). The BEI is primarily an index of exposure and not a level at which health effects might occur from exposure to benzene. Positive correlations have been made between benzene workplace air levels and urinary catechol and hydroquinone in exposed workers (Inoue et al. 1988a, 1988b; Rothman et al. 1998).

Hemoglobin and albumin adducts of the benzene metabolites, benzene oxide and 1,4-benzoquinone, have been used as biomarkers of exposure to benzene (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Smith and Rothman 2000; Yeowell-O'Connell et al. 1998, 2001). Furthermore, DNA adducts with benzene metabolites have been found after benzene exposure (Hedli et al. 1991; Lutz and Schlatter 1977; Reddy et al. 1989).

Ong et al. (1995) evaluated various biomarkers of benzene exposure for their relationship with environmental benzene levels. Muconic acid in the urine correlated best with environmental benzene concentrations. Urinary hydroquinone levels were the most accurate biomarker of exposure for the phenolic metabolites of benzene, followed by phenol and catechol. No correlation was found between environmental benzene levels and unmetabolized benzene in the urine, although other studies suggest that benzene in the urine may be a useful biomarker of occupational exposure (Ghittori et al. 1993).

The biomarkers discussed in the preceding paragraphs appear to be adequate indicators of exposure to benzene at relatively high occupational exposure levels, and may serve as biomarkers in acute exposure scenarios involving relatively high levels of benzene. However, some of these biomarkers do not appear to be reliable indicators of environmental exposure to benzene (concentrations below the common industrial standard of 1 ppm TWA). For example, results from data collected on 152 chemical workers showed a linear relationship between the concentration of benzene in breathing zone air (when greater than 10 ppm) and urinary concentrations of catechol and hydroquinone (Inoue et al. 1988a). Workers who had an average work-site exposure of 10 ppm benzene showed no significant differences in the concentration of urinary catechol or hydroquinone when compared to a group of unexposed subjects. In a study of pharmacy workers exposed to benzene levels measured in the parts per billion (ppb) range, there

3. HEALTH EFFECTS

was no significant difference in urinary levels of *trans,trans*-muconic acid between subjects exposed to 1.5 ppb and those exposed to 2.5 ppb (Sanguinetti et al. 2001). Recent reports indicate that urinary benzene may serve as the most sensitive biomarker of exposure to benzene concentrations well below 1 ppm (Farmer et al. 2005; Fustinoni et al. 2005).

Several additional factors must be taken into account when assessing the reliability of biomarkers of exposure to benzene. High and variable background levels of phenol and its metabolites result from ingestion of vegetables, exposure to other aromatic compounds, ingestion of ethanol, and inhalation of cigarette smoke (Nakajima et al. 1987). Relatively high urinary phenol levels (5–42 mg/L) have been found in persons with no known exposure to benzene (NIOSH 1974). Although muconic acid is used as a marker for benzene exposure, muconic acid in the urine can also result from ingestion of sorbic acid, a common food preservative (Ducos et al. 1990). Inoue et al. (1989b) suggested that individual urinary *trans,trans*-muconic acid content was not a useful index of benzene exposure due to large variations in measured individual background urinary *trans,trans*-muconic acid values.

In summary, several benzene metabolites may serve as biomarkers of exposure to benzene. Urinary benzene appears to be the most sensitive biomarker for low-level exposure to benzene. Refer to Tables 7-1 and 7-2 for information regarding analytical methods for determining benzene in biological samples and benzene metabolites in urine.

3.8.2 Biomarkers Used to Characterize Effects Caused by Benzene

In addition to using levels of benzene and benzene metabolites for monitoring purposes, various biological indices might also be helpful in characterizing the effects of exposure to benzene. As with monitoring for benzene exposure, monitoring for effects may best be accomplished through the use of a series of biomarkers with correlation of the results. Decreases in erythrocyte and leukocyte counts have been used as an indicator of high occupational exposures. Monitoring of benzene workers has included monthly blood counts, with workers being removed from areas of high benzene exposure when leukocyte counts fell below 4,000/mm³ or erythrocyte counts fell below 4,000,000/mm³ (ITII 1975; OSHA 1987). Hayes (1992) indicates that benzene-related leukopenia, commonly thought of as an intermediate end point in the process of developing benzene-related leukemia, is considered a biomarker of benzene poisoning in China, not necessarily related to leukemia. Leukocyte alkaline phosphatase (LAP) activity was increased in benzene workers exposed to about 31 ppm for a chronic time period (Songnian et al. 1982). Increased LAP activity is an indicator of myelofibrosis and is associated with both decreased

3. HEALTH EFFECTS

white blood cell counts and changes in bone marrow activity. The change in LAP activity could be used in the diagnosis of benzene poisoning since it was more sensitive than the change in the leukocyte count, although it is not a biomarker that is specific for benzene. Additionally, it seems reasonable that chromosomal aberrations in bone marrow and peripheral blood lymphocytes and sister chromatid exchanges could be used to monitor for benzene effects (Eastmond et al. 1994; Van Sittert and de Jong 1985). Benzene metabolites have also been found to form adducts with DNA (Chenna et al. 1995; Lutz and Schlatter 1977; Norpoth et al. 1988; Rushmore et al. 1984; Snyder et al. 1987).

Exposure to benzene causes toxic effects in the bone marrow via its metabolites and possibly by benzene via solvent effects (Eastmond et al. 1987; Gad-El-Karim et al. 1985; Hedli et al. 1991; Irons et al. 1980). Therefore, it is possible that hematological tests could be used as markers of hematotoxicity. To date, surveillance and early diagnosis of benzene hematotoxicity rely primarily on the complete blood count, including hemoglobin, hematocrit, erythrocyte count, leukocyte count, and differential and platelet counts. In effect, complete blood counts and marrow exams should be good for early detection of preleukemic lesions. Additionally, cytogenetic tests of marrow cells are being used. Workers exposed to benzene in the air have shown elevated levels of delta-aminolevulinic acid in erythrocytes and elevated coproporphyrin in the urine (Kahn and Muzyka 1973). These may be biomarkers for disruption of porphyrin synthesis and may be early indicators of adverse hematological effects. These effects are not specific for benzene. Hedli et al. (1991) observed that in rats, benzene metabolite-DNA adducts were observed in the bone marrow at doses that did not affect bone marrow cellularity, and suggested that monitoring of the bone marrow DNA adducts might be a sensitive bioassay of genotoxic effects of benzene exposure. Work by other researchers also suggests that monitoring DNA adducts or products of DNA damage might be useful (Bodell et al. 1993; Chen et al. 1994; Lagorio et al. 1994a; Reddy et al. 1989).

3.9 INTERACTIONS WITH OTHER CHEMICALS

Studies have been conducted on the interaction of benzene with other chemicals, both *in vivo* and in the environment. Benzene metabolism is complex, and various xenobiotics can induce or inhibit specific routes of detoxification and/or activation in addition to altering the rate of benzene metabolism and clearance from the body. Toluene, Aroclor 1254, phenobarbital, acetone, and ethanol are known to alter the metabolism and toxicity of benzene. Interactions reported in *in vivo* studies occurred at relatively high benzene exposure levels, which would not likely be encountered near hazardous waste sites.

3. HEALTH EFFECTS

Ethanol and benzene induce the formation of the hepatic cytochrome P-450 isoenzyme, CYP2E1, in rabbits and rats (Gut et al. 1993; Johansson and Ingelman-Sundberg 1988), although benzene derivatives, such as toluene and xylene, can inhibit the enzymatic activity of the isozyme (Koop and Laethem 1992). Ethanol enhances both the metabolism (*in vitro*) and the toxicity (*in vivo*) of benzene in animals (Baarson et al. 1982; Nakajima et al. 1985). Administration of ethanol (5 or 15% in drinking water, 4 days/week for 13 weeks) to mice exposed to benzene vapors at a concentration of 300 ppm, 6 hours/day, 5 days/week for 13 weeks) resulted in greater severity of benzene-induced hematological effects (anemia, lymphocytopenia, bone marrow aplasia, transient increases in normoblasts and peripheral blood atypia) relative to benzene-exposed mice not given ethanol (Baarson et al. 1982). The modulating effects of benzene were dose dependent. The enhancement of the hematotoxic effects of benzene by ethanol may be of particular concern for benzene-exposed workers who consume alcohol (Nakajima et al. 1985), although the interactions demonstrated in the mice occurred at much higher benzene exposure concentrations than would likely be experienced in workplace air. Benzene can interfere with the disappearance of ethanol from the body. Accordingly, increased central nervous system disturbances (e.g., depression) may occur following concurrent exposure to high levels of benzene and ethanol.

Other chemicals that induce specific isoenzymes of cytochrome P-450 can increase the rate of benzene metabolism and may alter metabolism pathways favoring one over another. Ikeda and Ohtsuji (1971) presented evidence that benzene hydroxylation was stimulated when rats were pretreated with phenobarbital and then exposed to 1,000 ppm of benzene vapor for 8 hours/day for 2 weeks. Additionally, phenobarbital pretreatment increased the rate of metabolism by 40% in rats and 70% in mice (Pawar and Mungikar 1975). In contrast, rats exposed to phenobarbital showed no effects on the metabolism of micromolar amounts (35–112.8 μmol) of benzene *in vitro* (Nakajima et al. 1985).

Co-administration of toluene inhibited the biotransformation of benzene to phenol in rats (Ikeda et al. 1972; Inoue et al. 1988b). This was due to competitive inhibition of the oxidation mechanisms involved in the metabolism of benzene. Phenobarbital pretreatment of the rats alleviated the suppressive effect of toluene on benzene hydroxylation by the induction of oxidative activities in the liver. This effect has been observed in other studies in rats (Purcell et al. 1990).

Mathematical models of benzene and phenol metabolism suggest that the inhibition by benzene of phenol metabolism, and by phenol on benzene metabolism, occurs through competition for a common reaction site, which can also bind catechol and hydroquinone (Schlosser et al. 1993). Flavonoids have been shown

3. HEALTH EFFECTS

to inhibit phenol hydroxylase or increase phenol hydroxylase activity in a dose-dependent manner, dependent on the oxidation potential of the flavonoid (Hendrickson et al. 1994).

SKF-525A and carbon monoxide are classic inhibitors of cytochrome P-450s. The binding between P-450 and carbon monoxide or SKF-525A is coordinate covalent. Carbon monoxide inhibits all cytochrome P-450 isoenzymes since it binds to the heme component of cytochrome P-450, whereas SKF-525A inhibits specific types. SKF-525A inhibited benzene metabolism in the rat (Ikeda et al. 1972). Injection of 80 mg/kg of SKF-525A in rats resulted in a depression of phenol excretion. It also prolonged phenol excretion and interfered in the conversion of benzene to glucuronides and free phenols. Carbon monoxide, aniline, aminopyrine, cytochrome C, and metyrapone inhibited benzene metabolism *in vitro* by mouse liver microsomes (Gonasun et al. 1973).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

Variability in human susceptibility to benzene toxicity may be related, at least in part, to genetic polymorphisms associated with metabolic processes. As discussed in Section 3.4.3, the flavoenzyme, NAD(P)H:quinone oxidoreductase (NQ01), catalyzes the reduction of 1,2- and 1,4-benzoquinone (reactive metabolites of benzene) to catechol and hydroquinone, respectively (Nebert et al. 2002), thus protecting cells from oxidative damage by preventing redox cycling. The NQ01*1 (wild type) allele codes for normal NQ01 enzyme and activity. An NQ01*2 allele encodes a nonsynonymous mutation that has negligible NQ01 activity. Approximately 5% of Caucasians and African Americans, 15% of Mexican-Americans, and 20% of Asians are homozygous for the NQ01*2 allele (Kelsey et al. 1997; Smith and Zhang 1998). Rothman et al. (1997) demonstrated that workers expressing negligible NQ01 activity were at increased risk of benzene poisoning (Rothman et al. 1997). In the same group of workers, those expressing rapid CYP2E1 activity were also at increased risk of benzene poisoning. Those workers with polymorphisms for both negligible NQ01 activity and rapid CYP2E1 activity exhibited greater than 7-fold increased risk of benzene poisoning than workers not expressing these polymorphisms. These

3. HEALTH EFFECTS

results indicate that individuals expressing rapid CYP2E1 activity may also be at increased risk for benzene toxicity.

Polymorphisms have been described for many of the glutathione genes (Cotton et al. 2000; Hengstler et al. 1998; Strange and Fryer 1999), the myeloperoxidase gene (Williams 2001), and the epoxide hydrolase gene (Omiecinski et al. 2000), which are known to be involved in benzene metabolism. However, the potential involvement of such polymorphisms in benzene toxicity have not been demonstrated in benzene-exposed workers.

Individuals with medical conditions that include reduced bone marrow function or decreased blood factors would be at increased risk for benzene toxicity. Treatments for certain medical conditions might result in decreases in particular blood factors, which could lead to increased susceptibility to benzene poisoning.

Ethanol can increase the severity of benzene-induced anemia, lymphocytopenia, and reduction in bone marrow cellularity, and produce transient increases in normoblasts in the peripheral blood and atypical cellular morphology (Baarson et al. 1982). The enhancement of the hematotoxic effects of benzene by ethanol is of particular concern for benzene-exposed workers who consume alcohol (Nakajima et al. 1985). Accordingly, increased central nervous system disturbances (e.g., depression) may be expected following concurrent exposure to benzene and ethanol.

Gender-related differences in susceptibility to benzene toxicity have been observed in animals. For example, Kenyon et al. (1998) exposed male and female mice to benzene vapor concentrations of 100 or 600 ppm and found increased benzene metabolism and associated genotoxicity in males, relative to females. Brown et al. (1998) used a PBPK modeling approach to assess potential gender-related differences in susceptibility to benzene. Their results suggest that women exhibit a higher blood/air partition coefficient and maximum velocity of benzene metabolism than men, and that women metabolize 23-26% more benzene than men under similar exposure scenarios. However, gender-related differences in susceptibility among benzene-exposed workers were not located in available reports.

At early stages of human development, metabolic pathways may not be fully functional, which might result in a lower level of susceptibility. Young children might experience increased susceptibility to benzene by inhalation due to increased breathing rates and potential for increased absorption, relative to

3. HEALTH EFFECTS

adults. However, no definitive human or animal data were located regarding age-related differences in susceptibility to benzene.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to benzene. 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 benzene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to benzene:

Goldfrank LR, Flomenbaum NE, Lewin NA, et al., eds. 1998. Goldfrank's toxicologic emergencies. 6th ed. Stamford: Appleton & Lange, 1384-1398.

Haddad LM, Shannon MW, Winchester JF. 1998. Clinical management of poisoning and drug overdose. 3rd ed. Philadelphia, PA: W.B Saunders Company, 940-941.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to benzene can occur by inhalation, oral, or dermal routes. General recommendations for reducing absorption of benzene following acute high-level inhalation exposure have included moving the patient to fresh air and monitoring for respiratory distress (Bronstein and Currance 1988; Haddad et al. 1998; HSDB 2007; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). The administration of 100% humidified supplemental oxygen with assisted ventilation, when required, has also been suggested (Bronstein and Currance 1988; Haddad et al. 1998; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). In the case of eye exposure, irrigation with copious amounts of water or saline has been recommended (Bronstein and Currance 1988; Haddad et al. 1998; HSDB 2007; Stutz and Janusz 1988). The removal of contaminated clothing and a thorough washing of exposed areas with soap and water have also been recommended (Bronstein and Currance 1988; Ellenhorn and Barceloux 1988; Haddad et al. 1998; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). Some sources suggest the administration of water or milk to the victim after ingestion of benzene (Stutz and Janusz 1988). Emesis may be indicated following recent substantial ingestion of benzene (Ellenhorn and Barceloux 1988); however, other sources do not recommend the use of emetics because of the risk of aspiration pneumonitis, especially once the patient loses consciousness (Bronstein and Currance 1988; Haddad et al. 1998). Some sources recommend gastric lavage if indicated (Haddad et al. 1998; Kunisaki and Augenstein 1994).

3. HEALTH EFFECTS

While lavage may be useful, induction of emesis should be regarded with great caution because the rapid onset of central nervous system depression may lead to aspiration. Although of unproven value, administration of a charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, has also been suggested as a way to stimulate fecal excretion of the chemical before it is completely absorbed by the body (HSDB 2007; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). Diazepam and phenytoin may be helpful in controlling seizures (Bronstein and Currance 1988; HSDB 2007; Stutz and Janusz 1988). Administration of epinephrine or other catecholamines has not been recommended because of the possibility of myocardial sensitization and subsequent arrhythmia (Haddad et al. 1998; HSDB 2007; Nahum and Hoff 1934).

3.11.2 Reducing Body Burden

Following absorption into the blood, benzene is rapidly distributed throughout the body. Since benzene is lipophilic, it is preferentially distributed to lipid-rich tissues. The initial stage of benzene metabolism is the formation of benzene oxide via P-450 mixed-function oxidases. Detoxification pathways generally involve the formation of glutathione conjugates of benzene oxide and glucuronide or sulfate conjugates of phenol or its subsequent metabolites, catechol, hydroquinone, and trihydroxybenzene. Other metabolites of benzene also have known toxic effects. Exhalation is the main route for excretion of unmetabolized benzene, while metabolized benzene is excreted primarily in the urine. Studies in humans and animals indicate that both exhalation and urinary excretion occur in several phases, with half-lives of minutes to hours. Hence, benzene and its metabolites have relatively short half-lives in the body, and while some of these metabolites are clearly toxic, accumulation of substantial body burdens are not expected.

No methods are currently used for reducing the body burden of benzene. It is possible that methods could be developed to enhance the detoxification and elimination pathways, such as ensuring sufficient glutathione stores in the body by the administration of N-acetyl-L-cysteine.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Administration of indomethacin, a nonsteroidal anti-inflammatory drug, has been shown to prevent benzene-induced myelotoxicity in mice and the accompanying increase in prostaglandin E in the bone marrow (Kalf et al. 1989; Renz and Kalf 1991). Co-administration of indomethacin also prevented an increase in the number of micronucleated polychromatic erythrocytes in peripheral blood. The authors suggest that these results suggest a role for prostaglandin synthetase in benzene-induced myelotoxic and genotoxicity, and a way to interfere with that process with substances such as indomethacin.

3. HEALTH EFFECTS

Prostaglandins have been shown to inhibit hematopoiesis (Kalf et al. 1989). Additionally, prostaglandin synthetase could be involved in the oxidation of phenol and/or hydroquinone to toxic metabolites (Kalf et al. 1989).

The use of indomethacin to block benzene toxicity has led to data that indicate that myelotoxicity may involve the destruction of stromal macrophages that produce IL-1, a cytokine essential for hematopoiesis (Renz and Kalf 1991). External administration of recombinant IL-1 to mice prior to benzene administration prevents the myelotoxicity, presumably by providing a source of the cytokine (Renz and Kalf 1991). Further indication that IL-1 is affected by benzene exposure comes from the work of Carbonnelle et al. (1995), who showed that exposure of human monocytes to micromolar amounts of hydroquinone for 2 hours resulted in significantly decreased secretion of IL-1 α and IL-1 β at concentrations of 5 μ M and above. RNA and protein synthesis were also inhibited. Additional research in this area indicates that tumor necrosis factor may provide protection against the inhibitory effects of hydroquinone on human hematopoietic progenitor cells (Colinas et al. 1995). The research of Shankar et al. (1993) determined that pretreatment with Protein A, a glycoprotein that acts as a multipotent immunostimulant, modulated the toxicity of benzene. Groups of six female albino rats (Swiss Wistar) were injected intraperitoneally with 1.0 mL/kg/body weight (879 mg/kg) benzene once daily for 3 consecutive days. Another group (six per group) was administered intravenously with 60 μ g/kg Protein A twice weekly for 2 weeks and then injected with 879 mg/kg benzene intraperitoneally once daily for 3 consecutive days. Controls were injected with normal saline. All of the animals were killed 24 hours after receiving the last benzene injection. Blood was collected from the jugular vein for enumeration of total leukocyte counts. Routine autopsy was performed on all animals and the liver, thymus, spleen, and kidney organs were collected for organ weights. In benzene-only treated animals, there was a significant decrease in the total leukocyte counts in the peripheral blood as well as a significant decrease in the number of lymphocytes, a decrease in the gross organ weights of thymus and spleen, a significant increase in the iron content and lipid peroxidation of the liver and bone marrow, and an increase in low molecular weight iron in the bone marrow. Pretreatment with Protein A prevented these parameters from changing.

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 benzene is available. Where adequate information is not

3. HEALTH EFFECTS

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 benzene.

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 Benzene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to benzene are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of benzene. 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.

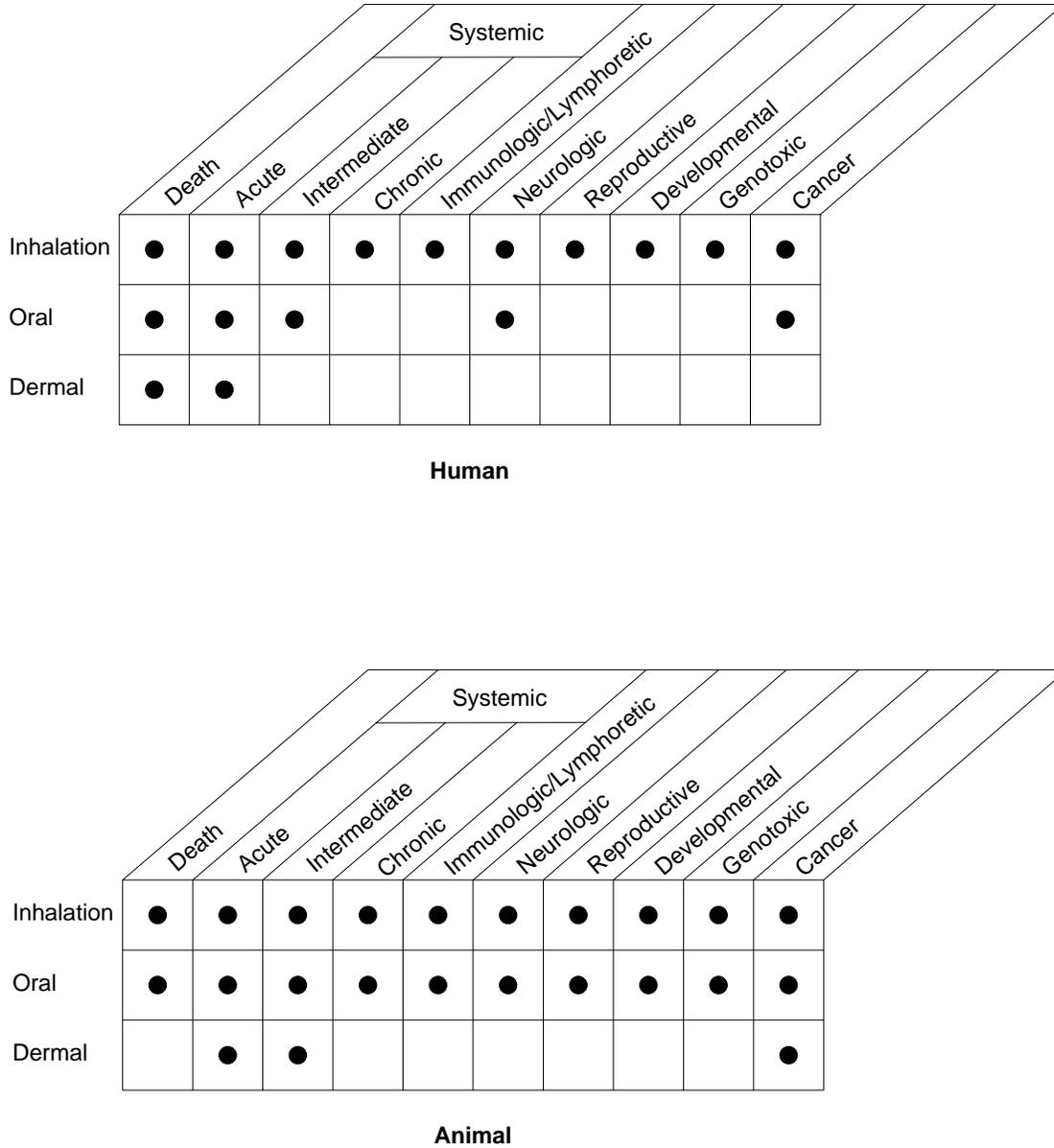
Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Virtually all of the information regarding health effects in humans comes from studies of workers exposed to benzene-containing solvents and/or adhesives. Exposures to benzene occurred at rotogravure printing shops; at shoe, rubber, and raincoat manufacturing plants; and during chemical manufacturing processes. Case reports and cohort studies describe both acute and chronic health effects. The predominant route of exposure in these studies is inhalation. Dermal contact is also suspected as a possible route of exposure in these studies.

As seen in Figure 3-6, inhalation information for humans is available regarding death; acute-, intermediate-, and chronic-duration systemic effects; immunologic, neurologic, reproductive, developmental, and genotoxic effects; and cancer. However, as mentioned above, human exposure to

3. HEALTH EFFECTS

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



● Existing Studies

3. HEALTH EFFECTS

benzene in specific work environments probably occurs not only by inhalation, but also by the dermal route. Limited information is available regarding direct skin contact with benzene by humans. Additionally, oral studies in humans are limited to isolated case reports of death and acute-duration systemic effects subsequent to accidental or intentional ingestion of benzene, although one study (Hunting et al. 1995) described effects in vehicle maintenance workers who siphoned gasoline by mouth. There is limited information on effects of dermal exposure of humans to benzene, including death, acute-duration effects, and cancer.

Inhalation and oral studies in animals provide data on death; systemic effects after acute-, intermediate-, and chronic-duration exposure; and immunologic, neurologic, reproductive, developmental, genotoxic, and cancer effects. Furthermore, data exist regarding acute- and intermediate-duration systemic effects and cancer in animals after dermal exposure to benzene.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. There are reports on the health effects resulting from acute exposure of humans and animals to benzene via the inhalation, oral, and dermal routes. The primary target organs for acute exposure are the hematopoietic system, nervous system, and immune system. Acute effects on the nervous system and immune system are discussed below under Neurotoxicity and Immunotoxicity. Information is also available for levels that cause death in humans (e.g., Cronin 1924; Flury 1928; Greenburg 1926; Tauber 1970; Thienes and Haley 1972) and in animals (e.g., Cornish and Ryan 1965; Drew and Fouts 1974; Smyth et al. 1962) following inhalation and oral exposures.

No acute human or animal data on hematological effects from oral or dermal exposure are available. However, there are acute inhalation data that characterize the effects of benzene on the hematological system in humans and animals. Data regarding effects on the human hematological system following acute inhalation exposure to benzene are scant, but indicate leukopenia, anemia, and thrombocytopenia after more than 2 days of occupational exposure to more than 60 ppm benzene (Midzenski et al. 1992). Data for hematological effects in animals after acute-duration inhalation exposure are more extensive. Changes in peripheral erythrocytes (Chertkov et al. 1992; Cronkite et al. 1985; Rozen et al. 1984; Ward et al. 1985), in peripheral leukocytes (Aoyama et al. 1986; Chertkov et al. 1992; Gill et al. 1980; Green et al. 1981b; Li et al. 1986; Ward et al. 1985; Wells and Nerland 1991), and in bone marrow cells (Chertkov et al. 1992; Corti and Snyder 1996; Cronkite et al. 1989; Dempster and Snyder 1991; Gill et al. 1980; Green et al. 1981b; Neun et al. 1992; Toft et al. 1982) were seen in rats and mice. An acute-duration inhalation

3. HEALTH EFFECTS

MRL of 0.009 ppm was determined based on the LOAEL for immunologic effects in the mouse (Rozen et al. 1984) (discussed below under Immunotoxicity). No acute-duration oral studies were suitable for deriving MRLs. Additional studies that include dose-response information on hematological effects following acute oral exposure could be designed to provide information that could be useful in deriving an acute-duration oral MRL for benzene. Such studies could be designed to serve as validation for existing PBPK models. Acute dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

Intermediate-Duration Exposure. There is sufficient information in humans and animals to identify the hematopoietic, nervous, and immunological systems as targets for benzene toxicity. The effects on the nervous system and immune system are discussed in the sections below titled Neurotoxicity and Immunotoxicity. Data on adverse hematological effects in humans are available following intermediate-duration exposures to benzene in the workplace (Aksoy and Erdem 1978; Aksoy et al. 1972). However, the exposure levels and durations were not well defined and, therefore, could not be used to calculate an MRL. Studies in rats and mice following inhalation exposure can be used to define NOAELs and LOAELs for hematological and immunological effects (e.g., Baarson et al. 1984; Cronkite et al. 1982, 1985, 1989; Dow 1992; Farris et al. 1993, 1997a, 1997b; Green et al. 1981a, 1981b; Luke et al. 1988b; Plappert et al. 1994a, 1994b; Rosenthal and Snyder 1987; Seidel et al. 1989; Snyder et al. 1978a, 1980; Toft et al. 1982; Vacha et al. 1990; Ward et al. 1985; Wolf et al. 1956). An intermediate-duration inhalation MRL of 0.006 ppm was derived, based on a LOAEL for immunologic effects in the mouse (Rosenthal and Snyder 1987) (discussed below under Immunotoxicity). Data on hematological effects from oral exposures in animals are also available (Hsieh et al. 1988b, 1990, 1991; NTP 1986; Shell 1992; Wolf et al. 1956), but no intermediate-duration oral MRL could be derived because the threshold for hematological (and immunological) effects could not be identified. Additional studies that include dose-response information on hematological effects following intermediate-duration oral exposure could be designed to provide information that could be useful in deriving an intermediate-duration oral MRL for benzene. Such studies could be designed to serve as validation for existing PBPK models. Intermediate-duration dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

Chronic-Duration Exposure and Cancer. The primary target for adverse systemic effects of benzene following chronic exposure is the hematological system. Hematotoxicity was reported in studies of humans chronically exposed to benzene in the workplace air (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974, 1987; Cody et al. 1993; Dosemeci et al. 1996; Doskin 1971; Erf and Rhoads 1939;

3. HEALTH EFFECTS

Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Lan et al. 2004a, 2004b; Li et al. 1994; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b; Townsend et al. 1978; Ward et al. 1996; Yin et al. 1987c). Several studies of occupational inhalation exposure to benzene did not find clinically-defined hematological effects (Collins et al. 1991, 1997; Tsai et al. 1983, 2004), but they had a high degree of uncertainty regarding estimations of benzene exposure levels. The study of workers of shoe manufacturing industries in Tianjin, China (Lan et al. 2004a, 2004b) identified the lowest LOAEL for hematotoxicity and was selected as the principal study for deriving a chronic-duration inhalation MRL of 0.003 ppm for benzene. Chronic-duration animal studies are available for hematological effects following inhalation exposure and provide support to the human data (Snyder et al. 1978a, 1980, 1982, 1984). No human data are available to evaluate hematological effects following oral exposure. Although chronic duration oral animal studies are available for hematological effects (Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986), the most extensive study (NTP 1986) did not conclusively define a NOAEL or less serious LOAEL for end points that could be used to derive an MRL. However, a chronic-duration oral MRL of 0.0005 mg/kg/day was derived for benzene based on route-to-route extrapolation of the same results (Lan et al. 2004a, 2004b) that served as the basis for the chronic-duration inhalation MRL. Additional chronic-duration oral animal data could be designed to provide support to the chronic-duration oral MRL and to assist in defining threshold levels for populations living near hazardous waste sites. Dermal data for humans and animals were not available. However, chronic-duration dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human carcinogen based on sufficient data in humans supported by animal evidence (IARC 2004; IRIS 2007; NTP 2005). Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and the occurrence of acute myelogenous leukemia (AML) (Hayes et al. 1997; IARC 1982, 1987; EPA 1995, 1998; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1996a, 1996b), as well as is suggestive evidence of associations between benzene and non-Hodgkin's lymphoma (NHL) and multiple myeloma (Hayes et al. 1997; Rinsky et al. 1987). Studies of workers in Ohio (the Pliofilm study) (e.g., Rinsky et al. 1981, 1987; 2002) and China (the NCI/CAPM study) (e.g., Hayes et al. 1997; Yin et al. 1996a, 1996b) provide the strongest data on the leukemogenic potential of benzene, including exposure-response information, and data from the Pliofilm study was used as the basis of inhalation and oral cancer risk values for benzene (EPA 1998; IRIS 2007). Additional studies on these and other cohorts could better characterize exposure level and exposure duration relationships for benzene

3. HEALTH EFFECTS

and leukemia, particularly at low levels of exposure, and clarify the potential of benzene to induce NHL and multiple myeloma.

Benzene is a multiple site carcinogen in rats and mice following inhalation exposure (Cronkite 1986; Cronkite et al. 1984, 1985, 1989; Farris et al. 1993; Maltoni et al. 1982a, 1982b, 1983, 1985, 1989; Snyder et al. 1980) and oral exposure (Huff et al. 1989; Maltoni et al. 1983, 1985, 1989; NTP 1986), inducing lymphomas and other neoplasms in numerous tissues not affected in humans. Although contributing to the weight of evidence for carcinogenicity, the animal studies do not identify a suitable model for leukemia in humans. An appropriate animal model would help to provide a better understanding of how benzene causes cancer, particularly the mechanism of benzene leukemogenesis. The exact mechanism of benzene carcinogenicity is not known, but it has been postulated that some benzene metabolites are capable of forming adducts with DNA and are responsible for reduced immune function which could potentially lead to cancer. The clastogenic properties of benzene may play a role in its carcinogenicity. DNA adduct formation could occur with both inhalation and oral exposures (Ding et al. 1983; Sasiadek et al. 1989). Questions that need to be answered with regard to the mechanism of benzene carcinogenesis include how benzene metabolites produce greater-than-additive effects, determination of the critical target genes, whether aplastic anemia is essential to the development of leukemia, and determination of the role of cytokines and growth factor pathways in benzene toxicity.

Genotoxicity. Evidence for the genotoxicity of benzene in humans comes from studies of chronically-exposed populations (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hallberg et al. 1996; Hartwich et al. 1969; Hedli et al. 1991; Karacic et al. 1995; Kašuba et al. 2000; Liu et al. 1996; Major et al. 1992, 1994; Nilsson et al. 1996; Picciano 1979; Pitarque et al. 1996, 1997; Popp et al. 1992; Qu et al. 2003a, 2003b; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Sellyei and Kelemen 1971; Smith et al. 1998; Sul et al. 2002; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Türkel and Egeli 1994; Van den Berghe et al. 1979; Yardley-Jones et al. 1990; Zhang et al. 1998b, 1999). These exposures have occurred primarily via inhalation, although some dermal exposure cannot be ruled out. In spite of the lack of accurate exposure data, exposure to multiple chemicals, and often inappropriate control groups, the association between benzene exposure and the appearance of structural and numerical chromosome aberrations in human lymphocytes suggests that benzene can be considered a human clastogen. Benzene-induced cytogenetic effects, including chromosome and chromatid aberrations, sister chromatid exchanges, and micronuclei, have been consistently found in *in vivo* animal studies (Anderson and Richardson 1981; Au et al. 1991; Chen et al. 1994; Chang et al. 2005; Eastmond et al. 2001; Erexson et al. 1986; Farris et al. 1996; Fujie et

3. HEALTH EFFECTS

al. 1992; Healy et al. 2001; Lee et al. 2005; Kolachana et al. 1993; Ranaldi et al. 1998; Siou et al. 1981; Toft et al. 1982; Ward et al. 1992). Binding of benzene and/or its metabolites to DNA, RNA, and proteins has been consistently observed in rats and mice (Arfellini et al. 1985; Creek et al. 1997; Lévy et al. 1996; Mani et al. 1999; Mazullo et al. 1989; Turteltaub and Mani 2003). Inhalation exposure of mice has yielded identifiable benzene-derived hemoglobin adducts (Sabourin et al. 1990). Ward et al. (1992) have shown that intermediate-duration exposure of mice to benzene by inhalation at levels below the current PEL may induce gene mutations in the lymphocytes.

Standard short-term *in vitro* assays indicate that benzene's genotoxicity is derived primarily from its metabolites, although benzene has been shown to produce DNA breaks in Chinese hamster ovary cells independent of metabolic activators (Douglas et al. 1985; Eastmond et al. 1994; Lakhansky and Hendrickx 1985; Zhang et al. 1993). There is good evidence that benzene affects cell cycle progression, RNA and DNA synthesis, as well as DNA binding (Forni and Moreo 1967, 1969; Hartwich et al. 1969; Sellyei and Kelemen 1971; Van den Berghe et al. 1979). Chen and Eastmond (1995) showed that benzene metabolites can adversely affect human topoisomerases (enzymes involved in DNA replication and repair); however, evidence exists that some repair of DNA binding with benzene metabolites occurs in human cells (Chenna et al. 1995).

The genotoxicity of benzene has been extensively studied and demonstrated in both humans and laboratory animals. Although some information is available regarding possible mechanisms of benzene genotoxicity and carcinogenicity, additional mechanistic studies would be helpful to more completely characterize mechanisms responsible for these effects.

Reproductive Toxicity. Reproductive data are available on women occupationally exposed to benzene (Mukhametova and Vozovaya 1972; Vara and Kinnunen 1946). The data suggest spontaneous abortions, menstrual disturbances, and ovarian atrophy. These studies are limited by the difficulty in identifying appropriate controls, problems in controlling for concomitant exposures to other chemicals, and inadequate follow-up. Only one study was found that described the reproductive effects on men exposed to benzene (Stucker et al. 1994). There are some data available from inhalation studies on reproductive effects of benzene in animals. Although there are data on adverse gonadal effects (e.g., atrophy/degeneration, decrease in spermatozoa, moderate increases in abnormal sperm forms) (Ward et al. 1985; Wolf et al. 1956), data on reproductive outcomes are either negative (Coate et al. 1984; Green et al. 1978; Kuna et al. 1992; Murray et al. 1979; Tatrai et al. 1980a) or inconclusive or conflicting (i.e., number of live fetuses, incidences of pregnancies) (Murray et al. 1979; Ungvary and Tatrai 1985).

3. HEALTH EFFECTS

Gofmekler (1968) showed infertility in female rats after intermediate-duration inhalation exposure to 210 ppm, but the results are poorly reported. Thus, they provide only suggestive evidence that benzene may have an adverse effect on reproductive outcomes. No data were located on reproductive effects following oral exposure to benzene in humans. Negative effects on reproductive outcome have been reported in one oral study in animals (Exxon 1986). NTP (1986) reported neoplastic changes in the reproductive tissues of female rats and male and female mice after chronic oral exposure. Given the paucity of available data across all exposure routes, it would be useful to have additional 90-day studies conducted by the oral and inhalation routes that assess reproductive organs histologically or cytogenetically. Although dermal contact is not likely to be the most relevant route of exposure for humans at hazardous waste sites, data on dermal exposure would also be useful. If the results of the suggested inhalation or oral studies indicate reproductive toxicity, multigeneration or continuous breeding studies for oral and inhalation exposures would help clarify the potential for benzene to cause reproductive effects in humans.

Developmental Toxicity. Based on epidemiological studies at hazardous waste sites, an increased susceptibility to benzene of pregnant women or their offspring has not been demonstrated (Budnick et al. 1984; Goldman et al. 1985; Heath 1983; Olsen 1983). However, these studies have several limitations that make it impossible to assess the effect of benzene on the human fetus. For example, the few studies that do exist are limited by a lack of control incidences for end points, problems in identifying exposed populations, a lack of data on exposure levels, and/or exposure to multiple substances (Budnick et al. 1984; Forni et al. 1971a; Funes-Cravioto et al. 1977; Goldman et al. 1985; Heath 1983; Olsen 1983). In the occupational setting, however, there may be stronger evidence of increased susceptibility to benzene of pregnant women and/or their offspring (Forni et al. 1971a; Funes-Cravioto et al. 1977). For example, severe pancytopenia and increased chromosomal aberrations occurred in a pregnant worker exposed to benzene (levels not reported) during the entire pregnancy. She gave birth to a healthy boy (Forni et al. 1971b). On the other hand, increased frequency of chromatid and isochromatid breaks and sister chromatid exchanges was found in lymphocytes from 14 children of female workers exposed to benzene (levels not reported) and other organic solvents during pregnancy (Funes-Cravioto et al. 1977). Irrespective of the hematological effects reported in the pregnant worker or the 14 children of female workers, the lack of complete and detailed medical records and the lack of follow-up limit the significance of effects with regard to post exposure morbidity.

A number of investigations have evaluated the developmental/maternal toxicity of benzene in animals following inhalation exposures. In these investigations, fetotoxicity was evidenced by reduced fetal

3. HEALTH EFFECTS

weight and/or minor skeletal variations at concentrations 47 ppm (Coate et al. 1984; Green et al. 1978; Kimmel and Wilson 1973; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). However, persistently altered fetal hematopoiesis occurred in mice at 20 ppm (Keller and Snyder 1986, 1988). Additional information designed to assess the hematopoietic system of the developing fetus (human or animal) following low-level *in utero* exposures to benzene is needed. Oral data are limited to two animal studies in which benzene was shown to reduce pup body weight when mice were administered a high single oral dose (Seidenberg et al. 1986) or have no effect after gestational exposure (Exxon 1986). Additional oral studies designed to assess the developmental effects (human or animal) of low-level exposures to benzene would be useful. No data are available on the developmental toxicity of benzene following dermal exposure. Although these data would be useful, the most likely routes of exposure for humans at hazardous waste sites are the inhalation and oral routes.

Immunotoxicity. The immune system is known to be a target for benzene toxicity. The effects do not appear to be route- or species-specific. The evidence for immunotoxicity in humans comes from workers exposed by inhalation of intermediate and chronic durations. A series of studies demonstrated decreases in numbers of circulating leukocytes (Aksoy et al. 1971, 1972, 1974), but levels of exposure were not well documented. In other studies, alterations in human serum immunoglobulins were observed, but there was concomitant exposure to xylene and toluene (Lange et al. 1973a, 1973b). There is a need to test for subtle alterations in the immune system and immune competence in workers with intermediate- and chronic-duration exposure to benzene. Animal studies support the findings of immune dysfunction and indicate that additional parameters of the immune system are affected by exposure to benzene in the air for acute (Aoyama 1986; Chertkov et al. 1992; Cronkite 1986; Cronkite et al. 1982, 1985, 1989; Gill et al. 1980; Green et al. 1981a; Li et al. 1986; Neun et al. 1992; Rozen et al. 1984; Rosenthal and Snyder 1985; Toft et al. 1982; Ward et al. 1985; Wells and Nerland 1991), intermediate (Baarson et al. 1984; Cronkite et al. 1982, 1985, 1989; Dow 1992; Gill et al. 1980; Green et al. 1981a, 1981b; Li et al. 1992; Plappert et al. 1994b; Rosenthal and Snyder 1987; Rozen and Snyder 1985; Seidel et al. 1990; Snyder et al. 1980; Stoner et al. 1981; Ward et al. 1985; Wolf et al. 1956; Songnian et al. 1982), and chronic (Snyder et al. 1980, 1984, 1988) periods. An acute-duration inhalation study found depressions of proliferative responses of bone-marrow-derived B-cells and splenic T-cells in mice at 10 ppm (Rozen et al. 1984), and an acute-duration inhalation MRL has been derived based on this study. An intermediate-duration inhalation study found delayed splenic lymphocyte reaction to foreign antigens evaluated by *in vitro* mixed lymphocyte culture following exposure of mice to benzene vapors at a concentration of 10 ppm (Rosenthal and Snyder 1987), and an intermediate-duration inhalation MRL has been derived based on this study. No human data on the oral route were available, but animal data showed immunological

3. HEALTH EFFECTS

effects after intermediate (Fan 1992; Hsieh et al. 1988b, 1990, 1991; Shell 1992; Wolf et al. 1956) and chronic exposures (Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986) via the oral route. Since the immune system is known to be a target organ, it is important to have information on subtle alterations in immune competence in people exposed to benzene in the air and in the drinking water. A decrease in cell-mediated immune functions, including alloantigen response and cytotoxicity, was reported in mice following intermediate inhalation exposure to 100 ppm of benzene (Rosenthal and Snyder 1987). This impaired cell-mediated immune function was also apparent in other *in vivo* studies (Stoner et al. 1981). Mice exposed to 100 ppm of benzene for a total of 100 days had reduced tumor resistance when challenged with syngeneic tumor cells and developed tumors that were lethal (Rosenthal and Snyder 1987). Further animal studies would also be useful in defining more NOAEL and LOAEL values. No data are available that document immunotoxicity in humans or animals exposed by dermal application. Dermal sensitization tests may also provide useful data on the likelihood of an allergic response in humans, since skin contact may occur in the workplace and at hazardous waste sites.

Neurotoxicity. In humans, the nervous system is a target of benzene toxicity following both inhalation and oral exposures. No data are available that demonstrate neurologic effects in humans or animals exposed dermally. There are sufficient data to suggest that it is the central nervous system which is affected following acute exposures. Neurological symptoms reported in humans following acute oral and inhalation exposures are similar and include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness (Cronin 1924; Flury 1928; Greenburg 1926; Midzenski et al. 1992; Tauber 1970; Thienes and Haley 1972). Acute- and intermediate-duration inhalation and oral animal studies provide supportive evidence that benzene effects the central nervous system (Carpenter et al. 1944; Cornish and Ryan 1965; Evans et al. 1981; Frantik et al. 1994). Effects observed include narcosis, hyperactivity, tremors, tonic-clonic convulsions, decreased evoked electrical activity in the brain, and slight nervous system depression. Behavioral changes were found in mice after 1 week of exposure to 300 ppm, and decreased grip strength was found after three exposures to 1,000 ppm (Dempster et al. 1984). An intermediate-duration inhalation study found increased rapid response in mice at 0.78 ppm (Li et al. 1992), but this study was limited due to apparent discrepancies between reported and actual benzene exposure levels, since neurological effects reported in other animal studies occurred only at much higher exposure levels. Acute-duration oral exposures of 950 mg/kg benzene altered neurotransmitter levels in the brains of rats (Kanada et al. 1994). Intermediate-duration oral exposures of 8 mg/kg/day resulted in changes in the levels of monoamine transmitters in the brain without treatment-related behavioral changes (Hsieh et al. 1988a).

3. HEALTH EFFECTS

One chronic-duration occupational study reported neurological abnormalities of the peripheral nervous system (global atrophy of lower extremities and distal neuropathy of upper extremities) in four of six benzene-exposed patients with aplastic anemia (Baslo and Aksoy 1982). Additional studies are needed to verify the peripheral nervous system effects that might occur following chronic exposures to low doses of benzene.

Although there are sufficient data to indicate that the nervous system is a target of benzene toxicity, the neurotoxicity of benzene has not been extensively studied. Additional studies in animals are needed to identify the thresholds of neurotoxicity following acute-, intermediate-, and chronic-duration inhalation and oral exposures.

Epidemiological and Human Dosimetry Studies. A large segment of the U.S. population is exposed to benzene. This exposure occurs primarily as a result of benzene emitted to the air from tobacco smoke, gasoline stations, and automobile exhaust. Benzene has been found in about a third of the NPL hazardous waste sites. The magnitude of exposure is greater for those occupationally exposed.

The predominance of available epidemiological data comes from occupational studies and associated analysis (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974; Baslo and Aksoy 1982; Ciccone et al. 1993; Cody et al. 1993; Dosemeci et al. 1994, 1996; Doskin 1971; EPA 1986, 1995, 1998; Erf and Rhoads 1939; Goldwater 1941; Greenburg et al. 1939; Hayes et al. 1997; IARC 1982, 1987; Infante 1978; Infante et al. 1977; IRIS 2007; Kipen et al. 1989; Lan et al. 2004a, 2004b; Li et al. 1994; Ott et al. 1978; Paustenbach et al. 1992; Paxton et al. 1994a, 1994b; Qu et al. 2002, 2003a, 2003b; Rinsky et al. 1981, 1987, 2002; Rothman et al. 1996a, 1996b; Townsend et al. 1978; Travis et al. 1994; Utterback and Rinsky 1995; Ward et al. 1996; Yin et al. 1987c, 1989, 1994, 1996a, 1996b). While these studies provide data on hematological and neurotoxic effects and evidence of carcinogenicity, they only offer information on the effects of inhalation exposure. Retrospective and prospective studies of populations that have been identified as being exposed to contaminated groundwater or drinking water could provide information on long-term health effects from oral exposures. The Benzene Subregistry Baseline Technical Report of the National Exposure Registry contains information on 1,143 persons who had documented exposure to benzene in their drinking water and were exposed for at least 30 days (Agency for Toxic Substances and Disease Registry 2001). No causal relationship has been proposed for health conditions identified in the base subregistry (Agency for Toxic Substances and Disease Registry 1995) or continued follow-up of the population (Agency for Toxic Substances and Disease Registry 2001).

3. HEALTH EFFECTS

Few well-conducted epidemiological studies of exposed populations exist. An epidemiological study of a population with chronic exposure to two NPL hazardous waste sites illustrates the problems inherent in assessing adverse health effects from waste site exposure (Dayal et al. 1995). Benzene was only 1 of 20 compounds or groups of compounds identified as being chemicals of concern in the waste site. The authors note that assessment of long-term exposure to a dumpsite is problematic because of a lack of history of materials deposited, the complexity of the waste components, the interaction of the components over time, and the emphasis on statistically significant versus biologically significant effects. The study had certain limitations, including lifestyle characteristics, the use of mailed-in responses, subjective symptom-reports without independent confirmation, and various problems with exposure estimation. A relationship was detected between exposed individuals and neurological symptoms, including learning difficulties, nervousness, numbness in fingers or toes, sleeping difficulties, unusual fatigue, and decreased sense of smell. Chest pains, irregular heartbeat, skin rashes, and detection of a peculiar odor or taste also occurred more often in the exposed population. However, no effort was made to relate the symptoms to particular chemicals in the waste site. Clearly, more studies of exposed populations surrounding waste sites would be warranted. Other studies include analyses of workers in China (Dosemeci et al. 1994, 1996; Lan et al. 2004a, 2004b; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b; Yin et al. 1987c, 1994), reanalysis of data from the Pliofilm cohort (Paxton et al. 1994a, 1994b; Utterback and Rinsky 1995; Ward et al. 1996), and assessment of clinical manifestations of benzene hematotoxicity in U.S. industries with potential for benzene exposure (Collins et al. 1991, 1997; Tsai et al. 1983, 2004). A publication by Lioy and Pellizzari (1995) outlines an appropriate framework for the National Human Exposure Assessment Survey, using benzene as a candidate compound. Other articles also address population-based exposure models and measurements (MacIntosh et al. 1995; Pellizzari et al. 1995).

Analysis of benzene dosimetry in animal studies suggests that the metabolic pathways leading to the production of the putative toxic metabolites appear to be low-capacity, high-affinity pathways that are saturated at relatively low-exposure concentrations (Henderson et al. 1992). The authors suggest that this could also be true for humans. Additional dosimetry studies are necessary to further define the role of toxic metabolites in the development of benzene-related leukemia.

Studies of DNA damage have primarily been performed on workers with preexisting hematological effects and cancer (Ding et al. 1983; Forni et al. 1971a; Picciano 1979; Yardley-Jones et al. 1988) or as biomonitoring of benzene exposure (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Hallberg et al. 1996; Kašuba et al. 2000; Liu et al. 1996; Nilsson et al. 1996; Pitarque et al. 1996; Sul et al. 2002; Surrallés et al. 1997; Zhang et al. 1999). Additional data on benzene-induced DNA damage could be useful in

3. HEALTH EFFECTS

monitoring possible adverse effects in individuals living near hazardous waste sites if more quantitative dose-response data were available for clastogenicity.

Studies to determine whether benzene causes solid tumors and hematological malignancies other than acute nonlymphocytic leukemia in humans have been suggested as a data need (Goldstein and Warren 1993). These data could be obtained by revisiting already established cohorts of exposed individuals, or by examining new cohorts.

Biomarkers of Exposure and Effect.

Exposure. There is no clinical disease state unique to benzene toxicity. However, the effects on the hematopoietic and immune systems are well recognized, and analytical methodologies exist for monitoring benzene levels in expired breath and blood (Brugnone et al. 1989, 1992; DeLeon and Antoine 1985; Pellizzari et al. 1988). Several biomarkers of exposure to benzene exist, including unmetabolized benzene in expired air and urine (Farmer et al. 2005; Fustinoni et al. 2005; Ghittori et al. 1993; Nomiya and Nomiya 1974a, 1974b; Sherwood 1988; Srbova et al. 1950; Waidyanatha et al. 2001), urinary phenol levels (Astier 1992; Inoue et al. 1986, 1988b; Jongeneelen et al. 1987; Karacic et al. 1987; Pagnotto et al. 1961; Pekari et al. 1992), and urinary *trans,trans*-muconic acid and *S*-phenylmercapturic acid levels (Boogaard and van Sittert 1995, 1996; Ducos et al. 1990, 1992; Farmer et al. 2005; Inoue et al. 1989b, 2000; Lee et al. 1993; Melikian et al. 1993, 1994; Pezzagno et al. 1999; Popp et al. 1994; Qu et al. 2005; Rothman et al. 1998; Ruppert et al. 1997; Sanguinetti et al. 2001; van Sittert et al. 1993; Weaver et al. 2000). Estimates of benzene exposure can be made by comparing the ratio of inorganic to organic sulfates in the urine (Hammond and Hermann 1960). However, urinary sulfate levels are variable, and they have not been used to identify exposure levels of benzene associated with minimal toxic effect. Furthermore, benzene-derived adducts with hemoglobin and albumin may be useful as biomarkers (Bechtold et al. 1992a, 1992b; Bechtold and Henderson 1993; Farmer et al. 2005; Fustinoni et al. 2005; Ghittori et al. 1993; Hedli et al. 1991; Lutz and Schlatter 1977; Nomiya and Nomiya 1974a, 1974b; Pekari et al. 1992; Reddy et al. 1989; Schad et al. 1992; Sherwood 1988; Smith and Rothman 2000; Srbova et al. 1950; Waidyanatha et al. 2001; Yeowell-O'Connell et al. 1998, 2001).

Phenol measurements have routinely been used for monitoring occupational exposures, and there is evidence that urinary phenol levels can be correlated with exposure levels (Astier 1992; Inoue et al. 1986; Pekari et al. 1992). Correlating urinary phenol with benzene exposure is complicated by high and variable background levels in nonexposed persons. The data suggest that variations in urinary phenol will

3. HEALTH EFFECTS

obscure phenol formed from low levels of benzene (Ong and Lee 1994; Perbellini et al. 1988). For exposures to 1 ppm or less, the current workplace standard, urinary phenol is not an adequate assay to determine the extent of benzene exposure. In addition, urinary excretion of phenol is lowered by coexposure to toluene (Inoue et al. 1988b). In many exposure situations (e.g., occupational settings and hazardous waste sites), toluene and other chemicals are present and could interfere with the metabolism and elimination of benzene. Studies that attempt to identify another biomarker that is specific to benzene, such as *S*-phenylmercapturic acid, the proposed urinary BEI (ACGIH 1996b), may be helpful for medical surveillance.

The metabolism of benzene to ring-opened compounds (e.g., muconic acid) may be pertinent to the development of a biomarker of benzene exposure and effect (Ducos et al. 1990, 1992; Inoue et al. 1989b; Lee et al. 1993; Melikian et al. 1993; Ong and Lee 1994; Witz et al. 1990b). However, the dose-response curve for metabolism of benzene to urinary muconic acid in humans needs to be determined. If urinary muconic acid increases in humans at low benzene exposures, as it does in mice, then it may be a valid biomarker of benzene exposure. Excretion of urinary muconic acid in humans has also been shown to be lowered by coexposure to toluene (Inoue et al. 1989b). The effect of coexposure to toluene and other chemicals on muconic acid formation needs to be assessed in order to determine the usefulness of muconic acid as a biomarker of benzene exposure.

Breath levels of benzene have been used as a measure of exposure (Brugnone et al. 1989; Money and Gray 1989; Nomiya and Nomiya 1974a, 1974b; Ong and Lee 1994; Pekari et al. 1992). However, the amount of benzene lost in expired air will vary not only with the dose, duration, and time from exposure, but also with the extent of metabolism in the body. Benzene levels in blood have been measured. However, blood levels rapidly decrease after exposure (Brugnone et al. 1989; DeLeon and Antoine 1985; Schrenk et al. 1941).

Benzene metabolites also form DNA adducts (Popp et al. 1992). The use of hemoglobin adducts formed from benzene metabolites as a biomarker of benzene exposure has been developed by Sun et al. (1990). Bechtold and colleagues have conducted further studies using this model (Bechtold et al. 1992a; Bechtold and Henderson 1993). Further refinement of this model would be useful to quantitate cumulative low-level exposures to benzene. Development of specific markers of exposure to use in epidemiological studies to clarify the shape of the dose-response curve in the low-dose region has been suggested as a data need (Goldstein and Warren 1993). Recent reports indicate that urinary benzene may serve as a sensitive

3. HEALTH EFFECTS

biomarker of exposure to benzene concentrations well below 1 ppm (Farmer et al. 2005; Fustinoni et al. 2005).

Effect. Monitoring of benzene workers has included monthly blood counts. Toxic effects occur in the bone marrow and arise either from benzene via a solvent effect, or via its metabolites (Gad-El-Karim et al. 1985; Irons et al. 1980). Hematological tests could be used as markers of hematotoxicity, but medical laboratories lack tests specific to benzene hematotoxicity. The tests cannot be relied upon to find preclinical disease but can identify the subtle changes that are early indicators of effects. Furthermore, these markers of effect may not be useful for long periods following cessation of exposure, nor do they distinguish between acute and chronic exposures. As stated above, additional studies are needed to define the role of benzene-related leukopenia in the disease process initiated by benzene exposure, to determine if it is a biomarker of effect, or an intermediate end point in the development of leukemia (Hayes 1992).

Absorption, Distribution, Metabolism, and Excretion. Data from both humans and animals consistently indicate that benzene is rapidly absorbed through the lungs (Eutermoser et al. 1986; Nomiya and Nomiya 1974a; Sabourin et al. 1987; Schrenk et al. 1941; Srbova et al. 1950; Yu and Weisel 1996). Although experimentally-acquired data are not available on oral absorption of benzene in humans, case reports of accidental or intentional poisoning suggest that benzene is rapidly absorbed from the gastrointestinal tract (Thienes and Haley 1972). The efficient absorption of oral doses in animals is well documented (Cornish and Ryan 1965; Parke and Williams 1953a; Sabourin et al. 1987). Benzene can be absorbed through the skin, but the rate of absorption is much lower than that for inhalation (Maibach and Anjo 1981; Susten et al. 1985; Tsuruta 1989). Following absorption into the body, benzene is widely distributed to tissues, with the relative uptake dependent on the perfusion of the tissue by blood, and the total potential uptake dependent on fat content and metabolism (Sato et al. 1975; Tauber 1970).

There is no evidence to suggest that the route of administration has any substantial effect on the subsequent metabolism of benzene, either in humans or animals. Benzene is metabolized primarily in the liver and to a lesser extent, in the bone marrow. Benzene is a preferential substrate of CYP2E1, which also metabolizes alcohol. The induction of CYP2E1 by benzene (and some of its metabolites) with subsequent generation of reactive metabolites, oxygen radicals, circulating lipid peroxides, and hydroxyl radicals could be associated with hematopoietic toxicity and carcinogenicity of benzene (Irons 2000; Parke 1989; Ross 1996, 2000; Smith 1996a, 1996b; Snyder 2000a, 2000b, 2002; Snyder and Hedli 1996; Snyder and Kalf 1994). CYP2E1 is not confined to the liver, but has also been detected in bone marrow. Andrews et al. (1979) demonstrated that rabbit bone marrow is capable of metabolizing benzene. Schnier

3. HEALTH EFFECTS

et al. (1989) subsequently found that rabbit bone marrow contains CYP2E1. Irons et al. (1980) demonstrated that benzene metabolism by rat bone marrow (*in situ*) was complete and independent of metabolism by the liver, with concentrations of phenol greater than catechol and hydroquinone. Although the total metabolism by bone marrow was limited (total metabolites present were 25% of those in blood), the concentration of metabolites in the bone marrow exceeded that in the blood. Similar studies have been conducted in mice (Ganousis et al. 1992). Benzene metabolism in bone marrow is not well understood; additional data regarding the initial oxidation step and the comparatively low levels of CYP2E1 activity in bone marrow would be useful in identifying the mechanisms of benzene's hematotoxicity. This aspect of metabolism may have implications for long-term exposures, which could be explored in chronic exposure studies. The intermediary metabolites of benzene are responsible for many of the toxic effects observed (Eastmond et al. 1987; Gad-El-Karim et al. 1985). Biotransformation is believed to be essential for benzene-induced bone marrow damage.

However, there is disagreement as to whether benzene is activated in the marrow, activated elsewhere and transported to the marrow, or metabolized in the liver and the metabolites activated in the marrow. Further studies on the metabolism of benzene would help define its mechanism of action. Additionally, more information is needed on the pathways of metabolism in humans, the chemical nature of the toxic metabolites, and the mechanism of toxicity. Recently published data comparing urinary metabolite profiles of orally administered benzene and phenol in mice suggest that zonal differences in metabolism in the liver may be responsible for relative differences in the production of hydroquinone, thus explaining the higher toxicity observed after benzene administration compared with phenol administration (Kenyon et al. 1995). Additional work in this area would aid in further understanding the kinetic determinants of benzene toxicity. Ethanol and dietary factors such as food deprivation and carbohydrate restriction enhance the hematotoxic effects of benzene. Therefore, more information regarding differences in metabolic pattern according to sex, age, nutritional status, and species, and correlation to differences in health effects would be useful.

Humans and animals both excrete inhaled benzene via expiration. Additionally, benzene metabolites are excreted primarily in the urine in both humans and animals. No studies in humans exist for excretion of oral doses of benzene. Studies in several animal species indicate that the route of excretion of benzene and/or its metabolites is a function of exposure level and the saturation of metabolic systems (Henderson et al. 1989). Data regarding excretion following dermal exposure in humans are limited. However, the major route of excretion in both humans and animals following dermal exposure is the urine.

3. HEALTH EFFECTS

Comparative Toxicokinetics. Qualitatively, absorption, distribution, metabolism, and excretion appear to be similar in humans and laboratory animals. However, quantitative variations in the absorption, distribution, metabolism, and excretion of benzene have been observed with respect to exposure routes, sex, nutritional status, and species. Further studies that focus on these differences and their implications for human health would be useful. Additionally, *in vitro* studies using human tissue and further research into PBPK modeling in animals would contribute significantly to the understanding of the kinetics of benzene and would aid in the development of pharmacokinetic models of exposure in humans. These topics are being addressed in ongoing studies (see Section 3.12.3).

Methods for Reducing Toxic Effects. Development of methods and practices that are specific for benzene is needed for reducing peak absorption, body burden and for interfering with the mechanism of action following benzene exposures. Since benzene metabolites are thought to play the major role in the toxicity and carcinogenicity, more information is needed about their covalent binding to nucleic acids and cellular macromolecules. This information would help the development of methods for possible prevention of benzene-induced toxicity. Related lines of investigation include the use of non-steroidal anti-inflammatory drugs to block prostaglandin and prostaglandin synthetase-mediated activity after benzene exposure, and the role of IL-1 cytokine activity in preventing depression of hematopoiesis.

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

No clear evidence of age-related differences in susceptibility to benzene toxicity was located. Benzene crosses the placenta (Dowty et al. 1976). Nursing infants can be exposed to benzene in the breast milk (Fabietti et al. 2004). Limited animal studies indicate that *in utero* exposure to benzene results in hematological changes similar to those observed in animals exposed only as adults (Corti and Snyder 1996; Keller and Snyder 1986, 1988). There is some indication that parental occupational exposure to benzene may play a role in childhood leukemia (Buckley et al. 1989; McKinney et al. 1991; Shaw et al. 1984; Shu et al. 1988). However, none of these studies indicate whether children may be at greater risk than adults for benzene toxicity. Children could potentially be at increased risk for benzene toxicity via the inhalation exposure route based on higher activity levels and ventilation rates than adults. Age-related differences in benzene metabolism could potentially affect susceptibility. Well-designed animal studies should be performed to adequately assess the potential for age-related increased susceptibility to benzene.

3. HEALTH EFFECTS

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

3.12.3 Ongoing Studies

Ongoing studies pertaining to benzene have been identified and are shown in Table 3-7 (FEDRIP 2005).

3. HEALTH EFFECTS

Table 3-7. Ongoing Studies on the Health Effects of Benzene

Investigator	Affiliation	Research description	Sponsor
Conaway CC	University of California Lawrence Livermore National Laboratory	DNA binding of radiolabeled benzene	National Center for Research Resources
French JE	National Institute of Environmental Health Sciences	Carcinogen inactivation of tumor suppressor genes in p53 mice	National Institute of Environmental Health Sciences
French JE	National Institute of Environmental Health Sciences	Mechanisms of leukemogenesis in genetically-altered mice	National Institute of Environmental Health Sciences
Sabri MI	Oregon Health and Science University	Biomarkers of exposure and effect in neurotoxicants including neurotic benzene derivatives	National Institute of Environmental Health Sciences
Monks TJ	University of Texas at Austin	Benzene metabolites and hematotoxicity	National Institute of Environmental Health Sciences
Ross D	Texas A&M University System	Protective effect of NQ01 against benzene toxicity	National Institute of Environmental Health Sciences
Smith MT	University of California Berkeley	Biomarkers of benzene exposure and genotoxicity	National Institute of Environmental Health Sciences

Source: FEDRIP 2005