

## 2. HEALTH EFFECTS

### 2.1 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 of 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.

### 2.2 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 lowestobserved-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt

at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user’s perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) 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 the carcinogenic effects (Cancer Effect Levels, CELs) of benzene are indicated in Figures 2-1 and 2-2. Because cancer effects could occur at lower exposure levels, the figures also show 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.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for benzene. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990h), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions,

asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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.

### **2.2.1 Inhalation Exposure**

Occupational or environmental exposure to benzene or benzene-containing materials usually occurs through the inhalation or dermal route. The main route of exposure is considered to be inhalation. Studies regarding human occupational or environmental inhalation exposure, in addition to experimental animal studies, are discussed in this section (Section 2.2.1).

#### **2.2.1.1 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 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). Death from various causes, including cancer, has been associated with chronic inhalation exposure to benzene (Li et al. 1994; Paxton et al. 1994a). In the study by Li et al. (1994), which was conducted during 1972-87 and was comprised of 74,828 benzene-exposed workers employed in 672 factories and 35,805 unexposed workers from 109 factories located in 12 cities in China, there were no significant differences found in the relative risks for total mortality and cancer mortality between male and female workers exposed to benzene. Relative risk estimates for total mortality were slightly higher for men than for women in the specific occupational categories but were not found to be significant in total mortality observed.

In animals, acute inhalation exposure to high concentrations of benzene has caused death. An inhalation LC<sub>50</sub> 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 per 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 per day, 5 days per 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 per day, 5 days a 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 per week, 6 hours per 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).

The LC<sub>50</sub> value and all reliable LOAEL values for each species and duration category are recorded in Table 2- 1 and plotted in Figure 2- 1.

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
<b>ACUTE EXPOSURE</b>							
<b>Death</b>							
1	Human	1 d 5-10 min				20000 (death)	Flury 1928
2	Rat (Sprague-Dawley)	4 hr				13700 (LC <sub>50</sub> )	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
<b>Systemic</b>							
5	Human	1-21 d 2.5 - 8 hr/d	Resp  Hemato  Dermal		60 M (mucous membrane irritation, dyspnea)	60 M (leukopenia, anemia, thrombocytopenia, MCV elevation)	Midzenski et al. 1992
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
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

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
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	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)		
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 wks 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 <sup>4</sup> 5 d/wk 6 hr/d	Hemato			400 M (decreased erythrocytes & leukocytes)	Cronkite et al. 1982

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
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 (CBA/Ca BNL)	2 d 5 d/wk 6 hr/d	Hemato		3000 M (neutropenia; reduced cellularity)		Cronkite et al. 1989
20	Mouse (C57BL/6BNL)	8 d 6 hr/d	Hemato		3000 F (decreased marrow cellularity)		Cronkite et al. 1989
21	Mouse (DBA/2J)	5 d 6 h/d	Hemato		10 M (50% decrease in CFU-E numbers)		Dempster and Snyder 1991
22	Mouse (C57B1/6)	2-8 d 24 hr/d	Hemato			100 M (leukopenia; decrease in marrow cellularity)	Gill et al. 1980
23	Mouse (CD-1)	5 d 6 hr/d	Hemato	9.9 M		103 M (decreased marrow cellularity; granulocytopenia, lymphocytopenia; decreased polymorphonucleocytes)	Green et al. 1981
			Bd Wt	4862 M			
24	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
25	Mouse (Hybrid)	5 d 5 d/wk 6 hr/d	Hemato	300 F	900 F (CFU-E depression)		Plappert et al. 1994a

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
26	Mouse (C57Bl/6J)	6 d 6 hr/d	Hemato		10 M (depressed lymphocyte counts; elevated RBC's)		Rozen et al. 1984
27	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
28	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
29	Mouse (NMRI)	1 wk	Hemato	14 M			Toft et al. 1982
30	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			
31	Mouse (Swiss-Webster)	5 d 6 hr/d	Hemato	3 M	25 M (decrease in WBC count)		Wells and Nerland 1991
32	Rabbit	Gd 7-20 24 hr/d	Bd Wt	156.5 F	313 F (reduced maternal weight gain)		Ungvary and Tatrai 1985
<b>Immunological/Lymphoreticular</b>							
33	Rat (Wistar)	7 d 8 hr/d		50 F	100 F (leukopenia; increased leukocyte alkaline phosphatase)		Li et al. 1986

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference	
					Less serious (ppm)	Serious (ppm)		
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 & thymus weights and WBC count)	Aoyama 1986	
36	Mouse (DBA/2)	2 wks 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
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. 1981

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
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 (C57B1/6J)	6 d 6 hr/d			10 <sup>b</sup> 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
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; splenic periarteriolar lymphoid sheath depletion, lymphoid depletion of mesenteric lymph nodes; thymic atrophy; myeloid hypoplasia of femoral marrow)	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

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Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference	
					Less serious (ppm)	Serious (ppm)		
<b>Neurological</b>								
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	
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 & blink reflex; pupillary contraction & involuntary blinking)	Carpenter et al. 1944
<b>Reproductive</b>								
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

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
60	Mouse (CF-1)	Gd 6-15 7 hr/d		500 F			Murray et al. 1979
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
<b>Developmental</b>							
63	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		40	100	(decreased fetal weight)	Coate et al. 1984
64	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d			100	(increased incidence of missing sternbrae)	Green et al. 1978
65	Rat (Sprague-Dawley)	Gd 6-15 7 hr/d		10	50	(decreased fetal weight)	Kuna and Kapp 1981
66	Rat (CFY)	Gd 7-14 24 hr/d			125	(decreased mean fetal weight; increased fetal weight retardation; skeletal retardation; 17% decrease in mean placental weight)	Tatrai et al. 1980a
67	Rat (CFY)	Gd 7-14 24 hr/d			47	(decreased fetal weight; signs of skeletal retardation)	141 (significant increase in fetal mortality) Tatrai et al. 1980b
68	Mouse (Swiss-Webster)	Gd 6-15 6 hr/d		10	20	(decreased circulating erythroid precursors, elevation of granulocytic precursor cells in neonates and 6-wk old offspring)	Keller and Snyder 1988

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
69	Mouse (CF-1)	Gd 6-15 7 hr/d			500	(decreased fetal body weight; delayed skeletal ossifications)	Murray et al. 1979
70	Mouse (CFLP)	Gd 6-15 12 hr/d			156.5	(decreased fetal body weight and skeletal retardation)	Ungvary and Tatrai 1985
71	Rabbit (New Zealand)	Gd 6-18 7 hr/d			500	(increased minor skeletal variants)	Murray et al. 1979
72	Rabbit (New Zealand)	Gd 7-20 24 hr/d		156.5	313	(decreased fetal weight; increased minor anomalies)	Ungvary and Tatrai 1985
<b>INTERMEDIATE EXPOSURE</b>							
<b>Death</b>							
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. 1981

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference	
					Less serious (ppm)	Serious (ppm)		
<b>Systemic</b>								
78	Human	4 mo -1 yr	Hemato			150	(pancytopenia)	Aksoy and Erdem 1978
79	Human	4 mo -1 yr	Hemato			210	(pancytopenia, hypocellular to hypercellular bone marrow)	Aksoy et al. 1972
80	Human	1 yr	Hemato		40		(decrease in WBC counts in first 4 months)	Cody et al. 1993
81	Human	3.5 mo -19 y	Hemato			29	(aplastic anemia)	Yin et al. 1987c
82	Rat (Sprague- Dawley)	3 wk 5 d/wk 6 hr/d	Hemato			500	(decreased WBC & lymphocytes; increased RBC & 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
86	Mouse (C57BL)	24 wk 5 d/wk 6 hr/d	Hemato		10 M		(depressed splenic red cells)	Baarson et al. 1984
87	Mouse (C57BL)	9 wk 5 d/wk 6 hr/d	Hemato		10 M		(depressed peripheral red blood cells, CFU-E)	Baarson et al. 1984

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
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 (Hale-Stoner)	9.5 wk 5 d/wk 6 hr/d	Hemato		400 M (decreased CFU-E, BFU-E & CFU-GM cells)		Cronkite et al. 1989
91	Mouse (CBA/Ca BNL)	20 d 5 d/wk 6 hr/d	Hemato			316 M (neutropenia; reduced cellularity)	Cronkite et al. 1989
92	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
93	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d	Hemato		300 M (granulocytic hyperplasia in bone marrow)*		Farris et al. 1993
94	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d	Hemato  Bd Wt	  302 M		302 M (decreased WBC, RBC; altered RBC morphology)	Green et al. 1981
95	Mouse (CD-1)	50 d 5 d/wk 6 hr/d	Hemato  Bd Wt	9.6 M  9.6 M			Green et al. 1981
96	Mouse (Kunming)	30 d , 6 d/wk 2 hr/d	Hepatic  Renal Bd Wt	12.52 M  12.52 M 12.52 M			Li et al. 1992

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
97	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
98	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d	Hemato	100 F	300 F (slight anemia; BFU-E & CFU-E depression in bone marrow)		Plappert et al. 1994a
99	Mouse (Hybrid)	8 wk 5 d/wk 6 hr/d	Hemato			300 F (decreased Hgb, Hct, erythrocyte counts)	Plappert et al. 1994b
100	Mouse (BDF1)	8 wk 5 d/wk 6 hr/d	Hemato		100 F (BFU-E and CFU-E depression)		Seidel et al. 1989b
101	Mouse (NMRI)	8 wk	Hemato	14 M			Toft et al. 1982
102	Mouse (Hybrid)	6 or 7 wk 5 d/wk 6 h/d	Hemato			300 F (decreased CFU-C, BFU-E & CFU-E)	Vacha et al. 1990
103	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d	Hemato  Bd Wt	30  300		300 (pancytopenia, bone marrow hypoplasia)	Ward et al. 1985
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, & increased erythroid cells)		Dow 1992
106	Gn pig (NS)	32 or 269 d 5 d/wk 7 hr/d	Hemato		88 (leukopenia)		Wolf et al. 1956

2. HEALTH EFFECTS

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference	
					Less serious (ppm)	Serious (ppm)		
<b>Immunological/Lymphoreticular</b>								
107	Human	4 mo -1 yr				210	(pancytopenia, hypoplastic to hyperplastic bone marrow. enlarged spleen)	Aksoy et al. 1972
108	Human	1 yr			40		(decreased lymphocytes)	Cody et al. 1993
109	Rat (Sprague-Dawley)	3 wk 5 d/wk 6 hr/d				500	(decreased myeloid & lymphoid cells)	Dow 1992
110	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		30	300		(leukopenia and lymphopenia)	Ward et al. 1985
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)	Baaron et al. 1984
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 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					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 (C57B1/6)	6 wk 5 d/wk 6 hr/d				1000 (leukopenia, granulocytopenia, lymphocytopenia)	Gill et al. 1980
118	Mouse (CD-1)	50 d 6 hr/d 5 d/wk			9.6 M (increased splenic CFU-S)		Green et al. 1981
119	Mouse (CD-1)	26 wk 6 hr/d 5 d/wk				302 M (reduced marrow and spleen cellularity; decreased spleen weight)	Green et al. 1981
120	Mouse (CD-1)	50 d 5 d/wk 6 hr/d			9.6 M (increased spleen weight, total splenic nucleated cellularity & NRBC)		Green et al. 1981
121	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d				302 M (lymphocytopenia, anemia, decreased spleen weight, decreased spleen & marrow cellularities)	Green et al. 1981
122	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
123	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d		100 F	300 F (increased T4/T8 ratio)		Plappert et al. 1994a

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
124	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
125	Mouse (C57B1/6)	20 d 5 d/wk 6 hr/d			10 M (delayed splenic lymphocyte reaction to foreign antigens evaluated in in vitro mixed lymphocyte reaction)		Rosenthal and Snyder 1987
126	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
127	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d		30		300 (leukocyte & lymphocyte depression; bone marrow hypoplasia; lymphoid depletion in mesenteric lymph node, plasma cell infiltration of mandibular lymph node, splenic periarteriolar lymphoid sheath depletion)	Ward et al. 1985
128	Gn Pig (NS)	32 or 269 d 5 d/wk 7 hr/d			88 (leukopenia, increased spleen weight)		Wolf et al. 1956
129	Rabbit (NS)	243 d 5 d/wk 7 hr/d			80 (leukopenia)		Wolf et al. 1956
130	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

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
<b>Neurological</b>							
131	Rat (Wistar)	3 wk 3-4 x 4 hr				929 M (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994
132	Mouse (H)	3 wk 3-4 x 2 hr				856 F (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994
133	Mouse (Kunming)	30 d 6 d/wk 2 hr/d			0.78 <sup>c</sup> M (increased rapid response)		Li et al. 1992
<b>Reproductive</b>							
134	Rat (Sprague-Dawley)	10 wk Gd 0-20 Ld 5-20 5 d/wk 6 hr/d		300 F			Kuna et al. 1992
135	Rat (Wistar)	93 d 5 d/wk 7-8 hr/d			6600 M (testicular weight increase)		Wolf et al. 1956
136	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
137	Rabbit (NS)	243 d 5 d/wk 7-8 hr/d			80 M (degeneration of germinal epithelium in testes)		Wolf et al. 1956
138	Gn pig (NS)	32 or 269 d 5 d/wk 7-8 hr/d			88 M (testicular weight increase)		Wolf et al. 1956

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference	
					Less serious (ppm)	Serious (ppm)		
<b>Cancer</b>								
139	Human	3.5 mo -19 y				29	(CEL: humanlymphocytic leukemia)	Yin et al. 1987c
140	Rat (Sprague- Dawley)	15 wk 4-5 d/wk 4-7 hr/d				200	(CEL: hepatomas)	Maltoni et al. 1982a, 1983, 1985
141	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
142	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				100 M	(CEL: leukemia)	Cronkite 1986
143	Mouse (C57BL/ 6BNL)	4-16 wk 5 d/wk 6 hr/d				300	(CEL: thymic and non-thymic lymphoma)	Cronkite et al. 1984, 1985
144	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				300	(CEL: hepatomata, lymphomatous & myelogenous neoplasms; Harderian & Zymbal gland, squamous cell & mammary carcinoma, papillary adenocarcinoma of the lung)	Cronkite et al. 1989
145	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				100 M	(CEL: hepatomata, lymphomatous & myelogenous neoplasms)	Cronkite et al. 1989
146	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M	(CEL: lymphoma in 12%)	Farris et al. 1993

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
147	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
<b>CHRONIC EXPOSURE</b>							
<b>Death</b>							
148	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (61% died, versus 46% in controls)	Maltoni et al. 1982a
149	Rat (Sprague-Dawley)	691 d 5 d/wk 6 hr/d				300 M (median lifespan 51 wk vs 65 wk in controls)	Snyder et al. 1978a
150	Mouse (AKR, C57Bl)	life 5 d/wk 6 hr/d				300 M (median lifespan 11-41 wk vs 39-75 wk in controls)	Snyder et al. 1978a, Snyder et al. 1980
<b>Systemic</b>							
151	Human	4 mo- 15 yr	Hemato			150 (pancytopenia)	Aksoy and Erdem 1978
152	Human	1-3 yr	Hemato			3 (anemia, lymphocytosis, thrombocytopenia, leukopenia, leukocytosis)	Doskin 1971
153	Human	NS	Hemato			24 (pancytopenia, hypoplastic bone marrow)	Erf and Rhoads 1939
154	Human	3-29 yr	Hemato		25 M (increased mean corpuscular volume)		Fishbeck et al. 1978
155	Human	0.5-5 yr	Hemato			11 (anemia, macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939
156	Human	1-25 yr	Hemato	20		75 (anemia and leukopenia)	Kipen et al. 1989

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
157	Human	1-21 yr	Hemato	0.53 M			Tsai et al. 1983
158	Human	>1 yr	Hemato		0.69 (leukopenia)		Xia et al. 1995
159	Human	>1 yr	Resp		33 M (sore throat; nasal irritation) 59 F (sore throat; nasal irritation)		Yin et al. 1987b
			Hemato	33 M 59 F			
			Renal	33 M 59 F			
			Ocular		33 M (eye irritation) 59 F (eye irritation)		
160	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)		
161	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)	

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference	
					Less serious (ppm)	Serious (ppm)		
<b>Immunological/Lymphoreticular</b>								
162	Human	3-5 yr				11	(macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939
163	Human	1-25 yr		20	75		(leukopenia)	Kipen et al. 1989
164	Human	>1 yr			0.69		(leukopenia)	Xia et al. 1995
165	Rat (Sprague- Dawley)	lifetime 5 d/wk 6 hr/d			100 M		(decreased lymphocyte counts, splenic hyperplasia)	Snyder et al. 1978a, 1984
166	Mouse (AKR, C57Bl)	lifetime 5 d/wk 6 hr/d			100 M		(lymphocytopenia, bone marrow hypoplasia )	Snyder et al. 1978a, 1980
167	Mouse (C57Bl, CD-1)	lifetime 6 hr/d 5 d/wk			300 M		(lymphopenia)	Snyder et al. 1988
<b>Cancer</b>								
168	Human	4-15 yr				150	(CEL: leukemia)	Aksoy and Erdem 1978
169	Human	28 mo- 40 yr				1	(CEL: leukemia, lymphoma)	Aksoy et al. 1987
170	Human	1-10 yr				10 M	(CEL: leukemia)	Infante 1978 (Follow-up to Infante 1977)
171	Human	18 mo				0.3 M	(CEL: leukemia)	Ott et al. 1978
172	Human	1-14 yr				16 M	(CEL: leukemia)	Rinsky et al. 1981 (Follow-up to Infante et al. 1977a)

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference	
					Less serious (ppm)	Serious (ppm)		
173	Human	1-30 yr				200	(CEL: leukemia)	Vigliani and Forni 1976
174	Human	>1 yr				2	(CEL: chronic erythroid leukemia)	Yin et al. 1989 (Follow-up to Yin et al. 1987a)
175	Rat (Sprague- Dawley)	104 wk 5 d/wk 4-7 hr/d				200	(CEL: hepatomas)	Maltoni et al. 1982a
176	Rat (Sprague- Dawley)	86 wk 5 d/wk 4-7 hr/d				200	(CEL: Zymbal gland carcinoma)	Maltoni et al 1982b
177	Rat (Sprague- Dawley)	104 wk 5 d/wk 4-7 hr/d				200	(CEL: hepatomas)	Maltoni et al. 1983, 1985
178	Rat (Sprague- Dawley)	lifetime 5 d/wk 6 hr/d				100 M	(CEL: Zymbal gland carcinoma, myelogenous leukemia, liver tumors)	Snyder et al. 1984
179	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 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to figure <sup>a</sup>	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
180	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

<sup>a</sup>The number corresponds to entries in Figure 2-1.

<sup>b</sup>Used to derive an acute inhalation minimal risk level (MRL) of 0.05 ppm. Concentration was converted to a human equivalent concentration and divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) and adjusted for intermittent exposure by multiplying by 6/24 (see Appendix A).

<sup>c</sup>Used to derive an intermediate inhalation MRL of 0.004 ppm. Concentration was converted to a human equivalent concentration and divided by an uncertainty factor of 90 (3 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) and adjusted for intermittent exposure by multiplying by 2/24 (see Appendix A).

AChE = acetylcholinesterase; Bd Wt = body weight; BFU-E = burst-forming colonies, erythroids; 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; CNS = central nervous system; d = day(s); F = female; Gd = gestational day; Hct = hematocrit; Hemato = hematological; Hgb = hemoglobin; hr = hour(s); LC<sub>50</sub> = 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; NS = not specified; occup = occupational exposure; RBC = red blood cell; WBC = white blood cell; wk = week(s); yr = year(s)

Figure 2-1. Levels of Significant Exposure to Benzene - Inhalation

Acute ( $\leq 14$  days)

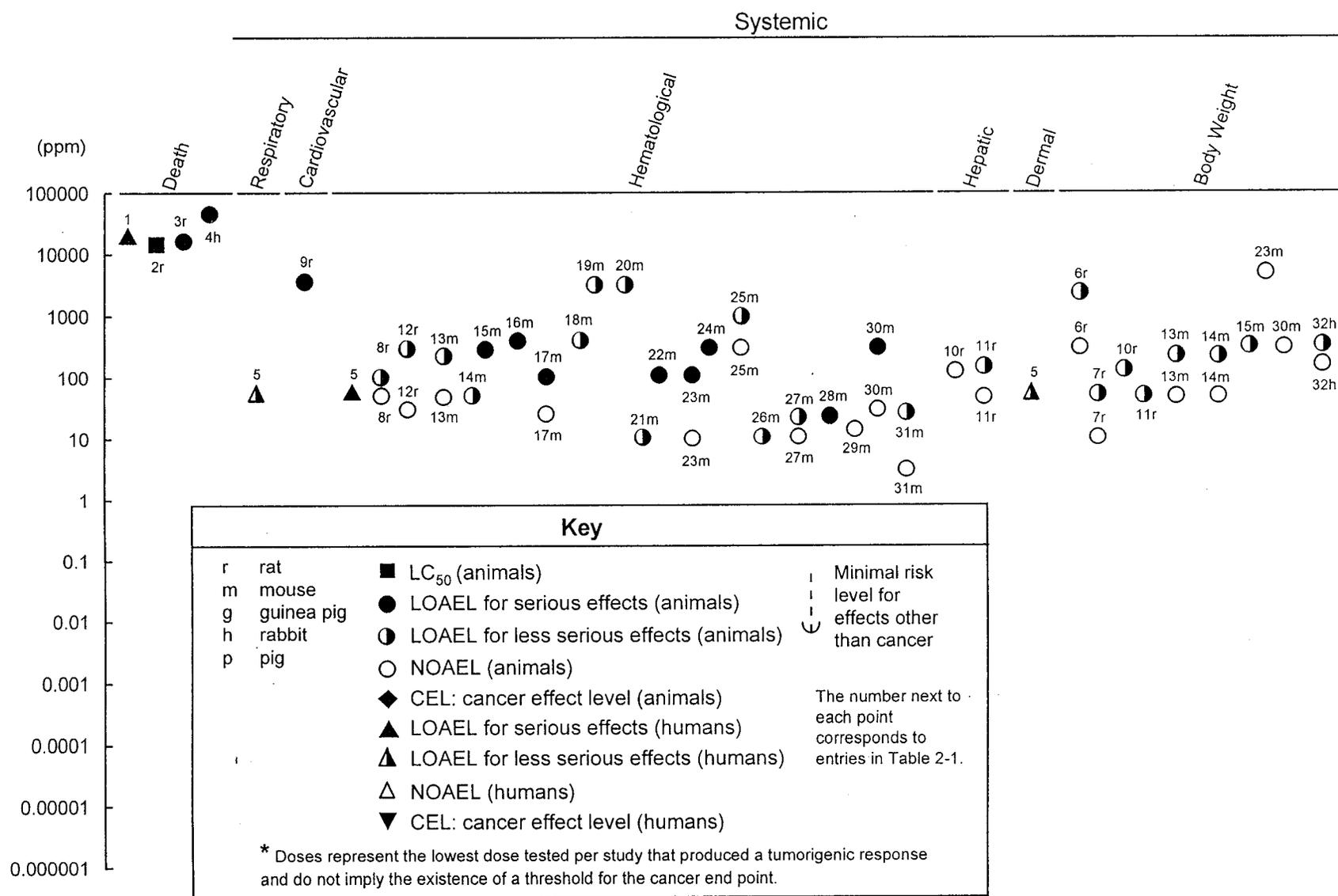


Figure 2-1. Levels of Significant Exposure to Benzene - Inhalation (cont.)

Acute ( $\leq 14$  days)

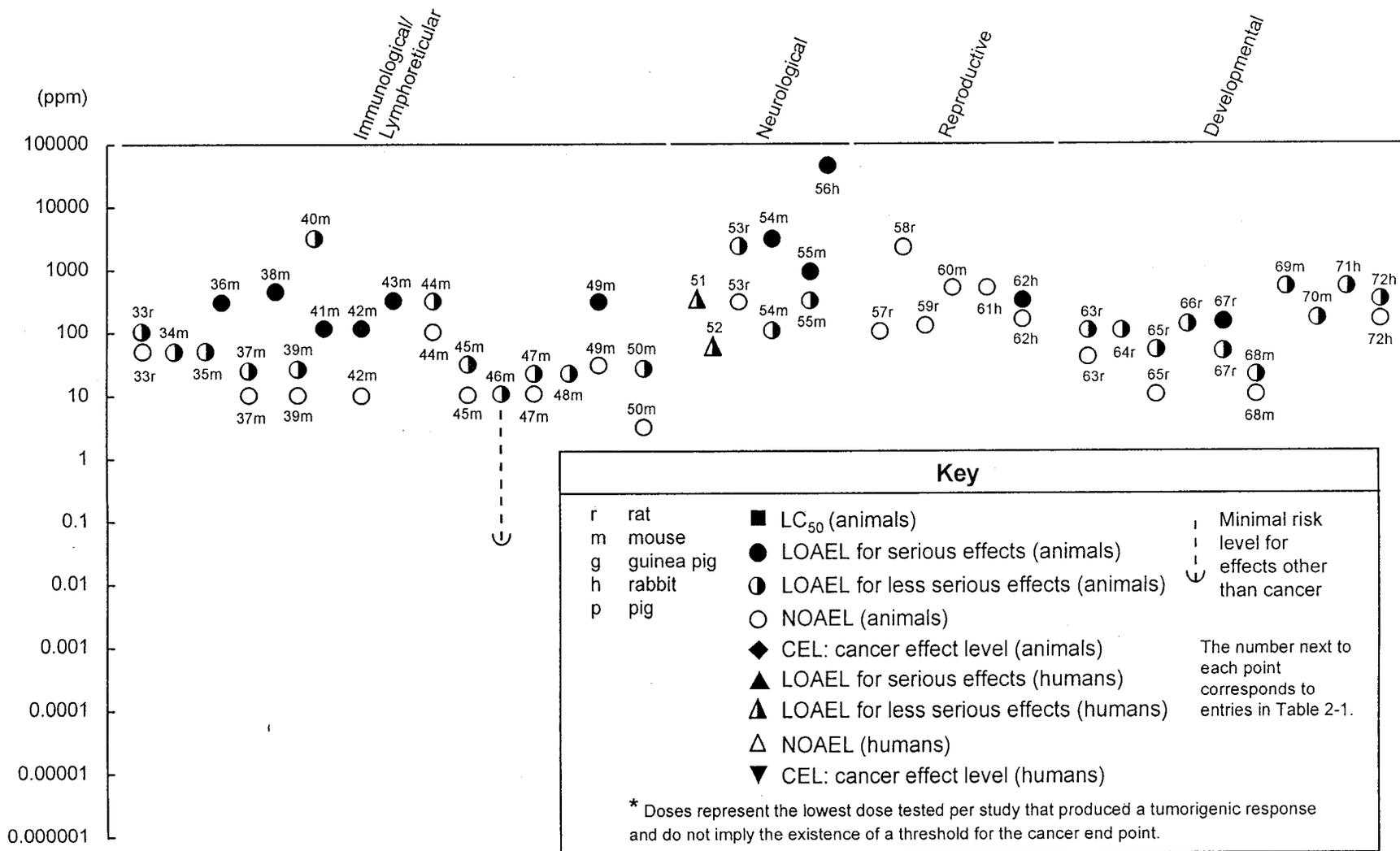


Figure 2-1. Levels of Significant Exposure to Benzene - Inhalation (cont.)

Intermediate (15-364 days)

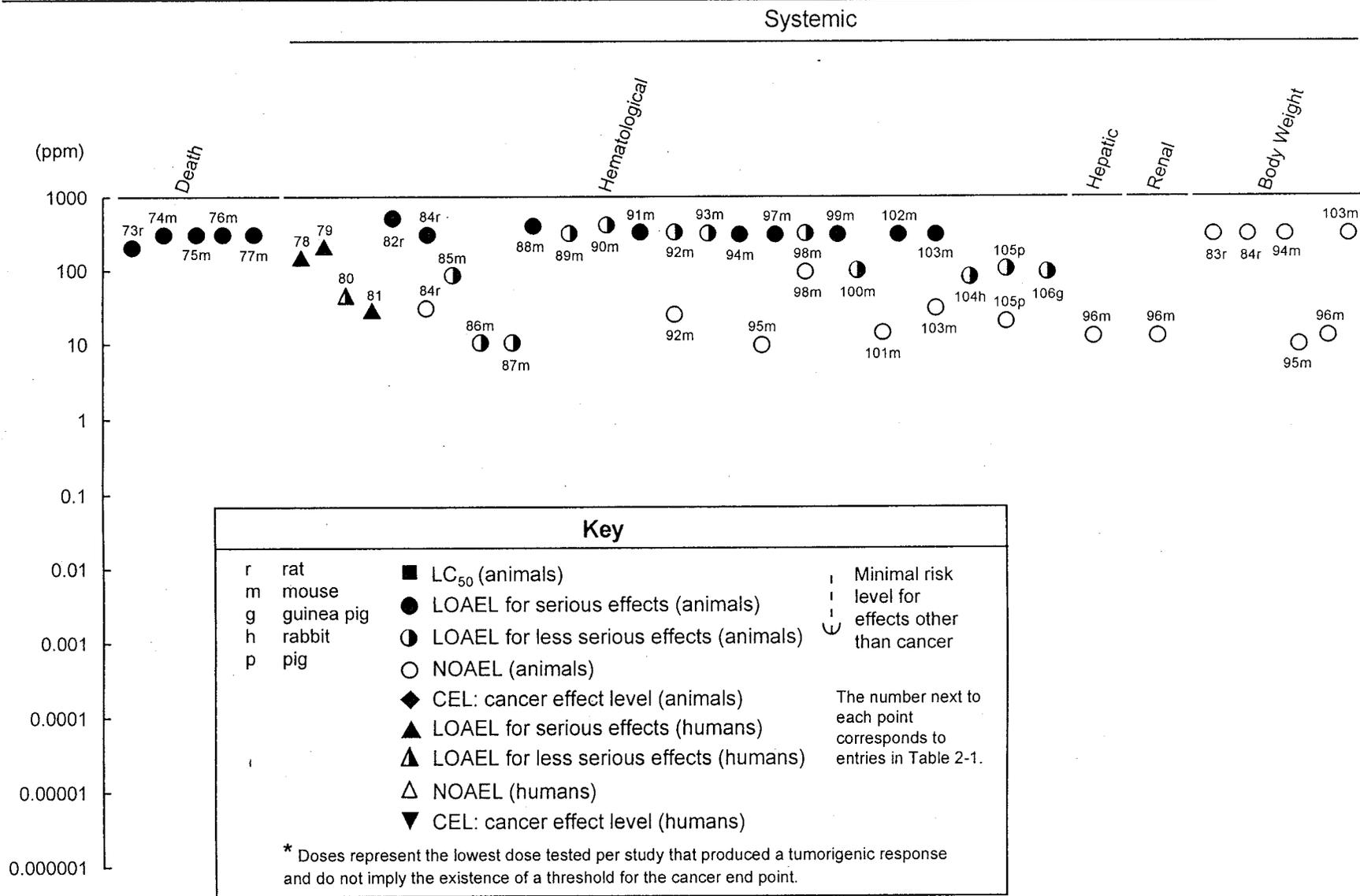
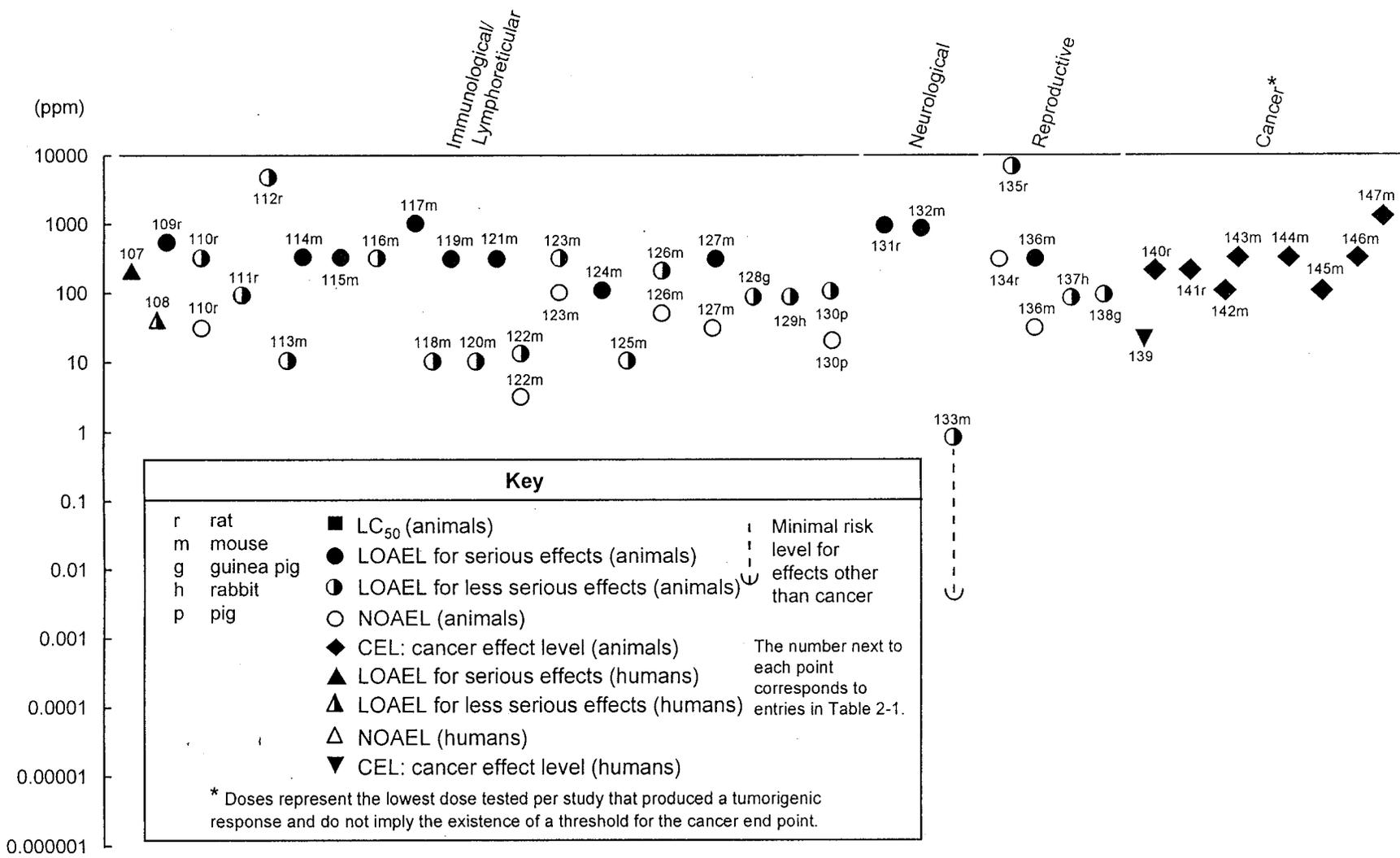
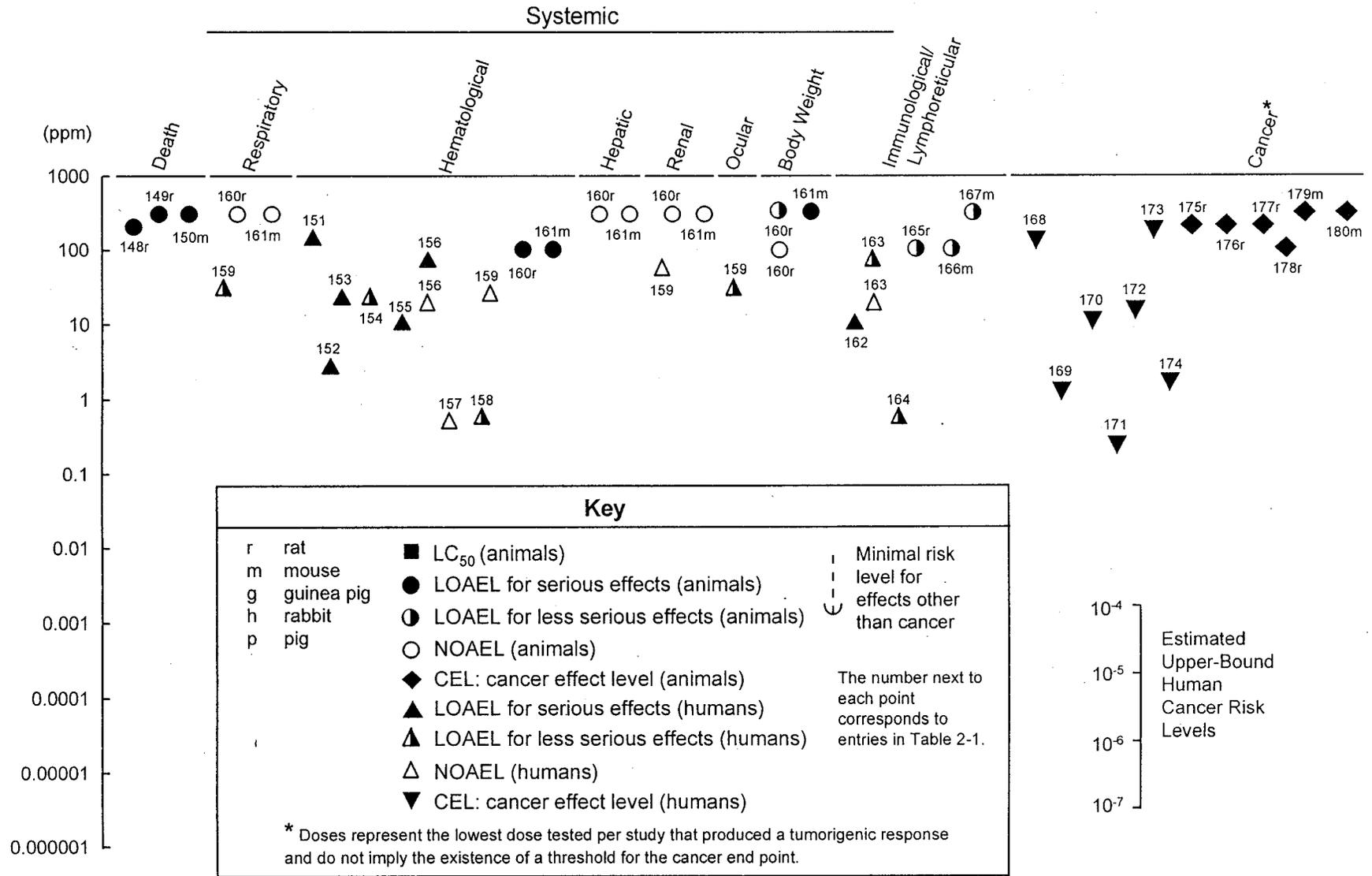


Figure 2-1. Levels of Significant Exposure to Benzene - Inhalation (cont.)  
Intermediate (15-364 days)



**Figure 2-1. Levels of Significant Exposure to Benzene - Inhalation (cont.)**  
 Chronic ( $\geq 365$  days)



### 2.2.1.2 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 2- 1 and plotted in Figure 2- 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 Collum 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 greater than 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 Collum 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 per week, 6 hours per 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).

**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. 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 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. These studies, described in detail below, generally have limitations, such as concomitant exposure to other chemicals and lack of appropriate control groups. In addition, a lack of adequate exposure data precludes a qualitative determination of the relationship between severity of effects and exposure levels. However, sufficient data are available to show that the hematopoietic system is a major target for benzene toxicity. Studies that were conducted well and that show effects linked to specific exposure levels are presented in Table 2-1 and Figure 2-1. Effects on leukocytes, lymphocytes, and bone marrow are also discussed in Section 2.2.1.3.

The human studies described below show that inhalation exposure to benzene for several months to several years results in pancytopenia or other deficits in the relative numbers of circulating blood cells. Continued exposure to benzene can also result in aplastic anemia or leukemia.

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. Aplastic anemia can progress to a type of leukemia known as acute myelogenous leukemia (Aksoy 1980), which is discussed in Section 2.2.1.8.

At least one study revealed that very low levels of benzene exposure produce no adverse hematological effects. This study of workers in a Texas refinery showed no changes in erythrocytes, leukocytes, thrombocytes, hemoglobin (Hgb), or hematocrit in workers who were exposed to benzene at 0.53 ppm for 1-21 years (Tsai et al. 1983).

Two studies of workers exposed to low levels of benzene in the workplace showed slight decreases in erythrocyte counts. A morbidity study of 282 workers in a chemical factory reported that 10 persons who were exposed to over 25 ppm of benzene in the workplace for an average of 9 years (range 3-29 years) had an increased mean corpuscular volume at the end of the high exposure period (1963), but normal values 11 years later (1974) (Fishbeck et al. 1978). Further study of these 282 workers revealed slight decreases in erythrocyte counts that were not correlated with levels of benzene exposure (2-35 ppm) or with duration of employment (1 month to over 20 years) (Townsend et al. 1978).

Persons employed in the rubber industry have also shown decreased blood cell counts that did not persist when benzene exposure decreased. Individual hematological records, demographic data, and chronological work histories of 459 workers employed between 1940 and 1975 at a rubber products manufacturing plant in Ohio were assessed (Kipen et al. 1989). Significant decreases in white and red

cell counts and hemoglobin were recorded for the period of 1940-48 when the exposure was 75 ppm. The trend was not apparent during later years (1949-75) when the exposure was decreased to 15-20 ppm. In a more in-depth study of the same workers, Cody et al. (1993) examined the blood counts of 161 workers for whom pre-employment counts were done prior to exposure in the rubber factory. The results indicated that during the first year of employment in the rubber factory, employees exposed to benzene levels higher than the median exposure (estimated at 40-54 ppm) had significantly lower white and red blood cell counts than employees exposed to benzene levels below the median exposure.

A series of studies was conducted on Turkish workers exposed to benzene-containing adhesives in various occupations. These studies 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 in small, shoe-manufacturing shops (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. Of these, 46 showed loss of leukocytes. The most common findings were leukopenia (21 cases, 9.7%), thrombocytopenia (4 cases, 1.8%), leukopenia associated with thrombocytopenia (10 cases, 4.6%), pancytopenia (6 cases, 2.8%), and eosinophilia (5 cases, 2.3%). 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.

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. Mild hematological abnormalities were noted in 14 workers including leukopenia in 9, thrombocytopenia in 4, and pancytopenia in 1. No LOAEL for the exposure to benzene alone could be defined in this study.

Another study revealed effects, ranging from mild to severe, of benzene exposure in factory workers in China (Yin et al. 1987c). The primary activities in these factories were the manufacture of paints, shoes, rubber, leather, and/or adhesives (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; about 1% had levels of 13-264 ppm. Benzene toxicity, as indicated by leukopenia (leukocyte <4,000/mm<sup>3</sup>), 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  $\geq 29$  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.

A study conducted by Li et al. (1994) during 1972-87 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. There were no gender differences in relative risks for mortality and cancer mortality, although risks tended to be somewhat higher in male workers than in female workers.

Another study suggested a correlation for hematotoxicity in workers exposed to benzene for 1-3 years at 3.2-12.8 ppm levels, which are 2-8 times the Russian standard (Doskin 1971). Included in the hematological findings were lymphocytosis, a biphasic leukocyte response (leukocytosis followed by leukopenia), or bone marrow hypercellularity for individuals that presented hematological abnormalities. Benzene levels and monitoring procedures were not clearly defined in this study. 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.

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).

In summary, benzene poisoning results in the development of pancytopenia, a condition characterized by decreased numbers of circulating erythrocytes, leukocytes, and thrombocytes. The potential mechanisms for the development of pancytopenia in humans include the destruction of bone marrow stem cells, the impairment of the differentiation of these cells, and/or the destruction of more mature hematopoietic cell precursors and circulating cells. Furthermore, pancytopenia can result from the combined destruction of the peripheral blood and bone marrow elements. In effect, individuals that develop pancytopenia and have continued exposure to benzene may develop aplastic anemia (i.e., pancytopenia associated with fatty replacement of functional bone marrow); others may exhibit both pancytopenia and bone marrow hyperplasia, a condition that suggests a preleukemic state. The data suggest that individual workers vary in their reactions to benzene.

More animal than human data are available from which to determine LOAEL or NOAEL values of benzene hematotoxicity. The data show that 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. 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. The following studies demonstrate these adverse hematological effects in animals. Effects on leukocytes, lymphocytes, and bone marrow are also discussed in Section 2.2.1.3.

Acute studies have shown that benzene exposure leads to anemia (any condition in which the number of erythrocytes in 1 mm<sup>3</sup> of blood, or the amount of hemoglobin in 100 mL of blood, or the volume of packed erythrocytes per 100 mL of blood is below normal levels). One study found changes in the levels of peripheral erythrocytes in C57BL/6 male mice that were acutely exposed to benzene by inhalation. Erythrocytes were elevated at 10 ppm, equal to levels in control animals at 31 ppm, and depressed at 100 and 301 ppm (Rozen et al. 1984). Decreased erythrocytes and lymphocytes were observed in mice exposed to 300 ppm intermittently for 6 hours a day, 5 days a week, over a period of 6 days to 23 weeks (Rozen and Snyder 1985; Ward et al. 1985). Ward et al. (1985) also noted decreased hemoglobin content, leukopenia, and decreased hematocrit after 2 weeks exposure at 300 ppm, progressing to pancytopenia. Decreased levels of leukocyte counts have also been noted in the blood and spleen of mice exposed to 211 ppm of benzene for 6 hours per day for 7 days or 48 ppm for 14 days (Aoyama 1986). Male Swiss-Webster mice were exposed via inhalation to benzene (3-2,290 ppm) for 6 hours per day for 5 days (Wells and Nerland 1991). Decreased leukocytes were observed at doses of 25 ppm and above. Green and colleagues examined the effect of benzene inhalation on the peripheral blood (Green et al. 1981b), 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 per day for 5 days. Green et al. (1981b) observed granulocytopenia and lymphocytopenia at  $\geq 103$  ppm, with no change in leukocytes differential; leukocytes decreased 43% compared to controls. Exposures at 306 to 4,862 ppm benzene decreased peripheral leukocytes to levels that were from 36 to 27% of control values. Lymphocytes and polymorphonucleocytes were also significantly depressed at doses of 103 ppm and above. Erythrocyte counts were depressed only at 2,416 and 4,862 ppm concentrations. Hematocrits of test animals were equivalent to control values at 1.1, 9.9, 603, and 1,276 ppm and were depressed at 103, 306, and 2,416 ppm showing no clear dose/response effect. Marrow cellularities were reduced at doses of 103 ppm and above (Green et al. 1981a, 1981b).

NMRI male mice exposed to benzene at concentrations ranging from 1 to 200 ppm, exhibited a reduction in bone marrow cellularity (nucleated cells per tibia), a reduction in the number of colonyforming granulopoietic stem cells (CFU-C) per tibia, and an increase in frequency of micronucleated polychromatic erythrocytes (MN-PCE) that varied with dose and duration of exposure as explained below (Toft et al. 1982). Mice exposed continually (24 hours/day) to 21 ppm or more benzene in air for 4-10 days showed significant changes in all of the parameters. No adverse effects on hematological parameters were noted in mice exposed to 14 ppm benzene continuously for 1-8 weeks (Toft et al. 1982). Intermittent exposure (8 hours a day, 5 days a week for 2 weeks) to 21 ppm, but not to 10.5 ppm, significantly reduced the number of CFU-C per tibia and elevated the frequency of MN-PCE, but did not affect bone marrow cellularity. Intermittent exposure to 50 ppm or more caused significant changes in all 3 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 to 95 or 201 ppm benzene for 2-8 hours per day, 5 days per week for 2 weeks, decreased cellularity and CFU-C per tibia were observed at 95 ppm after 6-8 hours per day exposure, whereas at 201 ppm benzene, decreased cellularity but no effect on CFU-C was noted after 2 hours per day exposure. A decrease in cellularity and CFU-C was observed after 4-8 hours per day exposure to 201 ppm benzene (Toft et al. 1982). DBA/2 mice exposed to 10 ppm benzene for 6 hours per day for 5 days exhibited a 50% reduction in CFU-E (erythroid progenitor cells) per tibia (Dempster and Snyder 1991). Decreases in bone marrow cellularity in the femur, hematocrit, and leukocytes were seen in DBA/2 mice exposed to 300 ppm benzene in air for 6 hours per day, 5 days per week for 2 weeks (Chertkov et al. 1992). Similar effects on bone marrow cellularity and CFU-E development were observed after a similar exposure of Swiss Webster and C57BL/6J mice (Neun et al. 1992, 1994). Neun et al. (1994) also extended these experiments, in which the bone marrow of Swiss Webster mice and C57BL/6J mice exposed to benzene under the same regimen (300 ppm, 6 hours per day, 4 days per week for 2 weeks) were cultured with benzene metabolites to assess bone marrow viability (see Section 2.5). Wistar rats exposed to doses of benzene up to 300 ppm for 8 hours per day for 7 days exhibited leukopenia at 100 ppm and greater, but not at 50 ppm (Li et al. 1986). Leukopenia was also observed in C57BL/6J mice exposed continuously to 100-1,000 ppm for 2-8 days (Gill et al. 1980). Sprague-Dawley SD/Tex rats were exposed to benzene vapor at 0 or 500 ppm for 5 day per week, 6 hours per day for 3 weeks (Dow 1992). Blood from the animals was evaluated for hematologic changes and the bone marrow for the presence of multinucleated erythroblasts. Animals exposed to 500 ppm showed decreased lymphocyte and leukocyte counts. Erythrocytes and hemoglobin values increased. In the bone marrow differential counts, rats showed a

relative decrease in lymphoid and myeloid cells at the 500 ppm dose level and an increase in erythrocytic cells. In a companion study, purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours per day, 5 days per week for 3 weeks (Dow 1992). Decreased peripheral leukocytes, total lymphocytes and T-cells, and increased erythroid cells were observed at 100 ppm. Exposure to 300 ppm for 6-9 weeks caused a reduction in erythroid progenitor cells in the femurs of C57BL/6 or hybrid mice (Baarson and Snyder 1991; Vacha et al. 1990). Depression of CFU-E was also noted in C57BL/6 mice from exposure to as little as 10 ppm of benzene for 9 weeks (Baarson et al. 1984).

Female BDF<sub>1</sub> mice (C57BL/6xDBA/2F<sub>1</sub> hybrids) were exposed to 100, 300, and 900 ppm benzene 6 hours per day, 5 days per week for up to 8 weeks (Plappert et al. 1994a). Benzene concentrations in the chamber were monitored 2 times per day by gas chromatography (GC). Hematological studies included peripheral blood data, T4 and T8 lymphocyte counts in the blood and spleen, and hemopoietic stem and progenitor cell assays in the marrow (spleen colony-forming units [CFU-S], colony-forming unit-culture [CFU-C], and erythroid burst-forming unit [BFU-E], erythroid colonyforming unit [CFU-EI]). No significant changes were observed in the peripheral blood data of mice exposed to 900 ppm benzene. Some perturbation of the reticulocyte numbers was observed, but values at days 3 and 5 did not differ from controls. Absolute numbers of lymphocytes and neutrophils did not differ from controls (no data shown). A slight anemia was observed at 4 and 8 weeks of treatment with 300 and 900 ppm benzene. Results showed minor changes in the stem and progenitor cells. CFU-E depression after 4 days of exposure was significant. There was a dose-dependent depression of colony forming cell numbers present at 4 and 8 weeks of exposure with a maximal effect at the level of CFU-E. Decreased CFU-E and BFU-E were observed in a companion study in which hybrid mice were exposed to 300 or 900 ppm benzene for the same period of time (Plappert et al. 1994b).

Granulocytic hyperplasia has been detected in the bone marrow of mice exposed to 300 ppm benzene in air for 6 hours per day, 5 days per week for 16 weeks, and held 18 months after the last exposure (Farris et al. 1993). Sprague-Dawley rats received 100 or 300 ppm benzene vapor for 6-hours per day, 5 days per week for 691 days (Snyder et al. 1978a, 1984). Anemia and leukopenia were observed at both concentrations. In addition, at both concentrations, hemosiderin was found in the spleen, which could be due to erythrocyte hemolysis. AKR mice were exposed to 100 or 300 ppm benzene vapor for 6 hours per day, 5 days per week for life (Snyder et al. 1978a, 1980). C57BL mice were exposed to 300 ppm benzene vapor for 6 hours per day, 5 days a week for life (Snyder et al. 1980). At

100 ppm, anemia, lymphocytopenia, and bone marrow hypoplasia were observed in AKR mice; the same effects at greater severity were observed in both strains at 300 ppm. Stem cell depression in the bone marrow was noted in several studies in mice exposed intermittently to benzene for 2 days to 16 weeks (Cronkite et al. 1982, 1985, 1989). Prolonged exposure to lower levels had a greater hematotoxic effect than exposure to higher levels for a shorter period of time (Cronkite et al. 1989). These studies also revealed that recovery could occur within a few weeks after cessation of exposure. Intermittent exposure of Hale-Stoner mice to 400 ppm benzene over 11 days caused decreased erythrocytes and leukocytes (Cronkite et al. 1982). Lymphopenia (at 25 ppm), anemia, and stem cell depression in the bone marrow (at 100 ppm) were reported in C57BL/6 mice after 2 weeks of exposure (Cronkite et al. 1985). Recovery of the bone marrow occurred following cessation of exposure. In addition, C57BL/6 mice or Hale-Stoner mice were exposed to 10, 25, 100, 300, or 400 ppm benzene for up to 16 weeks (Cronkite et al. 1982, 1985). At 2 weeks, exposure to 10 or 25 ppm did not result in adverse effects, but exposure to 100 ppm or higher concentrations for two or more weeks significantly reduced overall cellularity and the number of pluripotent stem cells of the bone marrow. Stem cell recovery was studied in the 300 ppm exposure group (Cronkite et al. 1985). In the animals exposed for 2 and 4 weeks, the stem cell numbers had returned to control levels by 2 weeks after exposure. Those exposed for 8 weeks had recovered by 16 weeks, and those exposed for 16 weeks had recovered incompletely to 92% of control values by 25 weeks. In CBA/Ca male mice, inhalation of 100, 300, or 400 ppm benzene for 6 hours per day, 5 days per week for up to 16 weeks produced dose-dependent decreases in bone marrow cellularity and marrow content of spleen colony-forming units (CFU-S) (Cronkite et al. 1989). In addition, a comparative study on effect and recovery of bone marrow cellularity and CFU-S was conducted on CBA/Ca mice exposed to the same total amount of benzene over different exposure regimens. Although the decrease in marrow cellularity of mice exposed to 3,000 ppm for 8 sessions (Regimen 1) was similar to that of mice exposed to 300 ppm for 80 sessions (Regimen 2), bone marrow cellularity in mice exposed for only 8 sessions recovered by 30 days after exposure, whereas recovery took longer (60 days) for mice exposed for the longer period of time (80 sessions). The number of CFU-S decreased to 62% and 38% of controls for Regimens 1 and 2, respectively. The number of CFU-S had recovered by 30 days post exposure for mice in Regimen 1; recovery was slower and incomplete by 178 days post exposure for mice in Regimen 2. Thus, prolonged exposure to lower levels of benzene had a greater hematotoxic effect than exposure to higher levels of benzene over a shorter duration. These findings were further confirmed in separate experiments on two groups of CBA/Ca mice following benzene inhalation exposure. One group was exposed 19 times to 316 ppm of benzene, 6 hours per day,

5 days per week and the second group 2 times to 3,000 ppm, 6 hours per day (Cronkite et al. 1989). Although the total amount of inhaled benzene was the same for both groups (6,000 ppm), the effects on lymphocytes, neutrophils, eosinophils, and the bone marrow content of CFU-S were much more pronounced and persisted for up to 214 days in the lower exposure group (316 ppm). These results suggest that longer exposure to 316 ppm of benzene may cause irreparable hematologic injury, while the hematotoxic effects caused by short exposure to 10 times higher benzene concentrations are reversible.

Green et al. (1981b) examined the effect of benzene inhalation on the peripheral blood and bone marrow of CD-1 mice following exposures to 0 or 9.6 ppm for 6 hours per day, 5 days per week for 10 weeks (50 days), or to 0 or 302 ppm for 6 hours per day, 5 days per week for 26 weeks. Animals exposed to 9.6 ppm for 50 days showed no detectable changes in the peripheral blood or bone marrow. Exposure to 302 ppm benzene for 26 weeks resulted in lymphocytopenia and anemia. Total leukocytes decreased to 48% of control values. Differentials revealed that lymphocytes were reduced to 13% of control values while the number of circulating polymorphonuclear leukocytes (PMN) appeared to be elevated. Circulating erythrocyte levels of test mice were reduced to 63% of control values. Red cell morphology was characterized by polychromasia, anisocytosis, poikilocytosis, stippling, and numerous Howell-Jolly bodies. Marrow cellularity was reduced to 32% of control, which was due primarily to a reduction in lymphocytes and granulocytes. Morphologically, nucleated marrow cells displayed a variety of nuclear/cytoplasmic blebbing, vacuolization, and atypical mitotic figures. In addition, asynchronous nuclear/cytoplasmic maturation was observed in myeloid precursors.

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, B6C3F<sub>1</sub>, and C57BL/6) exposed to 300 ppm benzene for 13 weeks (6 hours per day) for either 5 days per week (Regimen 1) or 3 days per 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 = B6C3F<sub>1</sub> > DBA/2) and regimen (Regimen 1 > Regimen 2), whereas the increase in

frequency of MN-PCE was dependent on strain (DBA/2 > C57BL/6 = B6C3F<sub>1</sub>) but, for the most part, was not dependent on exposure regimen.

Damaged erythroblast-forming cells were also noted in bone marrow (Seidel et al. 1989b). A substantial decrease in erythroid colony-forming units and smaller decreases in erythroid burst-forming units occurred in BDF<sub>1</sub> mice intermittently exposed to 100 ppm of benzene for 8 weeks (6 hours/day, 5 days per 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.

The peripheral blood and bone marrow were altered in CD-1 mice and Sprague-Dawley rats exposed for 91 days (13 weeks) to up to 300 ppm benzene (Ward et al. 1985). Interim sacrifices were performed on days 7, 14, 28, and 56 of exposure. No hematological effects were observed at 1, 10, or 30 ppm. At 300 ppm, however, mice exhibited significant increases in mean cell volumes and mean cell hemoglobin values on day 14. However, on days 28, 56, and 91, decreases in hematocrit, hemoglobin, erythrocyte count, leukocyte count, and platelet count were observed. The most common compound-related histological findings in mice occurred at 300 ppm and included myeloid hypoplasia of the bone marrow and increased extramedullary hematopoiesis in the spleen. Lesions were present at early sacrifice time points and were detected throughout the study, increasing in severity and incidence with time. The effects were present more often in males than in females and were more severe in males. The only significant change in rats exposed to 300 ppm was a decreased leukocyte count that was first noted in males at day 14 and in females at day 91. There was also a significant decrease in the percentage of lymphocytes in both male and female rats exposed to 300 ppm of benzene vapors throughout the whole study up to day 91.

Decreased levels of circulating leukocytes were reported following intermediate-duration exposure to benzene at levels of 80 ppm in the rabbit and 88 ppm in the rat and guinea pig (Wells and Nerland 1991; Wolf et al. 1956). Depression of lymphocytes and numbers of nucleated red cells formed in the spleen was also noted in C57BL/6 mice from exposure to as little as 10 ppm of benzene for 24 weeks (Baarson et al. 1984).

**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 time-weighted average (TWA) concentration for gasoline station attendants was estimated to be <0.2 ppm. The occupational 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.

CFY rats (20/group) were exposed to pure air, benzene (125 ppm) or benzene (400 ppm) and toluene (265 ppm) for 24 hours per 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 per 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 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 per week, 6 hours per 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 is 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 per week, 6 hours per day for life (Snyder et al. 1978a, 1984) or in AKR/J or C57BU6J mice similarly exposed to 300 ppm (Snyder et al. 1978a, 1980).

**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 greater than 60 ppm for up to three weeks (Midzenski et al. 1992). These effects are due to direct contact of the skin with the vapor, and other dermal effects L resulting from direct contact of the skin are discussed in Section 2.2.3.2.

**Ocular Effects.** Three hundred solvent workers who had inhalation exposures for more than 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 per day, 5 days per week for 10 weeks exhibited lacrimation at concentrations  $\geq 10$  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 2.2.3.2

**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 per 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 per day, 5 days

per 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 per 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 per day, 6 days per 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 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 per 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 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 per day, 6 days per 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 per day, 5 days per 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 per day, 5 days per week for life had decreased weight gain at 300 ppm (Snyder et al. 1978a, 1980).

### **2.2.1.3 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. One of the problems with these studies is that the workers were exposed to multiple solvents; therefore, benzene alone may not be responsible for the noted effects.

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). The levels of benzene exposure ranged from 1 to 1,060 ppm. These studies are more fully described in Section 2.2.1.2. In one study, routine 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 and is associated with both decreased white blood cell counts and with changes in bone marrow activity (Yin et al. 1982). 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 1972-87 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 acute-duration exposure to benzene at 25 ppm, 6 hours per day for 5 days, the same dose levels 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 Band 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 inhalation MRL of 0.05 ppm (see footnote to Table 2-1 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 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 acute-duration 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 per 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); and decreased leukocytes in mice exposed to 300 ppm for 10 days (Ward et al. 1985). These studies are more fully described in Section 2.2.1.2. Decreased spleen weight and levels of B- and T-lymphocytes have also been noted in the blood and spleen of mice exposed 6 hours per day to 47-48 ppm of benzene for 7 or 14 days (Aoyama 1986). Decreased thymus weights were also noted at the 48 ppm dose after 14-day exposure (Aoyama 1986).

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 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 per day for 5 days. Intermediate studies consisted of two different exposure regimens and concentrations: 6 hours per day, 5 days per week for 10 weeks (50 days) to a mean concentration of 0 or 9.6 ppm benzene; or 6 hours per day, 5 days per week for 26 weeks to a mean concentration 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  $\geq 103$  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 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 per week, 6 hours per 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 per day, 5 days per week for 3 weeks (Dow 1992). Exposure to 500 ppm showed a statistically significant T-cell depression and leukopenic and erythropathic effects in cells of the pigs.

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 et al. 1985, 1986). 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 2 groups of male CBA/Ca mice exposed to a total of 6,000 ppm of benzene by inhalation using 2 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, indicating the importance of the exposure regimen. In both groups, the lymphocyte numbers had not returned to normal 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/2F<sub>1</sub> hybrids) exposed to 0, 300, or 900 ppm benzene 6 hours per 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. 1994). 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). For further information, see Section 2.2.1.2.

Intermediate-duration exposure to benzene also decreased leukocyte counts in Sprague-Dawley rats and CD-1 mice exposed to 300 ppm for 14-91 days (2-13 weeks) (Ward et al. 1985). Leukopenia, granulocytopenia, and lymphocytopenia were also observed in C57BL/6 mice exposed intermittently to 1,000 ppm for 16 weeks (Gill et al. 1980). 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 (Yin et al. 1982). Exposure of C57BL

mice to 10 ppm benzene for 6 hours per day, 5 days per week, for 24 weeks causes depressions in the numbers of splenic nucleated red cells and lymphocytes (Baarson et al. 1984). Leukopenia, granulocytopenia, and lymphocytopenia were observed in C57BL/6 mice exposed to 1,000 ppm benzene for 6 hours per day, 5 days per week for 6 weeks (Gill et al. 1980).

Li et al. (1992) observed a 26% decrease in spleen weight in male Kunming mice exposed to 12.52 ppm benzene 2 hours per day, 6 days per 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  $\geq 30$  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 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. This indicates a benzene-induced delay in immune response to *L. monocytogenes*. Concentrations of 200 or 400 ppm for 4-5 weeks (5 days per 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 per 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. A similar *in vitro* observation was made using T-cells from mice exposed to 100 ppm of benzene 5 days per 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).

Chronic exposure to benzene caused bone marrow hypoplasia, lymphocytopenia, and anemia in mice exposed to 100 ppm for a lifetime (Snyder et al. 1980). Sprague-Dawley rats received 100 or 300 ppm benzene vapor for 6 hours per day, 5 days per week for life (Snyder et al. 1978a, 1984). Leukopenia and lymphopenia were observed starting during the first year at 100 ppm. AKR mice were exposed to 100 or 300 ppm benzene vapor for 6 hours per day, 5 days per week for life and C57BL mice were exposed to 300 ppm benzene vapor using the same exposure regimen (Snyder et al. 1978a, 1980). At 100 ppm, anemia, lymphocytopenia, and bone marrow hypoplasia were observed in AKR mice; the same effects were observed to a greater extent in both strains at 300 ppm.

The highest NOAEL values and all reliable LOAEL values for immunological effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

#### **2.2.1.4 Neurological Effects**

Following acute inhalation of benzene, humans exhibited 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, included 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 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 transferred from the problem area (Kraut et al. 1988; Yin et al. 1987b). In reports of cases of benzene poisoning, subjects exhibited headaches, nausea, tremor, convulsions, and unconsciousness, among other neurological effects (Cronin 1924; Davies 1929; Greenburg 1926; Midzenski et al. 1992; Tauber 1970).

Chronic exposure to benzene has been reported to produce neurological abnormalities in humans. Of eight patients (6 with aplastic anemia and 2 with preleukemia) with previous occupational exposure to adhesives and solutions containing 9-88% benzene, 4 of the 6 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 or higher. 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/m<sup>3</sup>), while the toluene vapors did not exceed the 5 mg/m<sup>3</sup> level. Subsequently (the authors do not specify when), the air levels of both benzene and toluene have not exceeded the 5 mg/m<sup>3</sup> 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 (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 per 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 per day, 6 days per 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 were observed. This study is the basis for the intermediate-duration inhalation MRL of 0.001 ppm (see the footnote to Table 2-1 and Appendix A).

All reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

#### **2.2.1.5 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 because the studies are limited. 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. In another study, 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 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. In a more recently published study, reproductive competence of male workers in two organic chemical factories in France was evaluated (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: less than 5 ppm (low) and greater than or equal to 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 pure air, or benzene (125 ppm) for 24 hours per 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 per day on Gd 6-15 (Coate et al. 1984). Pregnant rabbits exposed 12 hours per 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, male and female CD-1 mice were exposed 5 days per week, 6 hours per day to benzene vapor for 13 weeks (Ward et al. 1985). Histopathological 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 a day, 5 days a 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 1 or 2 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 2- 1 and plotted in Figure 2-1.

#### **2.2.1.6 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). 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 sternalbrae 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 per 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 per 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 per 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 per 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 litter incidence of missing sternalbrae was significantly increased in the 100 ppm 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 sternalbrae.

Kuna and Kapp (1981) found decreased fetal weight after exposure to 50 ppm and demonstrated that there was only 1 exencephalic rat in a group of 151 pups examined after *in utero* exposure to 500 ppm benzene. In the same study, of 98 pups examined for skeletal effects after *in utero* exposures of 500 ppm, only 1 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 per day (Tatrai et al. 1980b) or 100 ppm for 10 days, 6 hours per 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 per 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.

In a follow-up study, pregnant Swiss Webster mice were exposed 6 hours per 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 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 ( $\approx 10$  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 2-1 and plotted in Figure 2-1.

#### **2.2.1.7 Genotoxic Effects**

The available evidence for benzene-induced genetic toxicity in humans comes from studies of occupational exposure to benzene. In most of these studies, chromosome abnormalities were detected at exposure levels sufficient to produce blood dyscrasias. Exposures reported in these studies were primarily via inhalation, although dermal exposure may have occurred in some cases. The limitations of occupational studies include lack of accurate exposure data, possible coexposure to other chemicals,

and lack of appropriate control groups. The majority of these studies suggest that benzene and its metabolites are genotoxic, specifically clastogenic (i.e., capable of breaking the deoxyribonucleic (DNA) molecule in a manner observable at the chromosome level).

The evidence that benzene is clastogenic in humans comes from observed chromosomal aberrations in peripheral lymphocytes (Ding et al. 1983; Forni et al. 1971a; Karacic et al. 1995; Major et al. 1994; Picciano 1979; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Turkel and Egeli 1994). In one study, 21 people with hematological signs of chronic benzene poisoning exhibited significantly more chromosomal abnormalities (including hypo- and hyperdiploidy, deletions, gaps, and breaks) than the controls (Ding et al. 1983). The levels of benzene in air were not reported. Another study investigated benzene's effects in 33 workers exposed to air concentrations below 0.1 mg/L (31 ppm) for 10-26 years. Thirtyone of the workers did not show any clinical or hematological symptoms of chronic benzene intoxication. Two cases of pancytopenia were observed. The researchers reported nonrandom breaks and gaps in the chromosomes of the exposed group: chromosomes two, four, and nine were nearly twice as prone to breaks, while chromosomes one and two were nearly twice as prone to gaps (Sasiadek et al. 1989). However, this study is limited because relatively few controls were used, and all participants smoked which pre-exposed them to toxic compounds, including various levels of benzene, a component of tobacco smoke. The incidence of chromosome damage was much lower among the controls, and in addition, the distribution of damage in the controls was found to be random. The implication is that benzene and/or its metabolites may preferentially attack certain chromosomes (Sasiadek et al. 1989).

The peripheral lymphocytes of 20 male factory workers exposed to ambient benzene (concentrations not specified) for 1-20 years were tested for chromosomal aberrations; the numbers of aberrant chromosomes were compared to those of 43 controls (Tough and Court Brown 1965). The researchers found a significantly higher number of unstable chromosomal aberrations, including acentric, dicentric, tricentric, and ring chromosomes, among the 20 exposed workers. Fourteen of the men had neutropenia. Inquiries were made regarding past exposure to radiation since radiation can cause similar effects. The researchers concluded that radiation exposure was not a causal factor (Tough and Court Brown 1965). In an update of this study (Tough et al. 1970), chromosomal aberrations in peripheral lymphocytes of groups of individuals from three different factories were examined. Group 1 workers from factory 1 consisted of 20 men exposed to benzene from 1-20 years and 5 men

not exposed to benzene and employed for similar periods as previously described (Tough and Court Brown 1965). Exposure ceased 2-3 years before the blood samples were obtained. Group 2 workers from factory 2 consisted of 18 men employed for 6-25 years (12 exposed and 6 not exposed to benzene) with intermittent exposure to benzene, ending 4 years before the blood samples were obtained. Group 3 workers from factory 3 consisted of 20 men employed in a closed distillation plant for 2-26 years where benzene exposure continued until the time of the study. Five additional men in Group 3 who worked in factory 3 but were never employed in the distillation plant were used as controls. Blood samples were obtained and analyzed. The number of cells were counted and those containing unstable aberrations (Cu cells) and stable aberrations (C8 cells) were scored. Results showed significant increases in the percentage of cells with unstable chromosome aberrations (Cu cells) in groups of men exposed to benzene as compared with their corresponding controls and controls randomly selected from the general population. There were significant increases in the percentage of Cu cells in the exposed workers in Groups 1 and 2. In Group 2, the frequency of aberrations observed in the exposed workers was higher than the controls from the general population but did not differ from their on-site controls. In Group 3, the level of aberrations was approximately the same in the exposed workers and their on-site controls and neither differed significantly from the general population. Although the results suggest a relationship between benzene exposure and chromosomal aberrations in peripheral lymphocytes, the relationship does not appear to be a simple one.

In a study evaluating 52 workers exposed to benzene for 1 month to 26 years, chromatid breaks, chromosome breaks, marker chromosomes (rings, dicentrics, translocations, and exchange figures), and abnormal cells were evaluated in 200 peripheral lymphocytes from each worker, and the results compared to those from 44 workers with no recent history of benzene exposure (Picciano 1979). Monitoring of workroom air level of benzene during a 4-year period prior to the study indicated TWA levels of 2.1 ppm. However, some of the workers who were employed for up to 26 years may have been previously exposed to much higher levels. The percentage of aberrant findings was 3.5% for the exposed workers, and 2.9% for the unexposed workers. With regard to individual cytogenetic findings, there was no difference in the percentage of chromatid breaks or abnormal cells, but significant increases in frequencies of chromosome breaks (2-fold), and marker chromosomes (3-fold) were found in the peripheral lymphocytes of the exposed workers as compared to 44 control subjects. A significantly higher proportion of exposed workers with either chromosome breaks, or chromosome breaks and marker chromosomes was found. The distribution of individuals with these cytogenetic

aberrations was not associated with age. The lack of blood work-ups precluded the diagnoses of any hematological disorders in these workers. Exposure to ionizing radiation and/or other chemicals may have contributed to the chromosomal aberrations observed in the exposed group.

Case reports involving individuals with long-term occupational exposure to benzene also suggest that benzene damages chromosomes in hematopoietic cells and lymphocytes (Forni and Moreo 1967, 1969; Hartwich et al. 1969; Sellyei and Kelemen 1971; Van den Berghe et al. 1979). One of these case studies reported benzene exposure levels of 200-1,640 mg/m<sup>3</sup> (62.6-513.4 ppm) for a worker employed for 18 months who developed severe anemia, neutropenia, and thrombocytopenia. The patient was treated with transfusions and corticosteroids and showed improvement; however, the benzene-induced hemopathy evolved into leukemia (Sellyei and Kelemen 1971). At the same time, bone marrow specimens taken from this worker revealed the emergence of a new cell line characterized by supernumerary D-group chromosomes.

In another study, metaphase chromosome spreads were evaluated from 48 of 66 benzene-exposed individuals and 29 of 33 controls. The incidences of chromatid deletions and gaps were slightly increased in workers exposed to benzene levels of 10-100 ppm compared with control groups (Yardley-Jones et al. 1990). No concurrent hematological disorders were reported. However, these results were not statistically significant because of the heterogeneity of those examined. A small fraction of the exposed individuals had a higher frequency of chromosomal aberrations. Benzene exposure in these individuals was >5 years, and most of the time, air concentrations were <1 ppm with peaks of 100 ppm. Eliminating the individual with the greatest number of aberrations from the study markedly reduced the significance of the comparison (Yardley-Jones et al. 1990). No mention was made of the presence of hematological disorders in these individuals.

Both numerical and structural aberrations were reported in 9 of 13 patients that worked in an environment with benzene concentrations ranging from 150 to 210 ppm (Erdogan and Aksoy 1973). All 13 patients had abnormalities of the blood (10 with pancytopenia, one with acute myeloblastic leukemia, one with acute monocytic leukemia, and one with preleukemia). Thirty-four workers exposed to high concentrations of benzene showed chromosome damage in peripheral lymphocytes and bone marrow cells that persisted after cessation of exposure (Forni et al. 1971a). Of the 34, 16 had severe hemopathy. Although persistent effects in peripheral lymphocytes were considered to be an indicator that damage had occurred, they provide no conclusive evidence linking chromosomal damage

to the development of leukemic changes. However, stable chromosome aberrations in bone marrow cells could give rise to leukemic clones (Forni and Moreo 1967). For example, a 38 year-old female with benzene exposure for approximately 22 years (level not reported) presented with anemia that evolved, after a long latent period, into acute myeloblastic leukemia. During the latent period, multiple cytogenic studies showed a high rate of unstable and stable chromosome aberrations in cultured peripheral blood lymphocytes (Forni and Moreo 1967).

Several studies looked for benzene's effects on sister chromatid exchange in humans. One of these reports involved workers exposed to benzene levels ranging from 10 to 100 ppm in excess of 5 years (Yardley-Jones et al. 1988). The average benzene concentration from another report was determined to be around 50 ppm (Seiji et al. 1990). The findings were negative in both studies. However, in a third study of occupational exposure, a slight but statistically significant (6.55 compared to 6.05) increase in sister chromatid exchanges was observed in peripheral lymphocytes from workers exposed for an 8-hour TWA of 1.3 ppm benzene (range 0.3-5 ppm), in combination with toluene (Popp et al. 1992).

Data from *in vivo* animal studies strongly support the genotoxicity of benzene. Analyses of chromosomal aberrations, sister chromatid exchange, and micronuclei in the bone marrow and lymphocytes of both mice and rats consistently showed positive results. Acute exposure of male rats to 750 and 7,500 ppm benzene for both single and multiple exposure periods resulted in a significantly increased frequency of chromosomal aberrations in bone marrow cells (Anderson and Richardson 1981). Similarly, male Wistar rats exposed to 100 and 1,000 ppm benzene for 6 hours had elevated frequencies of chromosomal aberrations in bone marrow cells (Styles and Richardson 1984). Male and female DBA/2 mice acutely exposed to 3,100 ppm showed significant increases in sister chromatid exchange among bone marrow cells, but the rise in chromosomal aberrations was not significant (Tice et al. 1980, 1982). Both DBA/2 and C57BL/6 mice exposed to benzene concentrations ranging from 28 to 5,000 ppm exhibited a significant linear increase in sister chromatid exchange frequencies starting at the lowest concentration. The response leveled at 3,000 ppm for the DBA/2 mice and at 2,000 ppm for the C57BL/6 mice (Tice et al. 1982).

The evaluation of micronuclei frequencies in peripheral blood polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) permits an assessment of both recently induced and chronically accumulated bone marrow damage. PCEs have a life span of about 24 hours and are good indicators

of acute damage. NCEs have a life span of about 30 days and are good indicators of chronic damage. A study involving male DBA/2 mice reported a significant dose-dependent increase in lymphocyte sister chromatid exchange frequency and in micronuclei of bone marrow PCEs following a 6-hour exposure to 10, 100, and 1,000 ppm benzene (Erexson et al. 1986). The same experiment also involved Sprague-Dawley rats; there were significant increases in sister chromatid exchange frequencies in peripheral blood lymphocytes following exposures to 3, 10, and 30 ppm and dose-dependent increases in micronuclei of bone marrow PCEs after exposures to 1, 3, 10, and 30 ppm benzene (Erexson et al. 1986). Dose-dependent increases in micronuclei frequencies in bone marrow PCEs were observed in male NMRI mice following both continuous and intermittent acute exposure to benzene; significant increases began at 21 ppm (Toft et al. 1982). One study compared the effects of the duration of benzene inhalation on peripheral blood PCEs and NCEs in male and female DBA/2 mice (Luke et al. 1988a). Experimental DBA/2 mice were exposed to 300 ppm benzene for 6 hours per day for 5 days per week (Regimen 1) or 3 days per week (Regimen 2) for a duration of 1-13 weeks. PCEs were affected by benzene inhalation independent of exposure duration and regimen, while NCEs were affected only following Regimen 1 exposure. Males were more sensitive to benzene inhalation than females (Luke et al. 1988a). In a similar experiment, the effects of benzene on three different strains of male mice (DBA/2, C57BL/6, and B6C3F<sub>1</sub>) were compared (Luke et al. 1988b). The male mice were exposed to 300 ppm benzene for 6 hours per day for 5 days per week (Regimen 1) or 3 days per week (Regimen 2) for a duration of 13 weeks. Micronuclei increases in PCEs were found to be strain specific (DBA/2 > C57BL/6 = B6C3F<sub>1</sub>). Again, the effects on the PCEs were independent of exposure and regimen. Micronuclei increases in NCEs were observed to be both strain and regimen dependent with C57BL/6 = B6C3F<sub>1</sub> > DBA/2 and Regimen 1 > Regimen 2. The authors concluded that genotoxic damage by benzene inhalation can be dependent upon sex, strain, exposure duration, and exposure regimen (Luke et al. 1988a, 1988b).

CD-1 mice were exposed to purified air or benzene by inhalation at 0.04, 0.1, or 1.0 ppm for 22 hours per day, 7 days per week for 6 weeks (Ward et al. 1992). The effects of *in vivo* exposure to benzene were evaluated by using an autoradiographic assay to determine the frequency of mutants which represent mutations at the hypoxanthine-guanine phosphoribosyl transferase (hprt) locus in spleen lymphocytes. At the end of the six weeks exposure period, lymphocytes were recovered from the spleens of the mice and cryopreserved prior to assay. Mutant cells were selected on the basis of their ability to incorporate tritiated thymidine in the presence of 6-thioguanine. The increased frequencies of mutant spleen lymphocytes were significant at the low and mid, but not the high dose, and the

method does not take into account possible clonal expansion. Further evaluation of the induction of gene mutation at these dose levels seems warranted.

Mutations have also been detected in tissue cells after inhalation exposure of animals to benzene. Male C57BL/6 mice were exposed to a target dose of 300 ppm benzene 6 hours per day, 5 days per week for 12 weeks (Mullin et al. 1995). Control animals were exposed to filtered conditioned air. Animals were sacrificed and genomic DNA was purified from the lungs, spleen, and liver tissues. . Increased frequencies of mutations in the lacI transgene were detected in lung and spleen, but not in the liver. In another study, female BDF1 mice (C57BL/6xDBA/2F<sub>1</sub> hybrids) were exposed to 0, 100, 300, and 900 ppm benzene 6 hours per day, 5 days per week for up to 8 weeks (Plappert et al. 1994a). An increase in DNA damage was noted in peripheral blood cells after exposure to 300 or 900 ppm benzene for 3 days up to 4 weeks. In the bone marrow, 100 and 300 ppm benzene produced an effect after 5 days of exposure. No accumulation of DNA damage was seen after 4 weeks. At 300 ppm benzene, the repair was not complete even after 48 hours. At 100 ppm benzene, significant changes were seen in the liver after 5 days, but not in the blood. The repair, which was measured 24 and 48 hours after the end of the exposure, was almost complete in the blood and the liver after the 5-day exposure to both concentrations. However, the repair was not complete in the blood after 4 weeks of exposure to 300 ppm in the liver after 4 weeks of exposure to 100 and 300 ppm. In a companion study, female BDF1 mice (C57BL/6xDBA/2F<sub>1</sub> hybrids) were exposed in inhalation chambers to benzene (300 or 900 ppm) and/or toluene (250 or 500 ppm) 6 hours per day, 5 days per week for up to 8 weeks (Plappert et al. 1994b). DNA damage was studied in peripheral blood cells, bone marrow, and liver using the single cell gel assay and pooled blood from 3 animals/group. At 300 or 900 ppm benzene exposure, significant increases in DNA damage were detected in cells sampled from blood, liver and bone marrow. Combined exposures of benzene and toluene did not induce significant increases in DNA damage.

Other evidence of genotoxicity induced by benzene includes inhibition of DNA synthesis in certain cell types, although inhibition of DNA synthesis is not always indicative of genotoxic damage. Acute 4-hour inhalation exposure of female ICR mice to 2,000 ppm benzene produced a 15% inhibition of DNA synthesis in bone marrow cells, and exposure to 3,000 ppm inhibited DNA synthesis by 33% (Lee et al. 1988). Delayed cell cycle in mouse bone marrow and DNA adducts with benzene metabolites in mouse and rat hemoglobin or rat liver cells were also evidence of benzene inhalation

exposure (Lutz and Schlatter 1977; Sabourin et al. 1990; Tice et al. 1980, 1982). Other genotoxicity studies are discussed in Section 2.5.

#### **2.2.1.8 Cancer**

Neoplastic diseases of the hematopoietic system differ with respect to morphologic and clinical manifestations and often in their response to therapy. Leukemia may be divided into granulocytic leukemias (which include myelocytic, monocytic, and erythroblastic cell types) and lymphocytic leukemias. Both granulocytic and lymphocytic leukemias may be separated into acute and chronic forms. In acute myeloid leukemia (AML), there is diminished production of normal erythrocytes, granulocytes, and platelets, which leads to death by anemia, infection, or hemorrhage. These events can be rapid. In chronic myeloid leukemia (CML), the leukemic cells retain the ability to differentiate and perform function; later there is a loss of ability to differentiate. Case reports and epidemiological studies of workers have established a causal relationship between benzene exposure and AML. While some studies have implicated other types of leukemia or even lymphomas, only the incidence of AML and its variants has consistently been increased in groups of workers with excess benzene exposure (Goldstein 1988).

The clinical and epidemiological evidence is summarized below. The deficiencies in the studies include the lack of appropriate sampling techniques, exposure determinations, mortality standards, and other aspects of experimental design or methodology. Additionally, intermittent exposures to benzene made it difficult to assume that the average concentrations of benzene measured in a workplace actually indicated the true exposure experienced by each worker (Goldstein 1985). A cause-effect relationship between benzene and leukemia is sufficiently clear; however, there are few data from which dose-response relationships can be established.

Aksoy et al. (1974) reported the crude incidence of leukemia between 1967 and 1973 among 28,500 shoe, slipper, and handbag workers exposed to benzene in Istanbul, Turkey, to be approximately 13.5 per 100,000, compared to an estimated annual leukemia incidence of 6 per 100,000 for the general population of Turkey, resulting in a relative risk of 2 for all cell types of leukemia combined. This risk estimate was based on the diagnosis of leukemia or preleukemia (acute erythroleukemia) in 26 shoe workers in Istanbul and did not take into consideration differences in the age structure of the population of shoe workers and the general population. Average air concentrations

of benzene in the factories were reported to be between 15 and 30 ppm during non-work hours and about 150 ppm during work hours, and reached a maximum of 210 ppm (and in rare instances, 640 ppm) when adhesives containing benzene were used (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974). The duration for exposure of these workers with leukemia ranged from 3 months to 17 years. The clear association between exposure to benzene and the development of pancytopenia, which may progress to leukemia, alerted officials of these industries in Turkey that worker exposure to benzene needed to be reduced. The number of cases of pancytopenia in Turkey was reduced following the prohibition of benzene in 1969; the persistence of aplastic anemia associated with exposure to benzene is due to the use of benzene as a solvent for rubber and dyes, or for cleaning in some workplaces; it also may be due to the exposure to this agent before its use as a solvent was regulated (Aksoy et al. 1984). In a more recent study of 231 workers in 40 workplaces in Turkey, Aksoy et al. (1987) reported that 76.4% of the solvents and 19.1% of the thinners contained benzene concentrations of more than 1%, ranging between 0.7% and 7.6% (mean, 2.88%) for the solvents and between 0% and 6.6% (mean, 0.73%) for the thinners. The air concentration of benzene bracketed around 1 ppm. However, the concentration of benzene in one part of the tire cord plant was 110 ppm. Five cases of cancer, including leukemia and malignant lymphoma, were found among the exposed workers. Results of the hematological analysis also showed that 14 of the 231 workers had mild abnormalities, including leukopenia, thrombocytopenia, and pancytopenia (Aksoy et al. 1987).

Subjects with benzene-induced hyporegenerative anemia or pancytopenia of a long-standing duration subsequently developed acute leukemia. Between 1942 and 1975, 66 cases of benzene hemopathy were seen at the Institute of Occupational Health in Milan, Italy (11 of these were leukemia) (Vigliani and Forni 1976). The affected individuals worked in rotogravure plants, shoe factories, and other industries where benzene was used as a solvent. Benzene concentrations in the air near rotogravure machines were calculated to range between 200 and 400 ppm, with peaks up to 1,500 ppm.

A cohort of chemical workers exposed to benzene from 1946 to 1975 was identified. A dose-response relationship between cumulative exposure to benzene and mortality (from all lymphopietic cancer combined and from leukemia) was reported for this population of 3,536 male chemical workers who had cumulative benzene exposures of <180 ppm-months (1,809 workers), 180-719 ppm-months (1,047 workers), or  $\geq 720$  ppm-months (680 workers) (Wong 1987b; Wong et al. 1983). Workers exposed to a cumulative benzene dose of  $\geq 720$  ppm-months were 4 times more likely (relative risk, 3.93) to develop leukemia or other lymphopietic cancers. Of the 3,536 workers that had continual

exposure to benzene, 7 died of leukemia. When compared to nonexposed workers, the excess lymphopoietic cancer was significant for all males (relative risk, 3.2). None of the 7 leukemias were of the acute myelogenous cell type. A dose-response relationship between cumulative exposure and non-Hodgkin's lymphopoietic cancer was of borderline statistical significance ( $p=0.06$ ). Although the dose-response relationship adds strength to the association between exposure to benzene and leukemia and lymphatic and hematopoietic cancer, the estimated historical exposure data were not precise enough for quantitative risk assessment. Additionally, when exposure to benzene is expressed as cumulative exposure (in ppm-months exposure), it is not possible to distinguish between concentration and duration of exposure.

A retrospective cohort study was done in 1982-83 on 28,460 benzene workers in China, all of whom had worked in various factories for at least half a year between 1972 and 1981; however, exposure and employment duration were not necessarily limited to those years (Yin et al. 1987a). Thirty cases of leukemia (23 AML, 7 CML) with a mean latency of 11.4 years (0.8-49.5 years) were found in the benzene cohort, as opposed to 4 cases in a matched control cohort. Twenty-five of the leukemic workers had already died. Information on exposure levels was collected from company records, but there is no indication of the extent of these records with the exception of a note that three of the reported levels were based on only one measurement. Mean benzene levels to which workers with leukemia were exposed ranged from 3 to 313 ppm, with the majority falling between 16 and 157 ppm. Exposure ranges from which the means were derived were rather wide, indicating the possibility of at least occasional high exposures. Only 4 upper-level measurements were less than 10 ppm, while half of the remaining measurements were between 10 and 100 ppm, and the other half were between 100 and 2,000 ppm. The authors observed that the cumulative mortality due to leukemia was proportional to the duration of the exposure to benzene for exposure of up to 20 years and then leveled off.

The retrospective cohort study was also used to examine all causes of mortality. The authors (Yin et al. 1989) reported that mortality from all causes was significantly higher in the exposed - group-265.46 deaths per 100,000 person-years for the exposed group as compared to 139.06 deaths per 100,000 person-years for the unexposed group. Additionally, incidences of malignant neoplasms for the exposed group were significantly higher than for unexposed workers-123.21 cases per 100,000 person-years for the exposed group compared to 54.7 cases per 100,000 person-years for the unexposed group. Increased mortality was also noted in benzene-exposed men as indicated by

standardized mortality ratios (SMRs), and SMRs were significantly elevated for leukemia (574) and lung cancer (231) and nonsignificantly elevated for primary hepatocarcinoma (112) and stomach cancer (122). (An SMR of 100 is the normal value if an excess is not observed. An SMR of 200 represents 100% excess risk over normal.) The authors emphasized that the estimated average and cumulative lifetime benzene exposure levels were based on relatively few measurements; therefore, their findings are to be interpreted cautiously. They do emphasize, however, that the study supports the conclusion of an excess of acute nonlymphocytic leukemia among benzene-exposed subjects.

The same group reported that between 1979 and 1981, Chinese workers using benzene or benzene-containing mixtures were examined. Nine cases of leukemia were found out of 4,602 exposed workers. Presumably, some of these may have been included in the study discussed above. Although one worker was exposed for only 2 years, the others were exposed for between 7 and 25 years. No estimate of exposure levels was given for the leukemia cases, but exposure estimates for aplastic anemia cases found in the same study were 29-362 ppm (Yin et al. 1987c).

A retrospective cohort study of incident cases of hematopoietic neoplasms and related disorders among 74,828 benzene-exposed workers employed between January 1, 1972 to December 31, 1987 in 672 factories in 12 Chinese cities was conducted (Travis et al. 1994). Workers (35,805) not occupationally exposed to benzene employed in 109 factories during the same period were used for comparison. Follow-up of both exposed and nonexposed workers was carried out using occupational and medical records, and histopathologic material were reviewed for all patients with hematopoietic malignancies to ensure correct classification. Among benzene-exposed workers, 82 patients with hematopoietic neoplasms and related disorders were diagnosed: 32 (39%) cases of acute leukemia, 9 (11%) aplastic anemia, 7 (9%) myelodysplastic syndrome (MDS), 9 (11%) chronic granulocytic leukemia (CGL), 20 (24%) malignant lymphoma (ML) and related disorders, and 5 (6%) others. Among the nonexposed group, 13 hematologic malignancies were diagnosed: 6 (46%) patients with acute leukemia, 2 (15%) CGL, 3 (23%) ML, and 2 (15%) others. The hematopathologic features of acute nonlymphocytic leukemia (ANLL) associated with benzene exposure resembled the hematological features following chemotherapy or radiotherapy. In addition, this study documented MDS in association with benzene exposure. ANLL in workers exposed to benzene may represent a distinct clinicopathologic entity and may display a preleukemic phase in some patients, as does therapy-related ANLL. This study also showed greater diversity of hematologic malignancies in benzene exposed workers than previously reported. No significant differences were found in the

relative risks for cancer mortality between male and female workers in this cohort as reported by Li et al. (1994).

A cohort of 748 white male workers occupationally exposed to benzene for varying time periods between 1940 and 1949 in 2 manufacturing facilities producing rubber hydrochloride (Pliofilm®) has been studied (Infante 1978; Infante et al. 1977). The initial report (Infante et al. 1977) included evaluation of mortality statistics up to 1975, and the follow-up study (Infante 1978) included evaluation up to 1977. A summary of the overall results is presented here. Included for the study were all white males assigned to Pliofilm® production who were hourly employees at any time between January 1, 1940 and December 31, 1949. Personnel employed in Pliofilm® operations but who were not assigned to production jobs were not studied. All study cohort members were followed from the time of termination of employment to June 30, 1975. The risk of lymphatic and hematopoietic malignancies was calculated. The vital status of approximately 75% of the 748 cohort members who were originally in the study was verified. Persons for whom vital status could not be verified were assumed alive. In order not to overestimate the true risk of hematopoietic and lymphatic malignancies which may have been associated with benzene exposure, causes of death were determined from death certificate reporting, and coded according to an international standard list. A modified life table technique was used to generate person-years at risk of dying according to 5-year age grouping and 5-year calendar time periods. Two populations were chosen as control groups for generating the number of expected deaths in the study population. The first group was U.S. white males, and the second group was made up of employees at a fibrous-glass-construction products facility over the same period of time as the test population (January 1, 1940 to December 31, 1949) and who had achieved 5 or more years of employment by June 1972, which was the cutoff date for that study. In the test population, 140 total deaths were observed in the time period ending April 13, 1977, compared to 187 expected on the basis of the U.S. white male control group. The deficit was probably due to lack of follow-up for the test population. For total lymphatic and hematopoietic cancer, there were 9 observed versus 3.4 expected deaths when compared to U.S. white males. This difference was statistically significant, and is attributed to an excess in the incidence of leukemia. A mortality study of rubber workers exposed to benzene between 1940 and 1949 revealed lymphatic hematopoietic cancer, myeloid leukemia, and monocytic leukemia with a Standard Mortality Ratio (SMR) of 260. There were 7 observed compared to 1.38 expected deaths from leukemia. The SMR was 506, indicating a 5-fold excessive risk. When compared to the fibrous glass factory population, there were 7 observed compared to 1.47 expected deaths from leukemia (SMR=474), essentially the

same risk. The authors indicated that this estimate was conservative. When the population was followed for an additional 3 months, the SMR for leukemia was 560. Identification of type of leukemia revealed 4 cases of acute myelogenous leukemia, 2 cases of monocytic leukemia, and 1 case of chronic myelogenous leukemia. An additional case of myelogenous leukemia was subsequently identified that did not fit into the definition of the cohort, since the person started employment at the plant in 1950. This person was not included in the cohort or in the statistical analysis. With regard to the specific cell type of leukemia, the increased risk of myelogenous leukemia is between 5- and 10-fold, depending on the group used for comparison. Comparison with the National Cancer Institute age-specific leukemia mortality rate or the Connecticut Tumor Registry yields an 8.5-fold or 10-fold increased risk, respectively. The average benzene exposure for workers between 1941 and 1949 ranged from 10 to 100 ppm. Furthermore, the industrial hygiene assessments led to the conclusion that the environment of the workers in Pliofilm ® production was not contaminated with other materials known to be associated with the induction of blood disorders.

Rinsky et al. (1981) reexamined the population at the Pliofilm ® factories used by Infante (Infante 1978; Infante et al. 1977). In another retrospective cohort mortality study, workers exposed to benzene in the manufacture of rubber hydrochloride at these two locations in Ohio were evaluated for possible association between exposure to benzene and subsequent death from leukemia. The study population was in two groups. Group 1 was the same as in the Infante et al. (1977) study. In this later follow-up study, vital status of 98% of the 748 men in the cohort was ascertained (Rinsky et al. 1981), compared to 75% by Infante (Infante 1978; Infante et al. 1977). The remaining 2% were considered alive as of the study end date. Group 2 consisted of 258 individuals who first worked in a department with benzene exposure between January 1, 1950 and December 31, 1959. No examination was attempted of persons whose first exposure to benzene occurred after 1959 because the number of individuals was small and because one of the locations ceased production of rubber hydrochloride in 1965. Vital status follow-up was conducted to June 30, 1975. In Group 1, there were 180 deaths from all causes, compared with an expected number of 161 (SMR=113). Ten cases of malignancies of the hematopoietic and lymphatic systems were observed, versus 3.03 expected, which was a significant increase. With regard to leukemia, 7 cases were observed compared 1.25 expected (SMR=560). All 7 cases were of the myeloid or monocytic cell types. The 7 cases of leukemia were described. The ages of the men ranged from 29 to 65 at the time of death. Length of exposure ranged from 1 month to 20 years. Average exposures for these men ranged from 35 to 100 ppm benzene over the course of their employment. The time interval between first exposure and death ranged from 2 years to

22 years. In Group 2, there were 49 observed deaths from all causes compared to 56 expected (SMR=87). One case of leukemia was observed (as described as case 8 in Infante et al. 1977). Four additional cases of leukemia were discovered, but were not included in the later study since they did not comply with the cohort description of Group 2. The major findings of the later report confirm those of the previous report. Forty more deaths from all causes were reported in the Rinsky study (Rinsky et al. 1981). No additional cases of leukemia were noted among persons fitting the cohort description. There is a suggestion from the data that persons exposed at levels slightly higher than 10 ppm may exhibit a longer latency interval between exposure and the development of leukemia.

Rinsky et al. (1987) later published an epidemiological risk assessment in an update of the earlier studies (Infante 1978; Infante et al. 1977; Rinsky et al. 1981). In this update, cumulative benzene exposures were calculated for each member of the cohort in ppm-years. A total of 1,165 white males with at least 1 ppm-day of cumulative exposure to benzene through December 31, 1965, were included in the cohort. Causes of death were ascertained from death certificates up to December 31, 1981. SMRs were determined for leukemia by four cumulative exposure categories: 1 ppm-day to 40 ppm-year, 40-199.99 ppm-year, 200-399.99 ppm-year, and more than 400 ppm-year. A total of 1,165 white men included in the cohort contributed 31,612 person-years at risk. On December 31, 1981, a total of 819 (70.3%) were alive, 330 (28.3%) were dead, and 16 (1.4%) were lost to follow-up (total 98.6 accountability). Neither the mortality from all causes or the mortality from all malignant neoplasms combined was above the expected rate. There was a significant increase in deaths from all lymphatic and hematopoietic neoplasms (15 observed compared to 6.6 expected; SMR=227). This increase was due to an increase in the incidence of leukemia (9 cases observed versus 2.7 expected; SMR=337), and from multiple myeloma (4 observed versus 1 expected; SMR 409). In one location, 2 cases of leukemia were observed versus 0.58 expected (SMR=345); and in the other location, 5 cases were observed versus 0.67 expected (SMR=746). This follow-up study by Rinsky et al. (1987) revealed malignant neoplasias of the lymphatic and hematopoietic tissue (SMR=330) and both myelogenous and monocytic leukemias (SMR=560) following chronic benzene exposure (cumulative exposure levels were from <40 to >400 ppm over a 40-year working lifetime). The principle findings of this study were that there is a strongly positive exposure-response relation between benzene and leukemia. A question remains concerning the precise levels of benzene to which workers were exposed. Nevertheless, cumulative exposures categorized as <40, 40-200, 200-400, and >400 ppm-years had SMRs for leukemia of 109, 322, 1,186, and 6,637, respectively (a progressive increase in SMR with increasing cumulative dose).

The majority of benzene workers with hematologic disorders in the Rinsky studies (1981, 1987) were diagnosed before 1955. Thus, it is likely that diagnoses in the Rinsky studies of aplastic anemia, acute myelocytic anemia, and blood dyscrasia with secondary anemia and leukemoid reaction may actually represent myelodysplastic syndromes (i.e., patients whose stem cells had undergone malignant transformation but who died of anemia plus complications before frank acute leukemia became manifest). The myelodysplastic syndromes are disorders of stem cells and are characterized by maturation defects. Patients with myelodysplastic syndromes present with anemia and may die of infection or hemorrhage (due to platelet/clotting abnormalities), or their condition may progress to acute leukemia. Recently, the syndromes have been recognized in increasing numbers of younger patients who had received cytotoxic chemotherapy, radiation therapy, or both forms of treatment for a variety of malignancies. Diagnosis of myelodysplastic syndromes requires thorough examination of peripheral blood films and bone marrow preparations with precise application of diagnostic criteria. These diagnostic criteria changed between 1976 and 1982. Individuals with myelodysplastic syndromes should be included in the population at risk of developing leukemia following benzene exposure.

A more recent update and risk analysis of this cohort has been published (Paxton et al. 1994a, 1994b). The Pliofilm® cohort consisted of 1,868 individuals; 1,044 worked at the St. Marys plant location from 1940 to 1975 and 824 at the Akron plant site from 1936 to 1965. This cohort was classified into two groups: the wet side workers (1,291 workers) with direct benzene exposure and the dry side workers (577) with no direct benzene exposure. In the most recent update of these, NIOSH followed up the vital status of only the wet side workers through 1987. The updated vital status of the wet side workers from 1970 to 1981 resulted in the identification of an additional 123 deaths for a total of 481 deaths in the wet side subgroup. No new cases of multiple myeloma were reported. The results of this analysis support previous studies of this cohort.

A historical prospective study of mortality was conducted among 594 white males occupationally exposed to benzene in a chemical manufacturing facility and employed for varying time periods between 1940 and 1970 (Ott et al. 1978). The TWA exposures to benzene ranged from 0.3 to 35 ppm for various job categories. Three cases of myelogenous leukemia were identified (as compared to 0.8 expected cases); this is statistically significant based on incidence data from the Third National Cancer Survey (NOES 1990). Of the three cases, two were categorized as myelogenous leukemia, and one was categorized as bilateral bronchopneumonia, but myeloblastic leukemia was noted as an “other

significant condition.” A fourth cohort member died from aplastic anemia, while a fifth died from pernicious anemia. Bond et al. (1986b) performed an update of the Ott et al. (1978) study. This follow-up studied an additional 362 chemical workers potentially exposed between 1976 and 1982. Two additional deaths from myelogenous leukemia were identified, bringing the total number of deaths primarily from myelogenous leukemia to four in this cohort. Four deaths due to myelogenous leukemia represented a nonsignificant increase compared to the expected number of leukemia deaths (all types, 2.1 deaths expected, SMR=194), but it did represent a significant increase in the number of myelogenous leukemia deaths (versus 0.9 expected myelogenous leukemia deaths, SMR=444). The workers who died of leukemia had the potential for unquantified, brief, but potentially high, exposures to benzene (Bond et al. 1986b). The authors note that although the study provides support for the association between exposure to benzene and acute myelogenous leukemia, the data are complicated by small numbers of affected workers, concomitant exposure to other toxic compounds, and the uncertainty of the actual exposure level.

A similar study detected 3 cases of death from leukemia out of 6,520 workers in the fuel and steel industry who were followed for 20 years (Hurley et al. 1991). This epidemiologic evidence leads to the conclusion that leukemia risk exists at low benzene concentrations.

Another epidemiological study showed no leukemia deaths following benzene exposures to concentrations less than 1 ppm (Tsai et al. 1983). In this study, the cohort consisted of 454 employees at a Texas refinery who worked at aromatic distillate hydrogenation or cumene unit work stations between 1952 and 1978. The median benzene exposures were 0.14 ppm for refinery workers and 0.53 ppm for those in benzene-related units. Not all of the median exposures were TWAs. Within this cohort, the relative risk for all cancer was not significant as compared to case referents or to the general population of the United States. Furthermore, the evaluation of medical records of this cohort showed no significant changes in blood indices (leukocyte, erythrocyte, hemoglobin, hematocrit, platelet, clotting and bleeding time in minutes).

An evaluation of occupational chemical exposure, histological subtype, and cytogenetics was conducted on case studies of newly diagnosed AML or CML, or myelodysplastic syndromes (MDS) treated in the Main Hospital of Torino, Northern Italy, between October 1, 1989 and December 31, 1990 (Ciccone et al. 1993). There was a nonstatistically significant increased relative risk for exposure

to benzene. Data show 3 AML, 2 CML, and 4 MDS cases with regard to benzene exposures. No excess of clonal chromosome abnormalities was detected among occupationally exposed AML patients. Other studies also suggest increased risk of kidney, brain, bladder cancer, and soft-tissue sarcoma in addition to hematopoietic cancers after chronic-duration inhalation exposure to benzene (Greenland et al. 1994; Hunting et al. 1995; Lagorio et al. 1994b; Serraino et al. 1992; Steineck et al. 1990).

EPA (IRIS 1996) has reviewed the human and animal carcinogenicity data on benzene. A summary of the EPA calculations of unit risk values for leukemia based on human epidemiological studies is given below. It should be noted that these values are estimates of human risk since the true human risk at low doses cannot be accurately identified. The unit risk should not be used if the air concentration exceeds  $100 \mu\text{g}/\text{m}^3$  (0.031 ppm) since above this concentration the unit risk may not be appropriate. One ppm of benzene ( $3,190 \mu\text{g}/\text{m}^3$ ) exceeds the upper limit of  $100 \mu\text{g}/\text{m}^3$ .

The potency of benzene has been estimated by EPA based on three separate epidemiological studies (Ott et al. 1978; Rinsky et al. 1981, 1987; Wong et al. 1983). Equal weight was given to cumulative dose and weighted cumulative dose as well as to relative and absolute risk model forms. EPA estimated the lifetime probability of cancer to be  $2.9 \times 10^{-2} (\text{mg}/\text{kg}/\text{day})^{-1}$  or  $2.7 \times 10^{-2} (\text{ppm})^{-1}$  (IRIS 1996). Based on this value, the exposure levels associated with individual lifetime upper-bound risks of  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$  have been calculated to be  $4 \times 10^{-3}$ ,  $4 \times 10^{-4}$ ,  $4 \times 10^{-5}$ , and  $4 \times 10^{-6}$  ppm, respectively. These exposure level are indicated in Figure 2-1.

Crump (1994) published a risk estimate for benzene, using data from the Pliofilm® cohort through 1987, and applying a sensitivity analysis. The sensitivity of the risk estimates to model the assumptions was evaluated by applying dose-response models, and by investigating the types of cancer that were linked to benzene exposure. The analyses conducted by Crump (1994) were based on the incidence of acute myelogenous leukemia, and predicted a latency from benzene exposure to death of 7-20 years, compared to death from leukemia after radiation in 3-4 years. Multiplicative risk models were found to be better than additive risk models. Lifetime risks from 45 years of occupational exposure to 1 ppm benzene were between  $2 \times 10^{-5}$  and  $3.6 \times 10^{-4}$ . Unit risk (continuous lifetime exposure to 1 ppm benzene) ranged from  $9.7 \times 10^{-5}$  to  $1.7 \times 10^{-4}$ . Upper-bound risks for 45 years of occupational exposure were  $1.6 \times 10^{-3}$  to  $3.1 \times 10^{-3}$ , and from  $7.7 \times 10^{-3}$  to  $1.5 \times 10^{-2}$  for continuous lifetime exposure (unit risk). Wong (1995) also published an analysis of risk of acute myeloid

leukemia (AML) and multiple myeloma using the Pliofilm® worker data. No increased risk of AML was detected for cumulative exposure to benzene below 200 ppm-years. Above 200 ppm-years, risk of AML rose reaching an SMR of 98.37 for exposures of >400 ppm-years. No relation between multiple myeloma and benzene exposure was found. Wong and Raabe (1995) looked at the risk of cell-type specific leukemia in workers in both the United States and the United Kingdom. No increase was found in the risk for AML, chronic myelogenous, acute lymphocytic, or chronic lymphocytic leukemias.

Recent studies have shown benzene to be carcinogenic in animals by inhalation. However, all results are not universally accepted, primarily because of the difficulty in developing reliable animal models for benzene-induced leukemia. For example, inhalation of air concentrations of 300 ppm benzene for 4 weeks, 5 days per week, 6 hours per day by HRS/J mice did not alter the time of onset or the incidence of leukemia expected in HRS/J mice (Stoner et al. 1981). Stoner et al. (1981) suggested that perhaps a genetic shift has occurred in the HRS/J mouse during the past 10 years so that the (hr) gene locus no longer influences the previously observed greater susceptibility to leukemia in homozygous (hr/hr) animals.

Results from a series of ongoing studies in which Sprague-Dawley rats (13-week-old adults and 12-day-old embryos) were exposed 5 days per week, 4-7 hours per day to 200-300 ppm benzene via inhalation (for 15 or 104 weeks) and observed until spontaneous death suggest that benzene is a multipotential carcinogen (Maltoni et al. 1982a, 1982b, 1983, 1985, 1989). Exposure to benzene developed an increased incidence of Zymbal gland carcinomas, carcinomas of the oral and nasal cavities, hepatomas, and other malignant tumors (Maltoni et al. 1982b, 1983). An overall increase in incidence of total malignant tumors in benzene-exposed animals was observed, but there was no information on the significance of these findings. The incidence of certain carcinomas appeared to be dependent, to some extent, on exposure duration and the age of the treated animals (embryos were more susceptible than adults). The EPA Gene-Tox Carcinogenesis Panel (Nesnow et al. 1986), in an evaluation of the Maltoni et al. (1982b) study in which Zymbal gland carcinoma was induced, called results of the inhalation experiments inconclusive. In effect, the panel (after reevaluating the raw data, including the slides used for histopathology determinations) would not support a direct association between benzene and the malignant tumors reported by Maltoni et al. (1982b). However, in a study by Snyder et al. (1984), Sprague-Dawley rats exposed to 100 ppm benzene for 6 hours per day, 5 days per week for a lifetime exhibited not only Zymbal gland carcinoma, but also increased incidence of

myelogenous leukemia and liver tumors. It should be noted that Snyder et al. (1978a) found no indications of leukemia or preleukemic responses in Sprague-Dawley rats similarly exposed to 300 ppm.

A major study (Cronkite 1986; Cronkite et al. 1984, 1985) provides a basis for a reproducible animal model. In this study, mice were exposed to 300 ppm benzene by inhalation 6 hours per day, 5 days per week for 16 weeks. This exposure regimen was selected because the authors thought it most closely paralleled likely human exposure. Human epidemiological studies in the literature reported that workers were exposed for about 15% of their life span; 16 weeks represent  $\approx 15\%$  of the life span of mice. Although precise occupational exposures were not known, most studies indicated that workers had been exposed to concentrations up to 250-300 ppm during at least part of a working day; thus, 300 ppm was chosen. The C57BL/6 and CBA/Ca mouse strains were chosen for these studies because of their susceptibilities to ionizing radiation-induced thymic lymphoma. The strains are also recognized for their low spontaneous rates of AML, the disease most frequently associated with benzene exposure in humans.

Cronkite et al. (1984) reported a highly significant increase in thymic and nonthymic lymphomas in C57BL/6 mice exposed to 300 ppm of benzene by inhalation 6 hours per day, 5 days per week for 16 weeks. In a continuation of that study (Cronkite et al. 1985), a definite pattern for thymic and nonthymic lymphoma appearance and mortality was observed. Lymphoma was first observed at about 150 days after exposure. The mice began to die at 330 days, and mortality increased through 390 days, at which time it leveled off. An increased incidence of lymphoma and solid tumors was again observed 420 days after exposure, and mortality did not increase again until 570 days after exposure. Increased incidences of Zymbal gland and ovarian tumors were also found. In a subsequent study, CBA/Ca male mice that breathed 100 ppm of benzene 6 hours per day, 5 days per week for 16 weeks developed leukemia, while mice similarly exposed to 300 ppm had a significant increase in the incidence of myelogenous neoplasms (Cronkite 1986; Cronkite et al. 1989). Increased incidences of harderian and Zymbal gland, squamous cell and mammary carcinoma, papilloma, and adenocarcinoma of lungs were also seen. Lymphoma was observed in 12% (significant increase) of CBA/Ca mice exposed to 300 ppm benzene for 16 weeks (Farris et al. 1993). Acute granulocytic leukemia was not observed by Cronkite et al. (1989) or by Farris et al. (1993).

AKR mice were exposed to 100 or 300 ppm benzene vapor for 6 hours per day, 5 days a week for life (Snyder et al. 1978a, 1980). C57BL mice were exposed to 300 ppm benzene vapor for 6 hours per day, 5 days a week for life. No significant increase in leukemia or malignant lymphoma was found in AKR mice, whereas C57BL mice exhibited a significant increase (8/40) compared with 2/40 in controls in the incidence of hematopoietic neoplasms including 6 cases (15%) of thymic lymphoma. Thymic lymphoma is rare in C57BL mice. In a later study, CD-1 mice given an intermediate exposure (6 hours per day, 5 days per week for 10 weeks) to 1,200 ppm benzene and observed until death showed an elevated incidence of lung adenoma (Snyder et al. 1988). No increased incidence of lung adenomas was observed in C57BL/6 mice similarly exposed. Both CD-1 and C57BL/6 mice responded to intermittent lifetime exposures to benzene with elevated incidences of Zymbal gland tumors (Snyder et al. 1988). Neither of the protocols induced elevated incidences of leukemia/lymphomas in either strain (Snyder et al. 1988). The authors concluded that lifetime exposure to benzene, even when intermittent, is a more potent carcinogenic pattern of exposure than a short-term intense exposure pattern.

The cancer effect levels (CELs) for each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

## **2.2.2 Oral Exposure**

### **2.2.2.1 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  $\approx 125$  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 LD<sub>50</sub> 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 LD<sub>50</sub> (Cornish and Ryan 1965). The LD<sub>50</sub> for nonfasted rats was found to be 930 mg/kg. In 24-hour fasted rats, the LD<sub>50</sub> was 810 mg/kg. No increase in mortality was reported in Fischer 344 rats or B6C3F<sub>1</sub> mice treated with 600 mg/kg/day for up to 17 weeks (Huff et al. 1989; NTP 1986).

Sprague-Dawley rats (30-35 males, 3&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 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 per 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. B6C3F<sub>1</sub> mice given 100 mg/kg/day also had increased mortality compared to control mice.

The LD<sub>50</sub> values and ail reliable LOAEL values for death in each species following acute and chronic exposure are recorded in Table 2-2 and plotted in Figure 2-2.

#### **2.2.2.2 Systemic Effects**

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

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

Key to figure <sup>a</sup>	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
<b>ACUTE EXPOSURE</b>							
<b>Death</b>							
1	Human	once				126 (death)	Thienes and Haley 1972
2	Rat (Sprague- Dawley)	once (G)				930 M (LD <sub>50</sub> )	Cornish and Ryan 1965
3	Rat (Wistar)	once (GO)				5600 M (LD <sub>50</sub> )	Wolf et al. 1956
<b>Systemic</b>							
4	Rat (Sprague- Dawley)	Gd 6-15 daily (G)	Renal	1000 F			Exxon 1986
			Dermal		50 F (alopecia of hindlimbs & 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			1402M (increased liver weight, biochemical changes)	Pawar and Mungikar 1975
<b>Neurological</b>							
6	Human	once				126 (muscular incoordination, unconsciousness)	Thienes and Haley 1972

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure <sup>a</sup>	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
7	Rat (Sprague- Dawley)	1 d			88M (slight CNS depression)	1870 M (tremors)	Cornish and Ryan 1965
8	Rat (Sprague- Dawley)	once (G)			950M (altered neurotransmitter concentrations)		Kanada et al. 1994
<b>Reproductive</b>							
9	Rat (Sprague- Dawley)	Gd 6-15 daily (G)		1000 F			Exxon 1986
<b>Developmental</b>							
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

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure <sup>a</sup>	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
<b>INTERMEDIATE EXPOSURE</b>							
<b>Death</b>							
12	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1x/d (GO)				250 F (9/35 died)	Maltoni et al. 1983, 1985
<b>Systemic</b>							
13	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1x/d (GO)	Bd Wt	50	250	(body weight decreased 19%)	Maltoni et al. 1983, 1985
14	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)	
					25 F	(dose-related leukopenia at 120 days)	
			Musc/skel	600			
			Hepatic	600			
			Renal	600			
			Endocr	600			
			Bd Wt		200	(body weight decreased 14% in males & 16% in females)	

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to <sup>a</sup> figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
15	Rat (F-344/N)	<1 yr 5 d/wk (GO)	Hemato		50M (lymphocytopenia and leukocytopenia)		NTP 1986
			Bd Wt	100	25 F (lymphocytopenia and leukocytopenia) 200M (body weight decreased 11% or more in 25 weeks)		
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
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			

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure <sup>a</sup>	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
21	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)	Resp	600			NTP 1986
			Cardio	600			
			Gastro	600			
			Hemato	25 M	50 M (dose-related leukopenia)		
				200 F	400 F (dose-related lymphopenia)		
			Musc/skel	600			
			Hepatic	600			
			Renal	600			
			Endocr	600			
			Bd Wt	600			
22	Mouse (B6C3F1)	<1 yr 5 d/wk (GO)	Hemato		25 (lymphocytopenia)		NTP 1986
			Bd Wt	100			
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 consumption)		

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure <sup>a</sup>	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
<b>Immunological/Lymphoreticular</b>							
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 & 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
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)		25 M 200 F	50 M (dose-related 400 F lymphopenia)		Huff et al. 1989; NTP 1986

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure <sup>a</sup>	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL		Reference
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	
31	Mouse (B6C3F1)	30 d ad lib (W)			12 F (decreased leukocytes)	Shell 1992
<b>Neurological</b>						
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
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
<b>Reproductive</b>						
38	Rat (F344/N)	17 wk 5 d/wk (GO)		600		NTP 1986

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
39	Mouse (B6C3F1)	17 wk 5 d/wk (GO)		600			NTP 1986
<b>Cancer</b>							
40	Rat (Sprague- Dawley)	52 wk 4-5 d/wk 1x/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 1x/d (GO)				50 (CEL: Zymbal gland carcinoma)	Maltoni et al. 1989
						250 (CEL: nasal cavity carcinoma, fore-stomach liver angiosarcoma; Zymbal gland carcinoma)	
42	Mouse (RF/J)	52 wk 4-5 d/wk (GO)				500 (CEL: mammary pulmonary leukemias)	Maltoni et al. 1989

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure <sup>a</sup>	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
<b>CHRONIC EXPOSURE</b>							
<b>Death</b>							
43	Rat (F-344/N)	2 yr 5 d/wk (GO)				200 M (30/50 died) 50 F (14/50 died)	NTP 1986
44	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				100 (41/50 died M, 35/50 died F)	NTP 1986

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure <sup>a</sup>	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference				
					Less Serious (mg/kg/day)	Serious (mg/kg/day)					
<b>Systemic</b>											
45	Rat (F-344/N)	2 yr 5 d/wk (GO)	Resp	200 M 100 F			Huff et al. 1989; NTP 1986				
			Cardio	200 M 100 F							
			Gastro	100	200M (hyperkeratosis & acanthosis in nonglandular forestomach)						
			Hemato		50 M (lymphocytopenia and leukocytopenia) 25 F (lymphocytopenia and leukocytopenia)						
			Musc/skel	200 M 100 F							
			Hepatic	200 M 100 F							
			Renal	200 M 100 F							
			Endocr	200 M 100 F							
			Dermal	200 M 100 F							
			Ocular	200 M 100 F							
			Bd Wt	100		200 M (body weights decreased 23% in 103 wks)					
			46	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 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure <sup>a</sup>	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
47	Mouse (B6C3F1)	2 yr 5 d/wk (GO)	Resp	50 M 25 F	100	(alveolar hyperplasia)	NTP 1986
			Cardio	100	25	(epithelial hyperplasia and hyperkeratosis of forestomach)	
			Gastro		25	(lymphocytopenia; increased frequency of micronucleated normochromatic peripheral erythrocytes)	
			Hemato		25		
			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 wks to 19% in 103 wk in males) (mean body weight decreased 14-15% in week 99-103 in females)	
<b>Immunological/Lymphoreticular</b>							
48	Rat (F-344/N)	2 yr 5 d/wk (GO) <sup>1</sup>			50M	(lymphoid depletion of spleen & thymus)	Huff et al. 1989; NTP 1986
					25 F	(lymphoid depletion of spleen & thymus)	

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure <sup>a</sup>	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
49	Rat (Sprague- Dawley)	92 wk 4-5 d/wk 1x/d (GO)			500	(decreased WBCs after 84 weeks)	Maltoni et al. 1983, 1985
50	Mouse (B6C3F1)	2 yr 5 d/wk (GO)			25	(lymphopenia, hematopoietic hyperplasia in the bone marrow, splenic hematopoiesis)	Huff et al. 1989; NTP 1986
<b>Neurological</b>							
51	Rat (F-344/N)	2 yr 5 d/wk (GO)		200 M  100 F			NTP 1986
52	Mouse (B6C3F1)	2 yr 5 d/wk (GO)		100			NTP 1986
<b>Reproductive</b>							
53	Rat (F-344/N)	2 yr 5 d/wk (GO)		200 M  50 F			NTP 1986
54	Mouse (B6C3F1)	2 yr 5 d/wk (GO)			25M (preputial gland hyperplasia) 25 F (ovarian hyperplasia and senile atrophy)		NTP 1986

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure <sup>a</sup>	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
<b>Cancer</b>							
55	Rat (F-344/N)	2 yr 5 d/wk (GO)				50 M (CEL: squamous cell papillomas & carcinomas of the oral cavity) 25 F (CEL: Zymbal gland carcinomas)	Huff et al. 1989; NTP 1986
56	Rat (Sprague- Dawley)	92 wk 4-5 d/wk 1x/d (GO)				500 (CEL: Zymbal gland carcinoma; oral and nasal cavity carcinoma; angiosarcoma of the liver)	Maltoni et al. 1983, 1985
57	Rat (Wistar)	104 wk 4-5 d/wk 1x/d (GO)				500 (CEL: Zymbal gland and oral and nasal cavity carcinoma; angiosarcoma of the liver)	Maltoni et al. 1989
58	Rat (Sprague- Dawley)	104 wk 5 d/wk (GO)				50 (CEL: Zymbal gland carcinoma)	Maltoni et al. 1989
59	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				25 M (CEL: harderian gland adenoma, lymphoma) 25 F (CEL: lymphoma)	Huff et al. 1989; NTP 1986

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
60	Mouse (Swiss)	78 wk 4-5 d/wk 1x/d (GO)				500 (CEL: mammary tumors, lung tumors, Zymbal gland)	Maltoni et al. 1989

<sup>a</sup>The number corresponds to entries in Figure 2-2.

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 water; Hemato = hematological; 5-HT = 5-hydroxytryptamine; LD<sub>50</sub> = 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 = vanillyl mandelic acid; (W) = water; WBC = white blood cells; wk = week(s); yr = year(s); x = times

**Figure 2-2. Levels of Significant Exposure to Benzene - Oral**  
**Acute ( $\leq 14$  days)**

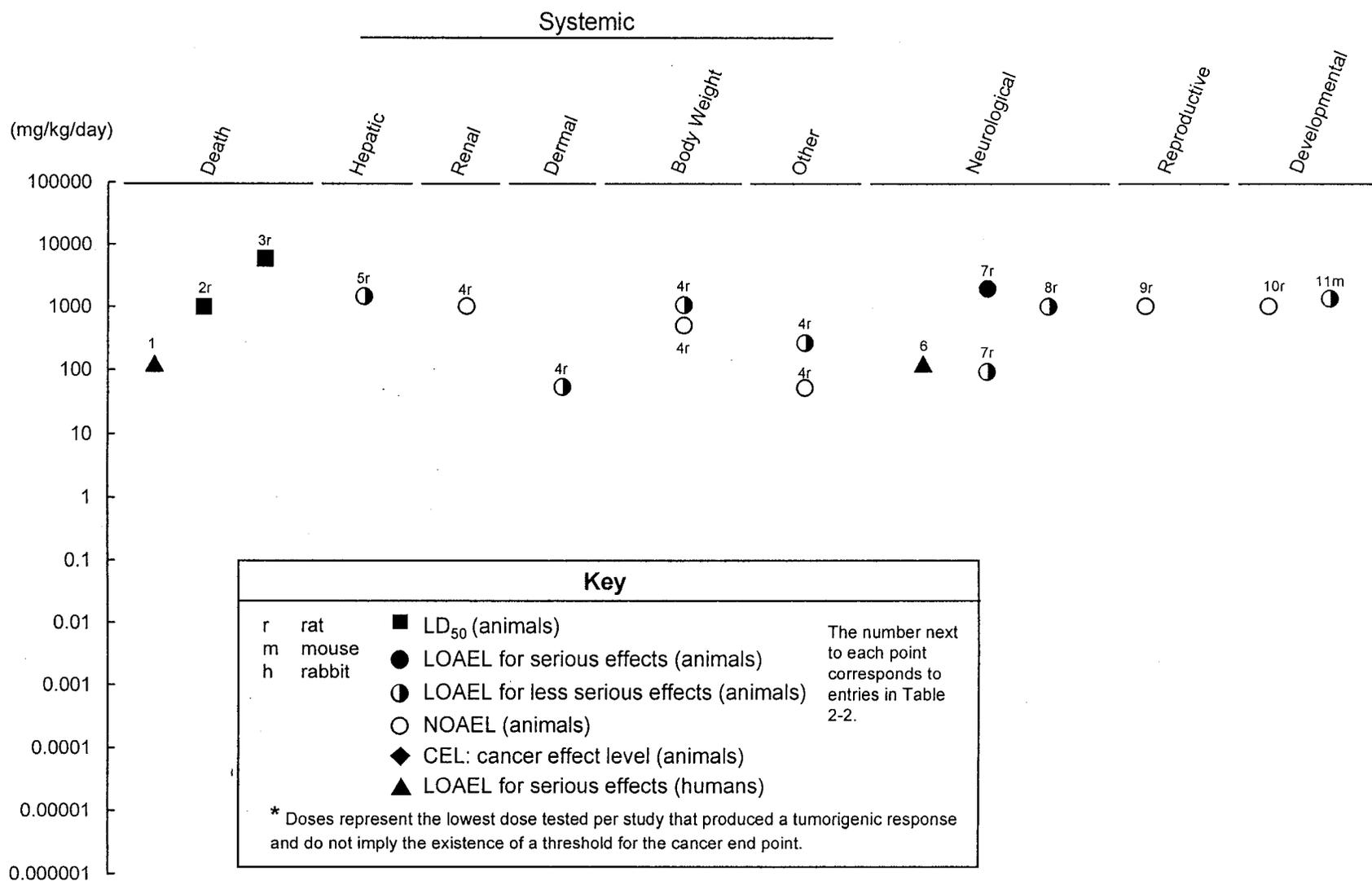
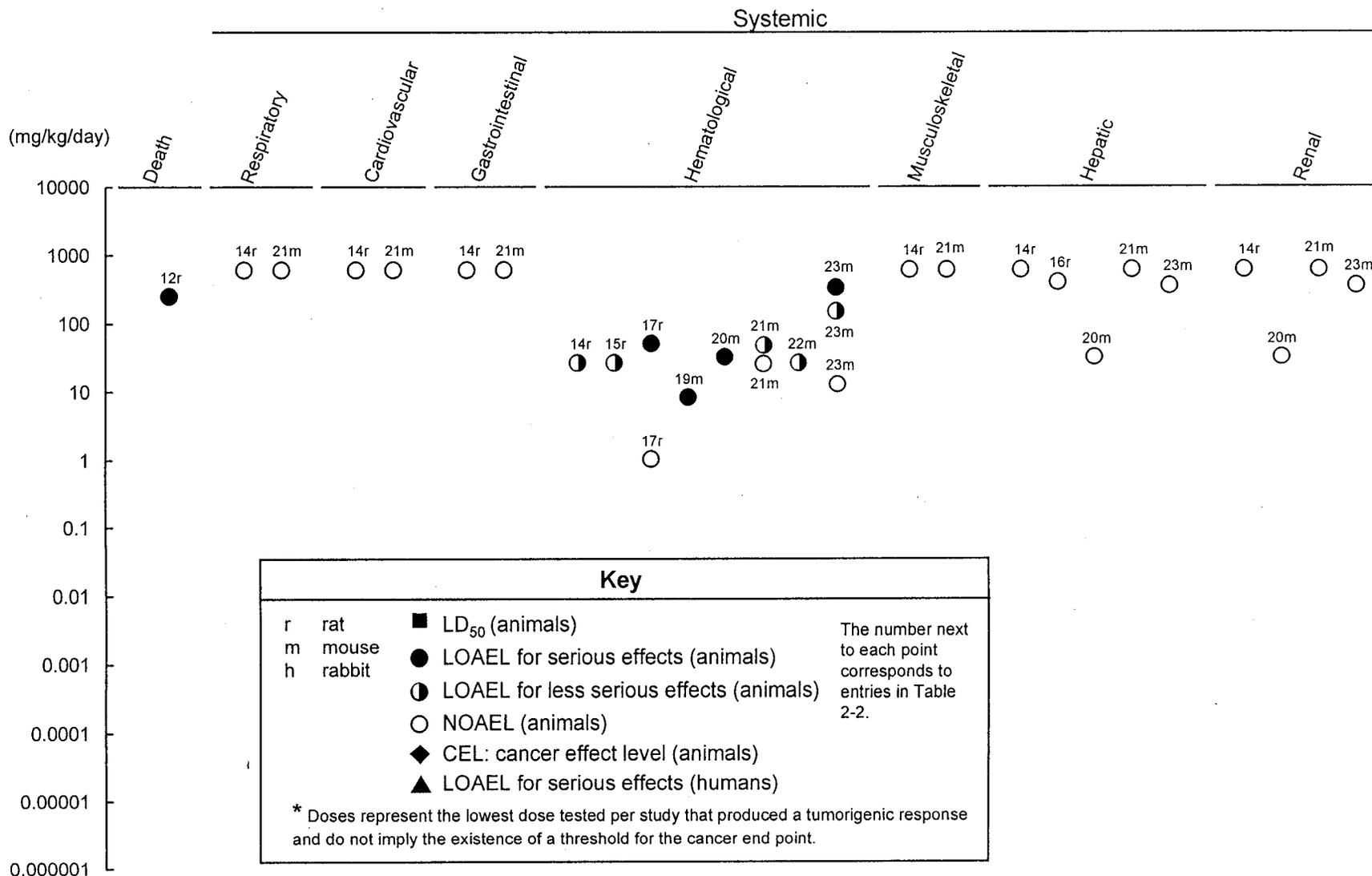
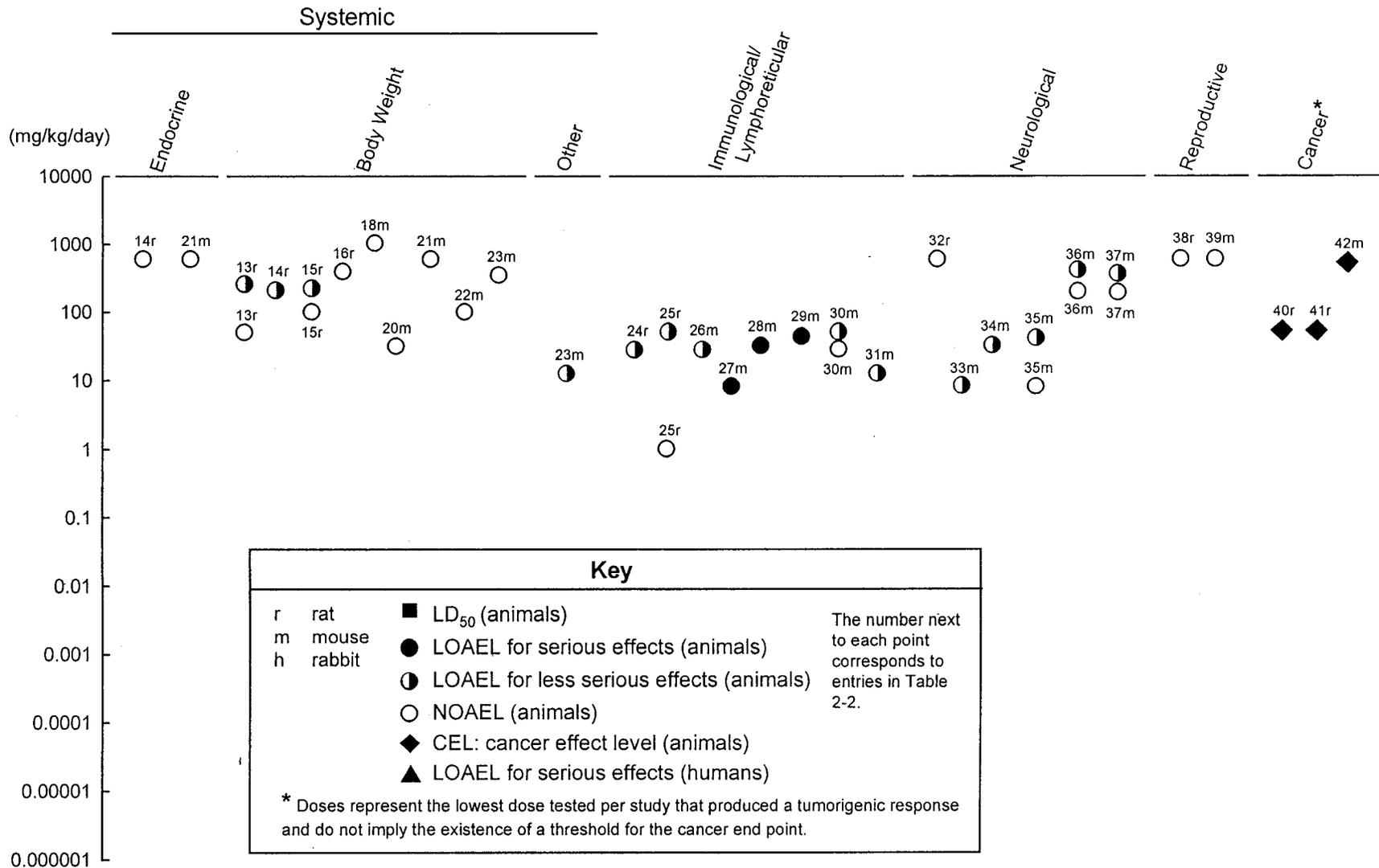


Figure 2-2. Levels of Significant Exposure to Benzene - Oral (cont.)

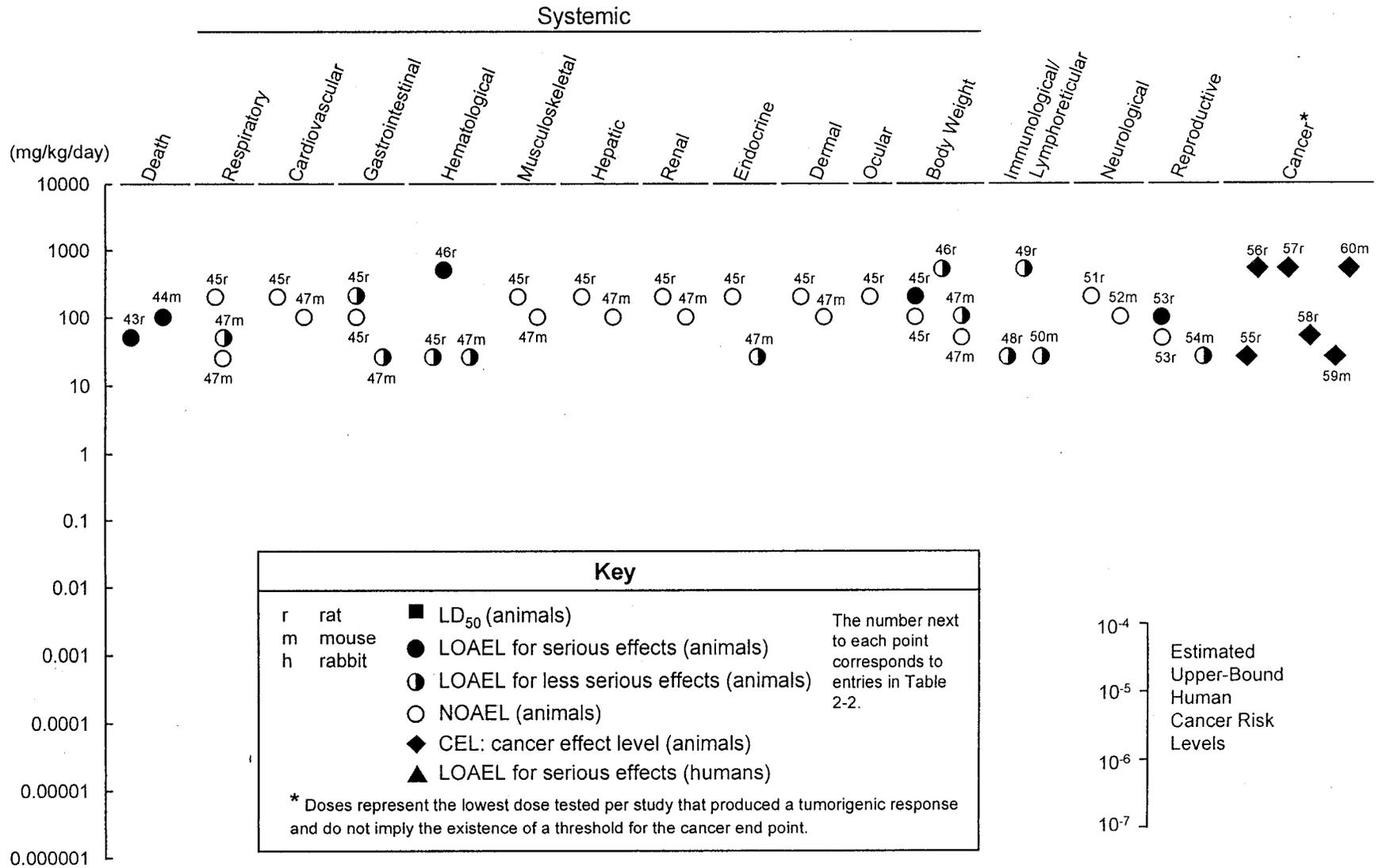
Intermediate (15-364 days)



**Figure 2-2. Levels of Significant Exposure to Benzene - Oral (cont.)**  
**Intermediate (15-364 days)**



**Figure 2-2. Levels of Significant Exposure to Benzene - Oral (cont.)**  
**Chronic (≥365 days)**



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

**Respiratory Effects.** Male and female Fischer 344 rats and B6C3F<sub>1</sub> 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 B6C3F<sub>1</sub> 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  $\leq 200$  mg/kg/day (male rats) or  $\leq 100$  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 B6C3F<sub>1</sub> 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  $\geq 25$  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 B6C3F<sub>1</sub> 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  $\geq 50$  mg/kg/day and female mice at  $\geq 400$  mg/kg/day for 120 days, but not for 60 days. Mice exposed to  $\geq 8$  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 B6C3F<sub>1</sub> 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 per 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  $\geq 25$  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 end of 2 years (Huff et al. 1989; NTP 1986). Increased frequency of micronucleated normochromatic peripheral erythrocytes was observed in mice at  $\geq 25$  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 per week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Decreased erythrocytes and leukocytes after were observed after 84 weeks.

**Musculoskeletal Effects.** No histopathological lesions were observed in femoral tissue from male and female Fischer 344 rats or B6C3F<sub>1</sub> 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 sternebrae, 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 per 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 B6C3F<sub>1</sub> 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 mice (Hsieh et al. 1990). No histopathological non-neoplastic lesions effects were observed in hepatic tissue from male and female B6C3F<sub>1</sub> 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 B6C3F<sub>1</sub> 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 B6C3F<sub>1</sub> 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 B6C3F<sub>1</sub> 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 2.2.2.8). In mice, Zymbal gland lesions showed epithelial hyperplasia in 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 B6C3F<sub>1</sub> 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 B6C3F<sub>1</sub> 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 per 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 B6C3F<sub>1</sub> 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 body weight in CD-1 mice (Hsieh et al. 1990). No adverse effect was observed on body weight of male and female B6C3F<sub>1</sub> 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 per 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 per 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 B6C3F<sub>1</sub> 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 B6C3F<sub>1</sub> 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  $\geq 12$  mg/kg.

### 2.2.2.3 Immunological and Lymphoreticular Effects

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

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 <sup>51</sup>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. For tracing gradual disappearance or delayed appearance of the toxicity after ending benzene administration, groups of mice were killed on day 7, 14, and 21 after the last administration of 27 mg/kg/day benzene for 28 days. 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 B6C3F<sub>1</sub> 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 B6C3F<sub>1</sub> 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 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-cell 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 significantly were 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 secretions were also noted. Similar results were noted at 40 mg/kg/day (Hsieh et al. 1991).

Oral administration of benzene to B6C3F<sub>1</sub> 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 per 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 2-2 and plotted in Figure 2-2.

#### **2.2.2.4 Neurological Effects**

In humans, symptoms of central nervous system toxicity (including giddiness, 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 5hydroxyindoleacetic 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 B6C3F<sub>1</sub> 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 B6C3F<sub>1</sub> mice with doses up to 600 mg/kg/day for 120 days (NTP 1986). In the same experiment, B6C3F<sub>1</sub> 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 in male and female Fischer 344 rats and B6C3F<sub>1</sub> 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 2-2 and plotted in Figure 2-2.

#### **2.2.2.5 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 B6C3F<sub>1</sub> 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 B6C3F<sub>1</sub> 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 2.2.2.8). 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 2-2 and plotted in Figure 2-2.

#### **2.2.2.6 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 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 2-2 and plotted in Figure 2-2.

#### **2.2.2.7 Genotoxic Effects**

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

Oral *in vivo* animal studies provide convincing evidence that benzene is genotoxic. Tests on bone marrow cells, lymphocytes, and erythrocytes in mice gave positive results for chromosomal aberrations and micronuclei increases. Studies clearly demonstrate that, concerning micronuclei and chromosomal aberrations, male mice are considerably more sensitive than females (Barale et al. 1985; Choy et al. 1985; Ciranni et al. 1988; Hatakeyama et al. 1992; Meyne and Legator 1980; Siou et al. 1981). Oral administration of 440 and 879 mg/kg/day benzene for 3 days to male and female CD-1 mice resulted in significantly elevated frequencies of chromosomal aberrations and micronuclei in bone marrow (Meyne and Legator 1980). A significant gain in chromosomal aberrations was observed at 440 mg/kg/day in male mice, while 879 mg/kg/day was needed to produce a similar effect in females. Significant positive responses for micronuclei also occurred after administration of 440 and 879 mg/kg/day to both males and females (Meyne and Legator 1980). In another study, male ICR mice given 36.6, 73.2, and 146.4 mg/kg/day for 14 days exhibited significant dose-dependent increases in chromatid aberrations in spleen lymphocytes and micronuclei in blood NCEs (Rithidech et al. 1987, 1988). Doses of 56.2, 141, 352, and 2,189 mg/kg/day for 2 days produced significant dose-dependent increases in chromosomal aberrations and micronuclei in bone marrow of both male and female Swiss mice, with males being again more sensitive (Siou et al. 1981). In the same study, Chinese hamsters (male and female) were given 2 doses of benzene of either 2,198 or 8,790 mg/kg/day. Neither males nor females showed significant positive responses for increased micronuclei, and only the males given 2 doses of 8,790 mg/kg/day exhibited a significant increase in chromosomal aberrations (Siou et al. 1981). Therefore, hamsters are less sensitive to these effects. In another study, male and female CD-1 mice were given benzene doses ranging from 26 to 440 mg/kg/day. An increase in MN-NCEs was only observed after 21 days in mice exposed to 220 mg/kg/day or more (Barale et al. 1985). Male B6C3F<sub>1</sub> mice exhibited dose-dependent increases of MN-NCEs for intermediate-duration exposures of

oral doses ranging from 25 to 600 mg/kg/day (Choy et al. 1985). Females were less sensitive, and significant dose-dependent increases were observed in male mice starting at 400 mg/kg/day. At each dose and sampling time, the frequency of MN-NCEs was higher in males than in females. Chronic administration of benzene to B6C3F<sub>1</sub> mice produced significant dose-dependent elevations in MN-NCEs at doses of 50 mg/kg/day or higher. At each dose and sampling time, the frequency was higher in males than in females (Choy et al. 1985). For bone marrow cells, increases in MN-PCEs were noted in mice after acute doses of 80 (Ciranni et al. 1988), up to 880 (Ciranni et al. 1991) 500 (Suzuki et al. 1989), 200 (Harper et al. 1984), 220 and 110 (Hite et al. 1980) mg/kg/day. A dose of 50 mg/kg/day for 40 weeks increased the frequency of MN-PCEs and MN-NCEs in both male and female hybrid mice (Armstrong et al. 1993). A comparison of the effects of benzene on MN-PCEs in bone marrow was also made between virgin females, pregnant mice, and their offspring (Ciranni et al. 1988). The researchers observed that virgin females had a higher frequency of micronuclei than pregnant females. Data from the fetuses suggest a benzene sensitivity comparable to that of adult female mice (Ciranni et al. 1988). Evaluation of spermatogonia from the testes of mice treated with doses of benzene up to 880 mg/kg revealed an increase in chromatid aberrations (Ciranni et al. 1991).

Male CD-1 mice were administered benzene dissolved in olive oil at gavage doses of 220 and 440 mg/kg (Chen et al. 1994). Control mice received olive oil. Bone marrow preparations were made 24 hours following benzene treatment. The characterization of the origin of micronuclei formed in the bone marrow erythrocytes and splenic lymphocytes following the *in vivo* exposure to benzene to mice was determined using two molecular cytogenetic approaches: (a) fluorescence *in situ* hybridization with a centromeric DNA probe and (b) immunofluorescent labeling with calcinosis-Raynaud's phenomenon-esophageal dismobility-sclerodactyly-telangiectasia syndrome of sclerodenna (CREST) antibody. Significant increases in the frequency of micronucleated PCE were observed at both 220 and 420 mg/kg. The mean of the polychromatic erythrocytes to normochromatic erythrocytes ratios per animal (PCE:NCE) showed a significant increase at 220 and 440 mg/kg benzene as compared to controls, indicating an increase in the presence of newly formed erythrocytes. *In situ* hybridization with a centromeric DNA probe or immunofluorescent staining with the CREST antibody indicated that the induced micronuclei resulted from both chromosome breakage and chromosomal loss. The frequency of CREST-positive micronuclei increased from approximately 3/1,000 binucleated cells in the controls to 11/1,000 cells in animals treated with 440 mg/kg benzene. The frequency of CREST-negative micronuclei increased from 1.3 of 1,000 binucleated cells in controls to 3 of 1,000 in

mice treated with 440 mg/kg benzene. The nuclear division index (NDI) for treated animals showed significant increases at 220 and 440 mg/kg benzene as compared to controls.

In an attempt to elucidate the *in vivo* adduct formation by benzene metabolites, Reddy et al. (1989b) developed a highly sensitive nuclease P<sub>1</sub>-enhanced <sup>32</sup>P-postlabeling assay for adduct detection at a frequency of one modification per 10<sup>9-10</sup> DNA nucleotides. When DNA was prepared from Zymbal glands of rats given 200 mg/kg of benzene, orally, 5 days per week for 10 weeks, 3 potential adducts were discovered. However, no adducts were detected in the DNA from the liver, kidney, or mammary gland of rats at any dose (Reddy et al. 1989a). The adduct levels corresponded to four lesions per 10<sup>9</sup> DNA nucleotides. The presence of adducts in the Zymbal DNA from benzene-treated rats may be related to the tumor formation in this organ following benzene treatment although this relationship is not clearly defined (see Section 2.2.2.8). Other genotoxicity studies are discussed in Section 2.5.

#### 2.2.2.8 Cancer

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

Lymphomas are sometimes, but not always, a variety of leukemia, and the fact that they are seen as the predominant form of benzene-induced hematological neoplasia in rodents may represent species specificity. Leukemia of the myeloid cell line, most frequently associated with benzene exposure in humans, has a low spontaneous rate in rodents. Lymphatic and hematopoietic cancers were seen in vehicle maintenance workers who occasionally siphoned gasoline by mouth, in addition to inhaling gasoline vapors and using gasoline to wash their hands and clean engine parts (Hunting et al. 1995)

Doses of 50, 100, or 200 mg/kg/day benzene in corn oil were administered by gavage to male Fischer 344 rats, 5 days per week for 103 weeks (Huff et al. 1989; NTP 1986). Doses of 25, 50, or 100 mg/kg/day benzene in corn oil were administered to female Fischer 344 rats and to male and female B6C3F<sub>1</sub> mice according to the same regimen (Huff et al. 1989; NTP 1986). Male rats treated with benzene for 2 years developed increased incidences of Zymbal gland carcinomas at 100 and 200 mg/kg/day (24% and 43%, respectively, compared to 6% in controls), squamous cell papillomas and squamous cell carcinomas of the oral cavity at all doses (18%, 32%, and 38%, respectively, compared to 2% in controls) and squamous cell papillomas and squamous cell carcinomas of the skin at the high dose (24 compared to 2% for controls). For female rats, benzene caused increased

incidences of Zymbal gland carcinomas at all doses (13, 14, and 33% compared to 0% for controls) and squamous cell papillomas and squamous cell carcinomas of the oral cavity at the mid and high doses (24% and 18%, respectively, compared to 2% for controls). Male mice treated with benzene for 2 years developed increased incidences of Zymbal gland squamous cell carcinomas at the mid and high dose (10 and 54%, respectively, compared to 0% in the controls), malignant lymphomas at all doses (21, 20, and 30% compared to 8% for controls), alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined) at the mid and high doses (38 and 43%, respectively, compared to 20% for controls), harderian gland adenomas at all doses (22, 27, and 29% compared to 2% for controls), and squamous cell carcinomas of the preputial gland at the mid and high dose (66% and 89%, respectively, compared to 0% for controls). For female mice, benzene caused increased incidences of malignant lymphomas, at all dose levels (56, 52, and 45% compared to 31% for controls), ovarian granulosa cell tumors and ovarian benign mixed tumors at the mid and high doses (49 and 40%, respectively, compared to 2% for controls), carcinomas of the mammary gland at the mid and high doses (12 and 28%, respectively, compared to 0% for controls), alveolar/bronchiolar adenomas and alveolar/bronchiolar carcinomas in the high dose (27% compared to 8% for controls) and Zymbal gland squamous carcinomas in the high-dose (10% compared to 0% for controls) group. The tumor incidence was dose-related for both rats and mice.

Maltoni et al. (1983, 1985, 1989) conducted a series of experiments on the carcinogenicity of benzene administered orally in olive oil to Sprague-Dawley rats, Wistar rats, RF/J mice, or Swiss mice. Sprague-Dawley rats were given 50, 250, or 500 mg/kg/day of benzene by stomach tube for up to 104 weeks (Maltoni et al. 1983, 1985, 1989). Animals were evaluated for carcinogenicity at different times during the experiment. At 52 weeks, carcinoma of the Zymbal gland was noted in 3% of the Sprague-Dawley rats exposed to 50 mg/kg/day and 11% of rats exposed to 250 mg/kg/day, while no carcinomas were present in controls fed olive oil (Maltoni et al. 1983, 1985, 1989). An increase in the incidence of carcinoma of the oral cavity (3%) was observed in animals receiving 250 mg/kg/day (3%). Carcinoma of the nasal cavities and forestomach, and liver angiosarcoma were also noted at 250 mg/kg/day. At 92 weeks, 15% of Sprague-Dawley rats exposed to 500 mg/kg/day benzene were found to have carcinomas of the Zymbal gland (Maltoni et al. 1983, 1985). Carcinoma of the oral (14%) and nasal (2.5%) cavities, and liver angiocarcinoma (2.5%) were also noted. At 104 weeks, Sprague-Dawley rats given 50-250 mg/kg/day exhibited a similar spectrum of tumors: carcinoma of the Zymbal gland at all doses, carcinoma of the oral cavity at 250 and 500 mg/kg/day, and carcinoma of the nasal cavity and forestomach, and angiosarcoma of the liver at 500 mg/kg/day. At the high

dose, incidence of oral carcinomas was 51%. A 7.5% incidence of forestomach carcinomas (compared to 0% in controls), 5% incidence of hepatomas (compared to 3% in the control group), and 6% incidence of angiosarcomas of the liver (compared to 0% in the control group) was also observed in these animals. Wistar rats exhibited a similar spectrum of tumor development. Sixteen percent of Wistar rats exposed to 500 mg/kg/day benzene for 104 weeks developed Zymbal gland carcinomas, but only 7.5% developed oral carcinomas, in contrast to the Sprague-Dawley rats (Maltoni et al. 1989). Nasal, forestomach, and hepatic tumors were also observed (Maltoni et al. 1989). Leukemia was also noted in the high-dose rats at 104 weeks. The incidences of leukemia in Sprague-Dawley and Wistar rats fed 500 mg/kg/day of benzene for 104 weeks were 5 and 7.5%, compared to 4 and 5% in the respective control groups. There were dose response relationships for the incidences of carcinomas of the Zymbal gland and carcinomas of the nasal and oral cavities. Maltoni also conducted oral carcinogenicity studies of benzene in mice (Maltoni et al. 1989). Carcinomas of mammary glands developed in 24% (compared to 3.7 for controls) and 11% (compared to 1.2% for controls) of Swiss and RF/J mice exposed to 500 mg/kg/day for 78 and 52 weeks, respectively (Maltoni et al. 1989). Increased incidence of tumors of the lung and Zymbal gland were also noted at 500 mg/kg/day in this study.

The 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. Tabulated data showed progression by benzene in rat hepatocarcinogenesis. Results indicated that benzene may have some initiating as well as progressor action at the dose tested; however, stereologic determination of the number of altered hepatic foci (AHF) detected with glutathione-S transferase and g-glutamyl-transpeptidase found very few AHF initiated with benzene and promoted by phenobarbital, which suggests that benzene is a poor initiator under these conditions. Since a single administration of benzene increased the incidence of hepatocellular carcinoma in both male and female rats compared with appropriate controls, benzene may possess progressor activity and increase the risk of cancer development through action at this stage of carcinogenesis. In a similar experiment, male Fischer 344 rats were divided into 4 groups (Taningher et al. 1995). Rats were given a 200 mg/kg

diethylnitrosamine (DENA) intraperitoneal injection in saline followed by gavage treatment with corn oil vehicle; a saline intraperitoneal injection without DENA and then daily doses of 400 mg/kg benzene in corn oil by gavage 5 days per week for 6 weeks; a single intraperitoneal injection of 200 mg/kg DENA in saline to initiate hepatocarcinogenesis, and then two weeks later, daily doses of 400 mg/kg benzene in corn oil 5 days per week for 6 weeks by gavage; or a single intraperitoneal injection of 200 mg/kg DENA in saline to initiate hepatocarcinogenesis then 2 weeks later followed by 500 ppm phenobarbital in their drinking water for 6 weeks. Benzene treatment did not significantly increase the foci number and area in rats treated with DENA alone compared to rats treated with DENA plus benzene. Practically no foci were observed in the liver of benzene treated rats. Thus, benzene exhibited no significant promoting activity.

The EPA Gene-Tox Carcinogenesis Panel categorizes benzene as having sufficient positive evidence for carcinogenicity in animal studies (IRIS 1996), and NTP (1986) concluded that there was evidence of carcinogenicity of benzene for the strains of rats and mice tested in their 2-year oral/gavage bioassay program.

EPA (IRIS 1996) reviewed human and animal carcinogenicity data on benzene. The following paragraph is a summary of the EPA calculations of unit risk values for leukemia based on human epidemiological studies. It should be noted that these values are estimates of human risk since the true human risk at low doses cannot be accurately identified. The unit risk for drinking water of  $8.3 \times 10^{-7}$  per  $\mu\text{g/L}$  should not be used if the water concentration exceeds 10,000  $\mu\text{g/L}$ , since above this concentration the slope factor may no longer be accurate.

EPA has used cancer risk data from human epidemiological studies to derive risk factors associated with oral exposure to benzene. Oral dose levels associated with specific carcinogenic risks have been extrapolated: the risk value of  $2.7 \times 10^{-2}$  for lifetime inhalation exposure to 1 ppm was converted to a slope factor of  $2.9 \times 10^{-2}$  for oral exposure of 1 mg/kg/day, assuming identical levels of absorption of benzene following both routes of exposure. Using the method described by EPA (IRIS 1996), the drinking water levels associated with individual upper-bound estimates of  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$  have been calculated to be  $1 \times 10^{-1}$ ,  $1 \times 10^{-2}$ ,  $1 \times 10^{-3}$ , and  $1 \times 10^{-4}$  mg/L, respectively, which are equivalent to dose levels of  $3 \times 10^{-3}$ ,  $3 \times 10^{-4}$ ,  $3 \times 10^{-5}$ , and  $3 \times 10^{-6}$  mg/kg/day, respectively. These dose levels are indicated in Figure 2-2.

The CELs for each species and duration category are recorded in Table 2-2 and Figure 2-2.

### 2.2.3 Dermal Exposure

#### 2.2.3.1 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 3 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.

#### 2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, or body weight effects in humans or animals or musculoskeletal effects in animals after dermal exposure to benzene. Available data pertaining to musculoskeletal, 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 2-3.

**Musculoskeletal 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

Table 2-3. Levels of Significant Exposure to Benzene - Dermal

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

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 hrs). 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 one time per 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 per day, 5 days per 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).

### **2.2.3.3 Immunological and Lymphoreticular Effects**

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

### **2.2.3.4 Neurological Effects**

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. 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.

### **2.2.3.5 Reproductive Effects**

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

### **2.2.3.6 Developmental Effects**

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

### **2.2.3.7 Genotoxic Effects**

A few case studies were found involving individuals whose job descriptions suggest dermal as well as inhalation exposure to benzene (Forni and Moreo 1967, 1969; Van den Berghe et al. 1979; Yardley-Jones et al. 1988, 1990). These cases are discussed under inhalation in Section 2.2.1.7.

No studies were located regarding genotoxic effects in animals after dermal exposure to benzene. Other genotoxicity studies are discussed in Section 2.5.

### **2.2.3.8 Cancer**

A cohort of 338 men were 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 3 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 and some workers would occasionally siphon gasoline by mouth. The men were employed for at least 1 year between January 1, 1977, and December 31, 1989. Cause specific SMRs were calculated and the relative risk of leukemia and other hematological cancers among all fleet maintenance workers and for the high exposure subgroup were estimated. The all-cause SMR was 0.50 with 33 deaths. The all-cancer SMR was 0.55 with 9 total deaths. The underlying causes of death from cancers are as follows: 1 from cancer of the digestive organs and peritoneum (SMR=0.22); 2 from cancer of the respiratory system (SMR=0.33); 2 from cancer of the

male genitalia (SMR=1.91); 3 from lymphatic and hematopoietic cancer (SMR=3.63); 1 from other and unspecified cancer (SMR=0.49). A subgroup analysis (n=297) reported 3 observed deaths for lymphatic and hematopoietic cancers (SMR=4.22); 2 observed deaths for leukemia and aleukemia (SMR=9.26); and 1 observed death for other neoplasms of lymphatic and hematopoietic tissue (SMR=2.57). The SMR of 9.26 for leukemia and aleukemia was statistically significant ( $p < 0.05$ ).

Dermal exposure to benzene did not induce skin tumors in mice (Bull et al. 1986). No papillomas developed in mice that were given a 2-week, 800 mg/kg/day topical application of benzene as the initiator and a 1 µg topical application of 12-*o*-tetradecanoylphorbol-13-acetate 3 times a week for 20 weeks and observed for 52 weeks (Bull et al. 1986). The authors concluded that it is difficult to estimate benzene-induced tumor incidence after dermal exposure, and that mouse skin may not be the optimal study system. This is because of the high rate of false-negative responses to chemicals, like benzene, with recognized carcinogenic activity.

### 2.3 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.

Absorption, distribution, metabolism, and elimination have been studied in both humans and animals. Investigations of the metabolism of benzene have led to the identification of toxic metabolites, and to hypotheses about the mechanism of toxicity.

#### 2.3.1 Absorption

##### 2.3.1.1 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. 1993; 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). Existing evidence indicates that benzene is rapidly absorbed by humans following inhalation exposure. Results from a study of 23 subjects that 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%. Respiratory retention (the amount of absorbed benzene that is not excreted via the lungs) was approximately 30% (Nomiyama and Nomiyama 1974a). In a similar study, 3 healthy non-smoking 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).

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 workphases were measured at 5 Finnish garages. Blood samples from car mechanics (8 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  $6 \pm 7 \text{ cm}^3/\text{m}^3$  ( $2 \pm 2$  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  $1.3 \text{ cm}^3/\text{m}^3$  (0.1-0.4 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 0.8-1.2 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  $0.5\text{-}1.1 \text{ cm}^3/\text{m}^3$  (0.2-0.3 ppm). During changing of the fuel filter to electronic fuel-injection system, benzene concentration ranged from 0.9 to  $3.4 \text{ cm}^3/\text{m}^3$  (0.3-1.1 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 8 workers had minimal exposure through the skin (0-1.1%). The other 6 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. 1993). 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  $\mu\text{g/L}$  (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 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 six 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 benzene levels were measured in the shower stall at 18-20 minutes (758-1,670  $\mu\text{g/m}^3$ ), in the bathroom at 10-25 minutes (366-498  $\mu\text{g/m}^3$ ), in the bedroom at 25.5-30 minutes (81-146  $\mu\text{g/m}^3$ ), and in the living room at 36-70 minutes (40-62  $\mu\text{g/m}^3$ ). The individual who took the 20-minute shower had inhalation doses of 79.6, 105, and 103  $\mu\text{g}$  (mean = 95.9  $\mu\text{g}$ ) for the 3 consecutive sampling days. These doses were calculated 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  $\mu\text{g/m}^3$ ) gave a total average shower-related inhalation dose of 113  $\mu\text{g}$ . An average dermal dose of 168  $\mu\text{g}$  was calculated 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/hr, an exposure duration of 0.33 hours, and a unit conversion factor of 11/1,000 cm<sup>2</sup>. 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.

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) report blood and urine levels of benzene for nonsmokers as 110.9 and 116.4 ng/L, respectively, and for smokers, 328.8 and 405.4 ng/L, respectively. The National Association of Medical Examiners Pediatric Toxicology (PedTox) Registry reported blood benzene concentrations ranging from 0.2 to 4.9 mg/L in 8 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 (range ND-0.055) µg/L, 0.18 (range 0.063-1.1) µg/L, and 0.066 (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.

Indoor and ambient air samples were measured from December 1992 to February 1993 by the Alaska Department of Environmental Conservation (Gordian and Guay 1995). Concentration levels for methyl tertiary butyl ether (MTBE), benzene, and formaldehyde were measured in ambient and indoor air samples. Blood samples were also taken from service station workers at the same time period. Blood samples from workers showed an increase of 300% in blood benzene concentrations after MTBE reformulated fuel was discontinued. Gasoline in Alaska has a benzene concentration of 5% (average U.S. national concentration is 1.5%).

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  $\approx$ 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 per day, 5 days per week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

### 2.3.1.2 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 ( $^{14}\text{C}$ ) 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 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. 1993).

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.

### 2.3.1.3 Dermal Exposure

Studies conducted *in vivo* in humans and *in vitro* using human skin indicate that benzene can be absorbed dermally. The data show that dermal absorption is not as substantial as absorption following inhalation exposure to benzene vapor or oral exposure. 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 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 (Loden 1986).

*In vivo* experiments on 4 human volunteers, to whom 0.0026 mg/cm<sup>2</sup> of <sup>14</sup>C-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 cm<sup>2</sup> of the forearm was exposed to approximately 0.06 g/cm<sup>2</sup> 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/cm<sup>2</sup>/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 workphases were measured at 5 Finnish garages. Blood samples from car mechanics (8 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 6 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. 1993). 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  $\mu\text{g}$  was calculated for a 20-minute shower using this water. The total benzene dose resulting from the shower was estimated to be approximately 281  $\mu\text{g}$  (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 2.3.1.1 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.

*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/cm<sup>2</sup> after 0.5 hours and 1.92 mg/cm<sup>2</sup> after 13.5 hours (Loden 1986). Following application of 5, 120, 270, and 520 µL/cm<sup>2</sup> of benzene to human skin, total absorption was found to be 0.01, 0.24, 0.56, and 0.9 µL/cm<sup>2</sup>, respectively. Thus, the total amount absorbed appears to increase linearly with dose. 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 µL/hour from inhalation and 1.5 µL/hour from whole-body (2 m<sup>2</sup>) dermal exposure (Blank and McAuliffe 1985). It was also estimated that 100 cm<sup>2</sup> of smooth and bare skin in contact with gasoline containing 5% benzene would absorb 7.0 µL/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).

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 exaggerated 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 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/cm<sup>2</sup> <sup>14</sup>C-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/cm<sup>2</sup>/hour (0.31 µg/cm<sup>2</sup>/hour); at 1,000 ppm the rate was 24.2 nmol/cm<sup>2</sup>/hour (1.89 µg/cm<sup>2</sup>/hour); and at 3,000 ppm the rate was 75.5 nmol/cm<sup>2</sup>/hour (5.90 µg/cm<sup>2</sup>/hour). The skin absorption coefficient was 0.619 cm/hour.

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 for 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. This value more closely correlates with the permeability constant of 1.5 derived by McDougal et al. (1990), than does the commonly used octanol/water partition coefficient of 134.9, as derived by Leo et al. (1971). 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 to be 0.037 for humans. 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.

## 2.3.2 Distribution

### 2.3.2.1 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).

Respiratory uptake (the amount of benzene absorbed from the lungs following inhalation of the vapors) in 6 male and female volunteers exposed to 52-62 ppm benzene for 4 hours was determined to be approximately 47% (Nomiyama and Nomiyama 1974a). Respiratory retention (the amount of benzene that is not excreted via the lungs following absorption) was determined to be about 30%. Although no sex differences in the final retention of benzene were observed in this study, another study in which male and female subjects were exposed to 25 ppm for 2 hours showed that benzene appeared to be retained longer in the females (Sato et al. 1975). The authors attributed these results to the higher fat content of females, but the difference in the total exposure period (4 hours as opposed to 2 hours) could have been responsible for the observed discrepancy in the results. -

Results from animal studies indicate that absorbed benzene is distributed throughout 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 to 2,000 ppm of benzene by pregnant mice, benzene and its metabolites were found to be present in lipid-rich tissues, such as brain and fat, and in wellperfused 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 per 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 per/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).

#### **2.3.2.2 Oral Exposure**

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

One animal study examined the distribution of benzene and its metabolites following oral exposure. In Sprague-Dawley rats administered a single dose of 0.15, 1.5, 15, 150, or 500 mg/kg of <sup>14</sup>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  $^{14}\text{C}$ -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  $^{14}\text{C}$ -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.

### 2.3.2.3 Dermal Exposure

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

A study of male rats treated dermally with  $0.004 \text{ mg/cm}^2$  of  $^{14}\text{C}$ -benzene, with and without 1 g of clay or sandy soil, revealed soil-related differences in tissue distribution following treatment. The  $^{14}\text{C}$  activity (expressed as a percentage of initial dose per g of tissue) 48 hours after treatment with soiladsorbed 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 3 groups, less than 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 carcass.

### 2.3.3 Metabolism

There is no available evidence to suggest that the route of administration has any substantial effect on the subsequent metabolism of benzene in humans. There is, however, information from animal studies to indicate that the exposure route affects the disposition and metabolism (Sabourin et al. 1987, 1988, 1989a, 1989b).

Data regarding metabolism of benzene in humans have come primarily from studies using inhalation exposures. Benzene is excreted both unchanged via the lungs and as metabolites 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).

Benzene metabolism in humans and animals follows many similar pathways. Benzene is metabolized by cytochrome P-450-dependent mixed-function oxidase enzymes. Cytochrome P-450s are ubiquitous in all tissues. However, since the predominant repository of cytochrome P-450 is the liver, benzene is primarily metabolized in the liver. The metabolism of benzene by hepatic cytochrome P-450 may play an important role in the bioactivation of benzene. Sammett et al. (1979) provided corroborative evidence for this role by showing that partial hepatectomy of rats diminished both the rate of metabolism of benzene and its toxicity, suggesting that a metabolite and/or metabolites formed in the liver are necessary for toxicity.

There are several toxification pathways and detoxification pathways (via conjugation) of benzene metabolism (Henderson et al. 1989). The first step in benzene metabolism is the formation of the epoxide benzene oxide via cytochrome P-450-dependent mixed-function oxidase (Jerina et al. 1968). After this occurs, two metabolic toxification pathways, one involving ring hydroxylation and the second involving ring opening, result in the formation of putative toxic benzene metabolites (Henderson et al. 1989). In the first pathway involving ring hydroxylation, acid-catalyzed opening of the epoxide ring is followed by aromatization resulting in the formation of phenol. Phenol is further converted into hydroquinone which is oxidized to benzoquinone. The conjugates formed from hydroquinone (hydroquinone glucuronide and hydroquinone sulfate) are markers for this toxification pathway leading to benzoquinone (Henderson et al. 1989). Phenol can also be metabolized to catechol

and trihydroxy benzene. Metabolism of benzene oxide by epoxide hydrolase leads to the formation of benzene dihydrodiol. Catechol can also be formed from benzene dihydrodiol via metabolism by cytosolic dehydrogenases (Henderson et al. 1989). The second pathway involving ring-opening leads to the formation of muconic acid via muconaldehyde. 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. 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).  $\beta$ -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. 1990). 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  $^{14}\text{C}$ -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. 1990). Other studies in animals support these results (Brondeau et al. 1992; Ducos et al. 1990; McMahon and Birnbaum 1991; 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  $0.22 \pm 0.03$  and  $0.24 \pm 0.02$  mg/g creatinine, or  $0.13 \pm 0.06$  and  $0.13 \pm 0.07$  mg/mg cotinine, respectively. Mean concentrations of muconic acid in groups of 63 pregnant and 53 nonpregnant female smokers were  $0.27 \pm 0.04$  and  $0.24 \pm 0.02$  mg/g creatinine, or  $0.24 \pm 0.06$  and  $0.13 \pm 0.07$  mg/mg cotinine, respectively. Because of its relative importance in benzene toxicity, 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).

There are two detoxification pathways. One pathway leads to the formation of mercapturic acid via glutathione conjugates of benzene oxide, which are subsequently metabolized to prephenyl mercapturic acid and phenyl mercapturic acid and eliminated via biliary excretion (Henderson et al. 1989; Sabourin et al. 1988; Schafer et al. 1993; Schlosser et al. 1993; Schrenk et al. 1992; van Sittert et al. 1993). The major portion of benzene oxide is nonenzymatically rearranged into phenol (Parke and Williams 1953a). The second detoxification pathway involves the formation of water-soluble urinary metabolites, which are glucuronide or sulfate conjugates of phenol (Henderson et al. 1989; Wells and Nerland 1991). Further metabolites of phenol and benzene dihydrodiol (e.g., catechol, hydroquinone, and trihydroxy benzene) are excreted as sulfate or glucuronide conjugates, which are considered detoxification products of benzene metabolism (Henderson et al. 1989; Schrenk and Bock 1990). Electroanalytical methods have been used to further elucidate the metabolism of benzene and phenol by both microsomal and peroxidase enzymes (Lunte and Lunte 1990). The pathways for biotransformation of benzene are shown in Figure 2-3.

The ability of bone marrow to metabolize benzene independently of the liver has been tested *in situ* in male Fischer 344 rats. Benzene metabolism by bone marrow 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. The concentration of total metabolites in blood was 0.215 nmol/g as compared to 81.3 nmol/g for bone marrow (Irons et al. 1980). 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 is known to play critical roles in modulating hydroquinone-induced toxicity; this suggests a metabolic basis for the observed increased susceptibility of mouse to benzene-induced hematotoxicity (Zhu 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 cytochrome P-450 (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 B6C3F<sub>1</sub> 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 one particular cytochrome P-450 family, namely cytochrome P-450<sub>ALC</sub> (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. 1990; Parke 1989; Snyder et al. 1993a). It is possible that hydroxy radical formation by cytochrome 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 P-450 in human 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.

Several studies examined the role of phenobarbital in benzene metabolism (Nakajima et al. 1985; Pawar and Mungikar 1975). Carworth Farms rats treated intraperitoneally with phenobarbital had higher activity levels of aminopyrine *N*-demethylase and acetanilide hydroxylase (determined in the liver extracts) than benzene-treated or control animals, indicating increased hepatic activity (Pawar and Mungikar 1975). However, when rats were orally pretreated with 1,400 mg/kg benzene and then treated intraperitoneally with phenobarbital, both the aminopyrine *N*-demethylase and acetanilide hydroxylase activity were similar to control levels. Other results indicate that phenobarbital

pretreatment of rats prior to treatment with benzene alters the drug metabolizing enzyme system producing more total and unconjugated phenol while decreasing benzene hematotoxicity (Gill et al. 1979). No increase in benzene metabolism was found in rats pretreated with phenobarbital and then exposed to a single inhalation dose of 500 ppm benzene (Nakajima et al. 1985). *In vitro* studies have also shown that phenobarbital may not increase metabolism of benzene and may have a protective effect against benzene-induced toxicity by enhancing the activity of conjugation reaction enzymes (Sato and Nakajima 1985).

Both NADPH-linked and ascorbate-induced lipid peroxidation activities induced *in vitro* were lowered 5.5% and 26%, respectively, in Carworth Farms rats following oral administration of 1,400 mg/kg/day of benzene for 3 days, followed by intraperitoneal injection of phenobarbital. It is concluded 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).

$\beta$ -Diethyl amino ethyl diphenyl propyl acetate hydrogen chloride (SKF-525A) and toluene have inhibited benzene metabolism in rats (Gill et al. 1979; Ikeda et al. 1972). Carbon monoxide, aniline, aminopyrine, cytochrome C, and metapyrene inhibit benzene metabolism in mouse liver microsomes (Gonasun et al. 1973). Ethanol ingestion as well as dietary factors such as food deprivation and carbohydrate restriction, enhance the *in vitro* metabolism of benzene in rat liver microsomes (Nakajima et al. 1987).

In rats exposed to ethanol and benzene, it was found that benzene interfered with the disappearance of ethanol from the body (Nakajima et al. 1985). The results of further studies showed that ethanol treatment increased the production of hydroxylated benzene metabolites, phenol and hydroquinone, suggesting induction of benzene metabolism (Nakajima et al. 1987). Ethanol is known to be an inducer of cytochrome P-450 (CYP2E1), the enzyme involved in the initial metabolism of benzene. The toxicity of alcohol, particularly necrosis of the gastrointestinal tract, is known to be associated with oxygen radical production. The possibility therefore exists of a synergism between-alcohol and benzene, and of the role of reactive oxygen and the formation of circulating lipid peroxides in the hematopoietic toxicity and carcinogenicity of benzene (Parke 1989).

Oral kinetic studies of pure, as opposed to soil-adsorbed, benzene were conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of radiolabeled benzene

alone, or radiolabeled benzene adsorbed to clay or sandy soil. Radioactivity was determined in plasma, stomach tissue, expired air, and urine. No difference in metabolism was detected with the treatments. However, the data suggested that loose binding of the benzene to the soils allowed more benzene to be available for metabolism, as opposed to being exhaled unchanged. This was especially true for the clay soil.

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. 1990).

Additional studies by Sabourin et al. (1987, 1988, 1989a, 1989b, 1992) showed that differences in species, routes of exposure, and dosing regimens will affect the disposition and metabolic fate of benzene. The metabolic fate of benzene follows two pathways that are currently thought to lead to toxic intermediates of benzene. Since they generate toxic products, they are also called toxification pathways. Each of the two pathways has its respective markers. One pathway of benzene metabolism involves the ring opening and is marked by the presence of *trans,trans*-muconaldehyde and muconic acid. Muconic acid has been postulated to be the marker of this pathway involving ring-opening. The second pathway involving ring hydroxylation includes the formation of ring-hydroxylated products such as phenol, hydroquinone, and hydroquinone conjugates. Consequently, hydroquinone and its conjugates have been postulated to be markers of this ring hydroxylation pathway. In addition, there are detoxification pathways involving mercapturic acid formation and phenyl conjugate formation. Therefore, markers of the detoxification pathways include phenyl conjugates such as prephenyl and phenyl mercapturic acid, phenyl sulfate, and phenyl glucuronide. The effect of species differences was evidenced by the fact that mice have a higher minute volume per kg body weight than rats ( $\square$  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). Rats and mice were 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 Fischer 344 rats and B6C3F<sub>1</sub> mice that were exposed to 50 ppm of radiolabeled (<sup>3</sup>H) 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.

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 <sup>14</sup>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 B6C3F<sub>1</sub> 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. B6C3F<sub>1</sub> 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).

A physiologically based pharmacokinetics (PBPK) model based on the ventilation rate, cardiac output, tissue blood flow rates, and volumes as well as measured tissue/air and blood/air partition coefficients has been developed (Medinsky et al. 1989a; Travis et al. 1990). Experimentally determined data and model simulations indicated that during and after 6 hours of inhalation exposure to benzene, mice metabolized benzene more efficiently than rats (Medinsky et al. 1989a). After oral exposure, mice and rats appeared to metabolize benzene similarly up to oral doses of 50 mg/kg, above which rats metabolized more benzene than did mice on a per kg body weight basis (Medinsky et al. 1989b). This model may be able to predict the human response based on animal data. Benzene metabolism followed Michaelis-Menton kinetics *in vivo* primarily in the liver, and to a lesser extent in the bone marrow. Additional information on PBPK modeling is presented in Section 2.3.5.

The effect of dosing regimen on benzene metabolism was studied in Fischer 344 rats and B6C3F<sub>1</sub> 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 ( $\approx 200$  ppm by inhalation) (Sabourin et al. 1989b), then the fraction of toxic metabolites formed relative to the amount administered will be reduced.

Kenyon et al. (1995) compared their urinary profile of metabolites in B6C3F<sub>1</sub> mice after oral dosing with phenol with the results of Sabourin et al. (1989a) who administered a comparable oral dose of benzene to B6C3F<sub>1</sub> mice. Their analysis 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. They 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).

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 have been found to form covalent adducts with nucleic acids and proteins in rats (Norpoth et al. 1988); to 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; Longacre et al. 1981a, 1981b; Sun et al. 1990); to bind to proteins in perfused bone marrow preparations (Irons et al. 1980) and in rat liver DNA *in vivo* (Lutz and Schlatter 1977); and to bind to DNA in rabbit and rat bone marrow mitochondria *in vitro* (Rushmore et al. 1984). The inhibition of ribonucleic acid (RNA) synthesis in liver and bone marrow mitochondria has been correlated with covalent binding of benzene metabolites to DNA *in vitro* (Kalf et al. 1982). *In vitro* studies showed that benzene metabolites inhibited RNA synthesis and formed adducts similar to benzene; *p*-benzoquinone was the most effective inhibitor of RNA synthesis. When  $^{14}\text{C}$ -hydroquinone was added to  $^3\text{H}$ -deoxyguanosine, two adducts were formed. Two adducts were also formed when  $^{14}\text{C}$ -hydroquinone was reacted with DNA from calf thymus and *M. lysodeikticus* (Snyder et al. 1987). Fischer 344 rats and B6C3F<sub>1</sub> mice were administered a single dose of 50, 100, 200, and 400 mg/kg  $^{13}\text{C}/^{14}\text{C}$ -radiolabeled benzene in corn oil via gastric intubation (McDonald et al. 1994). Animals were sacrificed 24 hours after administration of labeled benzene. Radiolabeled ( $^{14}\text{C}/^{13}\text{C}$ ) adducts in hemoglobin and bone marrow were monitored. Cysteine adducts of benzene oxide and 1,2- and 1,4-benzoquinone in the rats were assayed, and the proportions of cysteine-bound adducts to total protein binding were estimated. Increasing production of hemoglobin adducts was observed over all dose levels tested. Benzene oxide adducts with rat hemoglobin represented 27% of the total Hgb binding and 73% of the cysteinyl binding, whereas quinone adducts represented relatively small proportions. In the bone marrow, benzoquinone adducts were more abundant than those of benzene oxide. 1,2-benzoquinone adducts predominated in rat marrow with 9% of binding. The relationship between marrow adducts and dosage were linear. Protein adduct levels in the bone marrow were about 20% of the levels in rat hemoglobin. Comparisons of adduct production in the bone marrow indicated that 1,2-benzoquinone adducts were approximately 2.3-fold greater than those of 1,4-benzoquinone in the rat. In the mouse, increasing production of hemoglobin adducts was observed over all dose levels tested. With mouse hemoglobin, the 1,4-benzoquinone adducts accounted for 5.5% of the total hemoglobin binding and 12.2% of the cysteinyl binding, while 1,2-benzoquinone and benzene oxide each accounted for less than 3% of the total. In the bone marrow, benzoquinone adducts were more abundant than those of benzene oxide. 1,4-Benzoquinone adducts were more abundant in the mouse with 21% of binding. Protein adduct levels in the bone marrow were about 60% of the levels in mouse hemoglobin. Comparisons of adduct production in the bone marrow indicated that 1,4-benzoquinone adducts were approximately 17-fold greater than those of 1,2-benzoquinone in the mouse.

The use of  $^{13}\text{C}/^{14}\text{C}$ -radiolabeled benzene enabled McDonald et al. (1994) to show that some phenol and hydroquinone adducts (those containing non-radiolabeled  $^{12}\text{C}$ ) were present prior to experimental exposure of the animals to benzene, most likely from non-benzene sources, i.e., background levels. Background levels of adducts (non-radiolabeled) of 1,2-benzoquinone and 1,4-benzoquinone with hemoglobin and bone marrow proteins of both rats and mice were much greater than those of the radiolabeled benzene-specific adducts. In rat hemoglobin, background levels of 1,2-benzoquinone and 1,4-benzoquinone were 68-fold and 473-fold higher, respectively, than the adduct formed from the administered radiolabeled benzene of 50 mg/kg, and 11- and 25-fold higher at the 400 mg/kg dose. Similarly, mouse hemoglobin showed background levels of adducts that were 6-18-fold higher than the radiolabeled adducts at the low dose, and 2.7-5.2-fold higher at the high dose. When levels of benzoquinone adducts were expressed as ratios of the background levels, the levels of adducts from benzene exposure approached background levels at the two highest doses (200 and 400 mg/kg), but not at  $\leq 100$  mg/kg/day. The authors suggest that the background levels of quinone adducts accumulated over the life-span of the protein, and that a chronic steady-state exposure to benzene itself would result in levels of quinone adducts that would equal or exceed background levels. The existence of background levels of quinone adducts are important in assessing risk of exposure to benzene, especially at low doses.

In another study, Low et al. (1995) compared the benzene metabolizing activity of solid-tumor target and nontarget tissue homogenates from rats. Zymbal gland, nasal cavity, bone marrow, oral cavity, and mammary gland were the target organs; the liver, blood, brain and kidney were the nontarget organs. Target organs exhibited a greater ability to deconjugate but not to conjugate polar metabolites, to oxidize benzene to reactive intermediates, and to possess greater peroxidase activity than the nontarget organs. These results suggest that target organs may show increased susceptibility to the toxic effects of benzene through their ability to preferentially produce reactive intermediates.

Multiple mechanisms are involved in benzene toxicity and carcinogenicity. Such mechanisms may include synergism between metabolites (Eastmond et al. 1987; Snyder et al. 1989) or synergism between glutathione-depleting metabolites of benzene and oxygen radicals (generated by futile cycling of cytochrome P-450 or cycling of quinone metabolites) (Parke 1989).

The benzene metabolites hydroquinone and muconic dialdehyde can produce hematotoxic effects (Eastmond et al. 1987; Gad-El Karim et al. 1985; Latriano et al. 1986). The co-administration of

phenol (75 mg/kg/day) and hydroquinone (25-75 mg/kg/day) twice daily for 12 days to B6C3F<sub>1</sub> mice produced myelotoxicity similar to that induced by benzene (Eastmond et al. 1987). The proposed mechanism suggested that selective accumulation of hydroquinone occurred in the bone marrow after the initial hepatic conversion of benzene to phenol and hydroquinone. Additionally, phenol is thought to stimulate the enzymatic activity of myeloperoxidase, which uses phenol as an electron donor, thus producing phenoxy radicals. These radicals further react with hydroquinone to form 1,4-benzoquinone, a toxic intermediate that inhibits critical cellular processes (Eastmond et al. 1987).

Legathe et al. (1994) investigated the pharmacokinetics of hydroquinone and phenol in blood and recovery in urine after intraperitoneal administration of 75 mg/kg alone or in combination to B6C3F<sub>1</sub> mice. Combined administration resulted in a 2.6-fold increase in the area under the curve (AUC) for blood concentration of hydroquinone, and increased the half-life of hydroquinone from 9 to 15 minutes. The AUC of phenol was increased by a factor of 1.4, and clearance of phenol was decreased from 89 mL/min/kg when injected alone, to 62 mL/min/kg after co-administration. Recovery of conjugated metabolites in the urine indicated that both conjugation pathways were diminished. The authors suggest that enhanced myelotoxicity observed after co-administration is the result of reduced elimination of hydroquinone and phenol, leaving more phenol available for conversion to hydroquinone.

In studies on the erythroid cell line, Longacre et al. (1981b) demonstrated 60-80% decreases in the incorporation of iron (as <sup>59</sup>Fe) into bone marrow precursors of mice injected subcutaneously with 12-20 doses of 440 or 880 mg/kg of benzene. When given 48 hours prior to measuring <sup>59</sup>Fe uptake, benzene produced a dose-dependent decrease in erythroid cells (Snyder et al. 1989). Following intraperitoneal administration of benzene, hydroquinone, p-benzoquinone, and *trans,trans*-muconaldehyde, <sup>59</sup>Fe uptake was reduced. The combination of metabolites most effective in reducing <sup>59</sup>Fe uptake was hydroquinone plus *trans,trans*-muconaldehyde (Snyder et al. 1989).

### 2.3.4 Elimination and Excretion

#### 2.3.4.1 Inhalation Exposure

Evidence indicates that in humans 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). Respiratory uptake (the amount of benzene absorbed from the lungs following inhalation of the vapors) in 6 male and female volunteers exposed to 52-62 ppm benzene for 4 hours was determined to be approximately 47% (Nomiyama and Nomiyama 1974a). Respiratory excretion (the amount of absorbed benzene excreted via the lungs) in a total of six male and female volunteers exposed to 52-62 ppm benzene for 4 hours was determined to be approximately 17%. No differences in respiratory excretion were observed between men and women (Nomiyama and Nomiyama 1974a, 1974b). Results from a study of 23 subjects that 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 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 urines. Kok and Ong (1994) report blood and urine levels of benzene in nonsmokers as 110.9 and 116.4 ng/L, respectively, and in smokers, 328.8 and 405.4 ng/L, respectively. 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-phenyl-N-acetyl cysteine (PhAC) 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/m<sup>3</sup>.

As discussed in Section 2.3.3, 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±1.02 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). Data on excretion of benzene or its metabolites in human breast milk after inhalation exposure were not found.

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 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 <sup>14</sup>C-benzene appeared to be dependent on the inhaled concentration (Sabourin et al. 1987). At similar exposures to vapor 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), less than 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  $\leq 260$  ppm in rats, 85-92% of the radioactivity was excreted as urinary metabolites, while at exposures of  $\leq 130$  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 per day, 5 days per week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

#### 2.3.4.2 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 ( $\approx 340$  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 1953). 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 1953).

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  $^{14}\text{C}$ -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,  $\geq 90\%$  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  $^{14}\text{C}$  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  $^{14}\text{C}$  was eliminated in the expired air; in mice, 69% of the 150 mg/kg dose of  $^{14}\text{C}$  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.

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. Excretion patterns were altered by the presence of the soils. Expired air was the primary excretion route for benzene alone, with lesser amounts eliminated in the urine during 48 hours after exposure. Urine and expired air were equal routes of exposure with sandy soil, while clay soil produced the results opposite to those seen with pure benzene. The increase in urinary excretion in the presence of soil suggests that the soil allows greater absorption, and thus greater opportunity for metabolism of benzene, compared to benzene alone. For clay soil, particularly, the increased potential for metabolism is supported by the significantly lower amount of unmetabolized benzene in expired air. Less than 2% of the dose was eliminated in the feces for all treatments, although sandy soil treatment resulted in the highest amount of benzene associated with fecal excretion. These results further suggest that clay soil binds benzene.

### 2.3.4.3 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  $0.0024 \text{ mg/cm}^2$   $^{14}\text{C}$  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  $0.06 \text{ g/cm}^2$  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  $^{14}\text{C}$ -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  $0.004 \text{ mg/cm}^2$  of  $^{14}\text{C}$ -benzene, with or without one gram 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 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  $^{14}\text{C}$  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).

#### 2.3.4.4 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  $^{14}\text{C}$ -benzene (0.5 and 150 mg/kg) was injected intraperitoneally into rats and mice, most of the  $^{14}\text{C}$ -benzene and  $^{14}\text{C}$ -metabolites were excreted in the urine and in the expired air. A smaller amount of  $^{14}\text{C}$ -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 phenyl glucuronide but less sulfate conjugate. Both strains excreted similar amounts of phenyl mercapturic acid (Longacre et al. 1981a).

### **2.3.5 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: the external/exposure concentration and target tissue dose of the toxic moiety, and the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). 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: model representation, model parametrization, model simulation, and model validation (Krishnan and Andersen 1994). In the early 1990s validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The

numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is 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 concentrations were higher or were administered in different species. Figure 2-4 shows a conceptualized representation of a PBPK model.

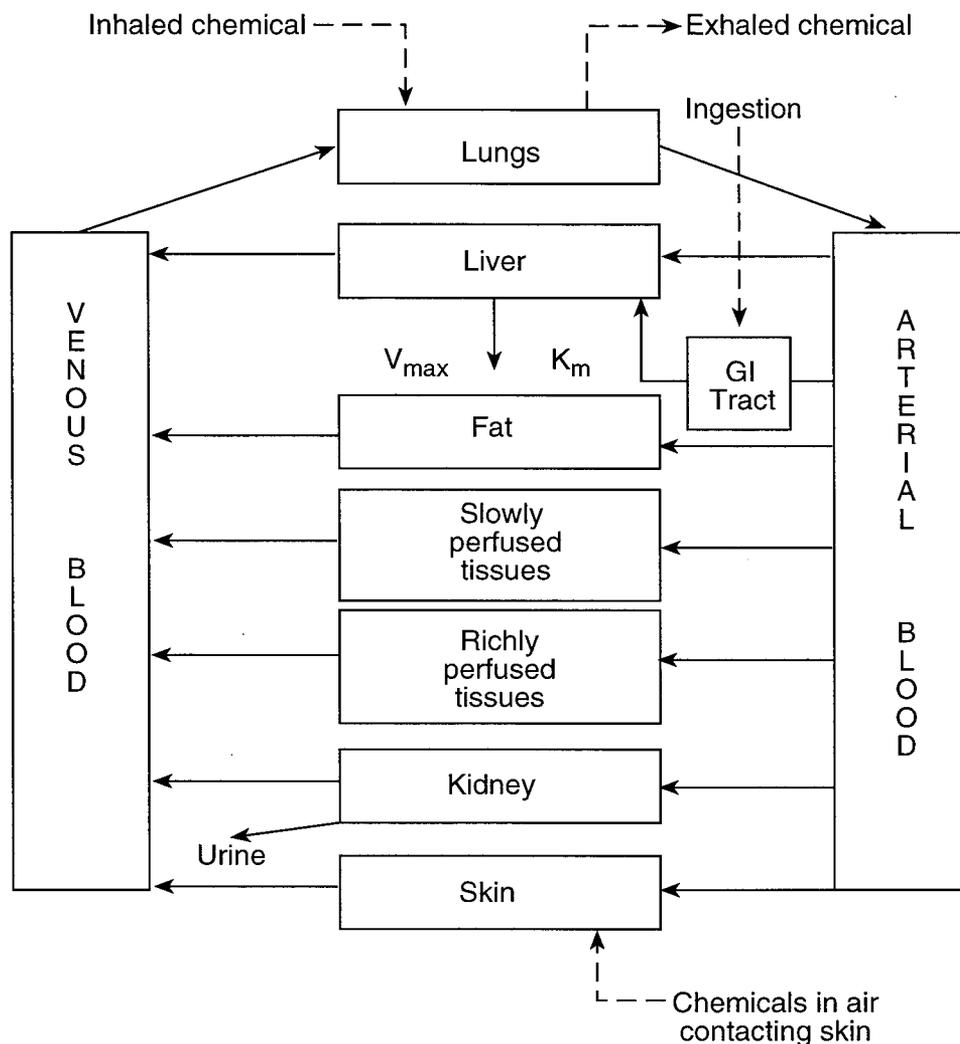
PBPK models for benzene exist, and the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

#### **2.3.5.1 Summary of PBPK Models.**

Four benzene PBPK models that describe the disposition of benzene in animals have been identified from the open literature since the late 1980s. These models are identified as the Medinsky model (Medinsky et al. 1989a, 1989b, 1989c), the Travis model (Travis et al. 1990), the Bois and Paxman model (Bois and Paxman 1992), and the Sun model (Sun et al. 1990). Based on the information presented in these four models, there appears to be sufficient evidence to suggest that PBPK models for benzene are fairly refined models which have a strong potential for use in human risk assessments. The PBPK model developed by Medinsky et al. (1989a, 1989b, 1989c) has provided a basic model for

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**Figure 2-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance**



Source: adapted from Krishnan et al. 1992

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.

the fate of benzene in laboratory animals. Using this model as a template, other more sophisticated and refined models have been developed that can be used in human risk assessment work.

#### **2.3.5.2 Benzene PBPK Model Comparison.**

The four PBPK models that are highlighted in this section of the profile have each contributed to the overall understanding of the pharmacokinetics of benzene. For instance, the Medinsky model addresses species differences in benzene kinetics using mice and rats. The Travis model specifically addresses human pharmacokinetics of benzene in comparison to experimental animal data, whereas the Bois and Paxman model addresses the effect of exposure rate on benzene metabolism. Finally, the Sun model addresses the formation of hemoglobin-benzene derived adducts in the blood, as a tool in monitoring benzene exposure. Each of these models is discussed in detail below.

#### **2.3.5.3 Discussion of Models.**

##### **The Medinsky Model.**

The Medinsky model (Medinsky et al. 1989a, 1989b, 1989c) is one of the original benzene PBPK models developed to describe and ultimately predict the fate of benzene in mice and rats and to determine if the observed differences in toxic effects could be explained by differences in pathways for metabolism of benzene or by differences in uptake of benzene.

**Risk Assessment.** This model successfully described the differences in disposition of benzene in mice and rats. Specifically, the model simulations indicated that for inhalation concentrations up to 1,000 ppm, mice metabolized at least 2-3 times as much benzene as did rats. Simulations of oral exposure indicated that rats metabolized more benzene on a kg body weight basis than did mice at doses greater than 50 mg/kg. Similarly, patterns of metabolites were different for the two species, with mice producing primarily hydroquinone glucuronide and muconic acid, metabolites-linked to toxic effects, and rats producing primarily phenyl sulfate, a detoxification product. These simulated results agree with experimental data, and provide a framework for understanding the greater sensitivity of the mouse to benzene toxicity.

**Description of the Model.** The Medinsky benzene PBPK model was based on an earlier PBPK model developed by Ramsey and Andersen (1984) to describe the disposition of styrene exposure in mice and rats. Disposition of benzene in humans was not originally represented in the Medinsky model (Medinsky et al. 1989a, 1989b), but human rate constants were later derived from the model (Medinsky et al. 1989c). Others have developed models that address human disposition of benzene (Travis et al. 1990). A schematic representation of the Medinsky model is shown in Figure 2-5, with oral and inhalation routes represented. The dermal route of exposure is not represented in this model, or in any other models found in the literature. Other models address the intraperitoneal route of exposure (Travis et al. 1990).

The tissue compartments included in the Medinsky model are: (a) the liver, presumed for the sake of the model to be the only organ where metabolism of benzene takes place; (b) a group of poorly perfused tissues including muscle and skin; (c) a group of richly perfused tissues including bone marrow, kidney, and intestines; and (d) a fat compartment. The poorly perfused tissues were important to the development of the model because of the large body mass they represent, the rapidly perfused tissues because of the large blood flow to these tissues, and the fat compartment because of its large partition coefficient. Large tissue mass, high blood flow, and increased solubility were assumed to result in significant amounts of benzene being distributed to that compartment, although the kinetics of distribution may be different for each compartment.

Physiologic, biochemical constants and partition coefficients used in the model are shown in Table 2-4. Physiologic constants (organ volume, blood flows, etc.) and tissue and blood coefficients were taken from Ramsey and Andersen (1984) or were taken from other literature sources. All metabolism of benzene, occurring only in the liver, and consisting of initial metabolism to benzene oxide, which is then further metabolized by one of four pathways, was modeled by Michaelis-Menten kinetic parameters. Metabolic rate constants were determined by fitting the results of model simulations to experimental data obtained by exposing mice and rats to benzene orally and by inhalation (Medinsky et al. 1989b; Sabourin et al. 1987). Human metabolic rate constants were derived by using the metabolic parameters obtained for mice, the more sensitive rodent species, and simulating 8-hour inhalation exposure over a range of concentrations. Model simulation results were compared to experimentally derived values for total metabolism of benzene and profiles of the individual major metabolites, including metabolism through benzene oxide to hydroquinone conjugates,

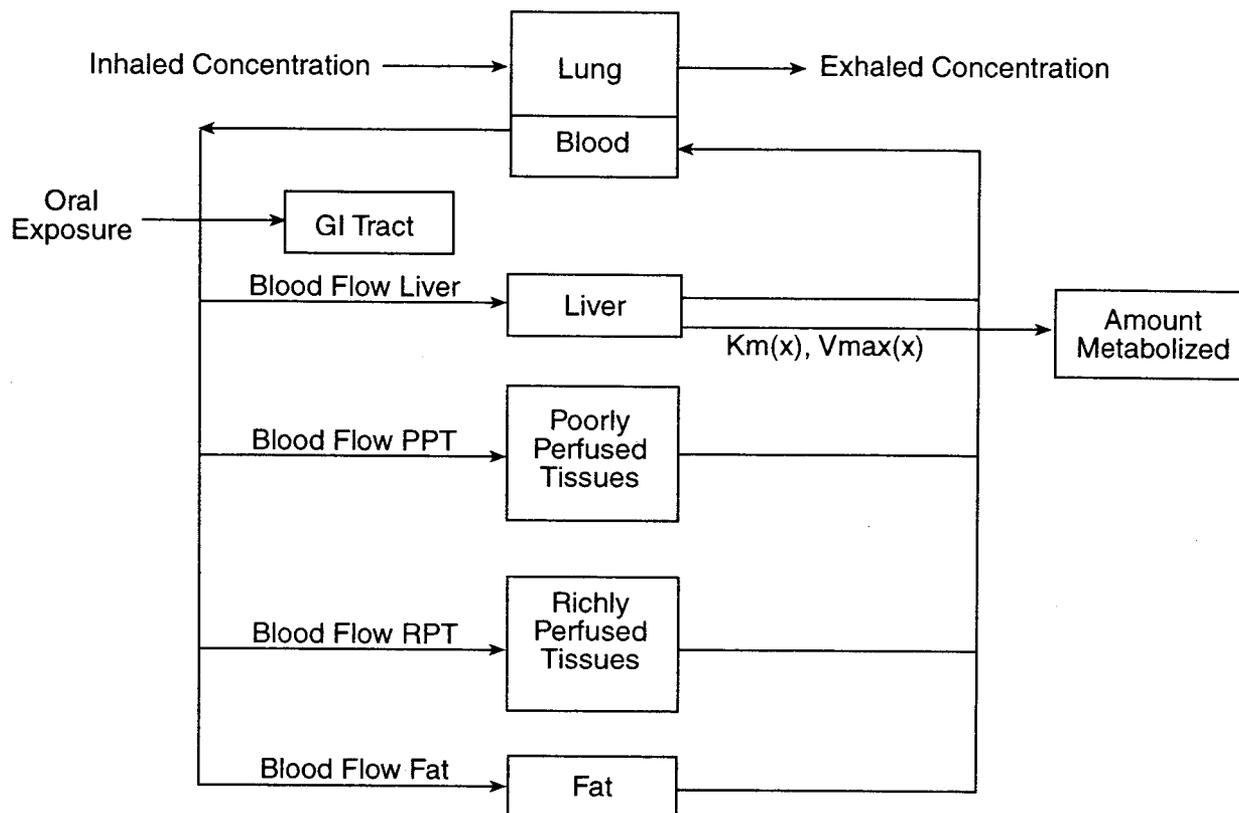
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**Table 2-4. Parameters Used in the Medinsky PBPK Model for Benzene**

Parameters	Mouse	Rat	Human
	<i>Weights (kg):</i>		
Body	0.030	0.288	70.0
	<i>Percentage of body weight</i>		
Liver	3.0	2.53	
Fat	11.0	6.30	
Rapidly perfused tissues	7.6	4.39	
Slowly perfused tissues	66.0	77.07	
Other <sup>a</sup>	12.4	12.4	
Gastrointestinal transfer (fraction/hr)	3.0	0.25	
	<i>Flows (L/hr/kg)</i>		
Alveolar ventilation	55.3	32.4	
Cardiac output	34.8	19.4	
	<i>Percentage of cardiac output</i>		
Liver	25.0	25.0	
Kidney	9.0	9.0	
Fat	2.0	5.0	
Rapidly perfused tissues	51.0	51.0	
Slowly perfused tissues	15.0	15.0	
	<i>Partition coefficients</i>		
Blood/air	18.0	18.0	
Liver/blood	1.0	1.0	
Fat/blood	28.0	28.0	
Rapidly perfused/blood	1.0	1.0	
Slowly perfused/blood	0.6	0.6	
	<i>Metabolic and macromolecular binding constants</i>		
$V_{\max, C}$ (mg/hr/kg)	10.55	5.79	1.02
$K_m$ (mg/L)	0.09	0.62	0.09
$V_{\max, phc}$ C (mg/hr/kg)	12.12	4.77	0.17
$K_{m, phc}$ (mg/L)	0.08	0.05	0.08
$V_{\max, pmc}$ C (mg/hr/kg)	7.04	1.25	0.67
$K_{m, pmc}$ (mg/L)	0.31	0.07	0.31
$V_{\max, hqc}$ C (mg/hr/kg)	1.48	0.10	0.15
$K_{m, hqc}$ (mg/L)	0.01	0.23	0.01
$V_{\max, muc}$ C (mg/hr/kg)	0.23	0.19	0.07
$K_{m, muc}$ (mg/L)	0.01	0.01	0.01

<sup>a</sup>Includes tissues that are not perfused with blood such as bone (not including marrow); body fluids such as gastrointestinal tract contents, urine, bile, blood in major vessels not included in any tissue group; and hair.

**Figure 2-5. Physiologic Model Used to Describe the Pharmacokinetics of Benzene in Mice and Rats During Inhalation and Oral Exposure**



Adapted from Ramsey and Anderson 1984

phenol conjugates, muconic acid, or metabolites of the mercapturic pathway. Various parameters of the model were adjusted until the simulated data agreed with the experimental data.

**Validation of the Model.** Since the model was developed by Medinsky et al. (1989a) using the data of Sabourin et al. (1987), the model accurately reflects these data. Bois et al. (1991a) applied the data of Rickert et al. (1979) to the Medinsky model and compared to results obtained using data from Sabourin et al. (1987). In general, the Medinsky model tended to overestimate data to which it was not specifically fitted. It did correctly predict the percentage of a gavage dose administered to rats that was exhaled unchanged, or excreted as metabolites within 48 hours.

**Target Tissues.** The model correctly described metabolic differences observed between mice and rats. For instance, mice metabolize benzene more efficiently than rats during and after a 6-hour inhalation exposure. After oral exposure, mice and rats metabolized doses of benzene up to 50 mg/kg in a similar manner. At oral doses above 50 mg/kg, rats metabolized more benzene than did mice on a per kg body weight basis. Similar results were seen with the excretion of individual metabolites. Excretion of major metabolites, the phenyl conjugates, was similar for both species for oral doses up to 20 mg/kg, after which rats produced more phenyl conjugates than did mice. After inhalation, mice produced more phenyl conjugates at all exposure levels compared to rats when normalized to body weight. For hydroquinone metabolites, however, mice produce far more after both oral and inhalation exposure than rats. For muconic acid, the model predictions indicate that after inhalation exposure, mice produced more than rats; after oral exposure, the relative amount of muconic acid produced by the two species was similar.

**Species Extrapolation.** The Medinsky model used species-specific information to outline the model parameters; little extrapolation of information between mice and rats was required. Based on the parameters derived for mice, metabolism of benzene after inhalation by humans was simulated. The results indicate that at concentrations below 10 ppm, metabolism and formation of metabolites is linearly related to inhaled concentration, and that hydroquinone conjugates are the predominant metabolite. At concentrations above 10 ppm, the model predicts a change in the metabolic profile, with increased proportion of phenyl conjugates, associated with detoxification processes.

**High-low Dose Extrapolation.** High-low dose extrapolation was not specifically addressed in the Medinsky model. It is important to note however that the experiments used to determine some of the

model parameters spanned a wide concentration range and involved benzene concentrations spanning *at least* 1 order of magnitude for most experiments.

**Interroute Extrapolation.** The Medinsky model used two routes of administration, oral and inhalation, in rats and mice to describe the disposition of benzene. This model was used to predict human formation of benzene metabolites after inhalation exposure.

**Strengths and Limitations of the Medinsky Model.** The Medinsky model has many aspects which are desirable in a PBPK model. It uses more than one route of administration, it uses a range of concentrations and doses, it uses more than one species, and it specifically addresses the production of toxic metabolites, the putative agents of damage in benzene toxicity. However, the model is limited by the fact that it does not predict data other than that on which it was modeled very accurately (Bois et al. 1991a). In addition, because the model focuses on the prediction of metabolite kinetics, the kinetics of benzene itself suffer. This could be a problem in risk assessment if unmetabolized benzene plays a part in carcinogenesis as suggested by some researchers (Bois et al. 1991a). A major limitation of the model is its lack of inclusion of bone marrow activation of phenolic metabolites by myeloperoxidase-catalyzed reactions. In addition, the model bases the formation of muconaldehyde on the formation of muconic acid. Since muconaldehyde is a very reactive compound, it is a direct-acting alkylating agent which rapidly reacts with GSH and other cellular nucleophiles. Using muconic acid formation grossly underestimates muconaldehyde levels in tissues.

#### **The Travis Model.**

**Risk Assessment.** Risk assessment was not specifically addressed for this model.

**Description of the Model.** Travis et al. (1990) developed a model to describe the pharmacokinetics of benzene in mice, rats, and humans. The model contained five compartments, consisting of liver, fat, bone marrow, and muscle, and organs such as brain, heart, kidney, and viscera, connected by the arterial and venous blood pathways. Michaelis-Menten kinetics was assumed in all species, and occurred primarily in the liver, and to a lesser extent in the bone marrow. The species-specific physiological and chemical parameters were taken from the literature. The metabolic parameters were obtained by fitting the empirical data to the model.

**Validation of the Model.** The Travis model was validated using mouse and rat inhalation and gavage data from Sabourin et al. (1987), inhalation data for mice and rats from Snyder et al. (1981b), subcutaneous injection data from mice found in Andrews et al. (1977), intraperitoneal injection data from rats in Sato and Nakajima (1979a), and inhalation data in rats from Rickert et al. (1979) and Sato et al. (1975). Human inhalation exposure data from Berlin et al. (1980) and Sherwood (1972) were used for low concentration (5 ppm) modelling. For intermediate concentrations (25-57 ppm), the human data from Sato et al. (1975), Sherwood (1972), and Nomiyama and Nomiyama (1974a, 1974b) were used. For high concentrations (99-100 ppm) the data of Sherwood (1972) and Teisinger and Fiserova-Bergerova (1955) were used. The model successfully replicated the data, as discussed below.

**Target Tissues.** The Travis model successfully predicted the total amount of benzene in expired air and total metabolite produced in 48 hours following gavage administration in mice and rats, and inhalation administration in mice, but slightly overestimated these parameters in rats after inhalation exposure. Blood concentrations of benzene following inhalation exposure in mice and rats were adequately predicted. Bone marrow concentrations of benzene following subcutaneous injection in mice and inhalation exposure in rats were accurately predicted, as were blood concentrations of benzene in rats following intraperitoneal injection. For humans, the model slightly overestimated the data for benzene in expired air at the low concentration of 5 ppm. For the mid concentration of 25 ppm, excellent fit was obtained from the model for both benzene in expired air and blood benzene levels. Model agreement for expired benzene was also good at concentrations of 57 and 99 ppm. The model was also accurate in predicting the amount of phenol in the urine after a 5-hour exposure to 31.3 or 47 ppm.

**Species Extrapolation.** The Travis model used many of the same physiologic parameters that the Medinsky model used (average body weights, organ percentage of body weight, blood flow, etc.). Species-specific parameters were used, so extrapolation across species was not addressed.

**High-low Dose Extrapolation.** For human exposure, the Travis model successfully-predicted benzene in expired air at low (5 ppm), intermediate (25-57 ppm) and high concentrations of inhaled benzene. The animal studies used to validate the model employed a wide range of concentrations, encompassing at least one order of magnitude in concentration.

**Interroute Extrapolation.** The Travis model successfully predicted results from inhalation, oral, subcutaneous, and intraperitoneal administration of benzene.

**Strengths and Limitations of the Travis Model.** The Travis model has many aspects which are desirable in a PBPK model. It uses more than one route of administration, it uses a range of concentrations, it uses more than one species, and it successfully predicts blood and bone marrow concentrations of benzene after different routes of administration. However, the model is limited by the fact that it does not predict data other than that on which it was modeled very accurately (Bois et al. 1991a). For instance, the concentration of benzene in fat is poorly predicted by the Travis model. This could be a problem in risk assessment since benzene is fat soluble and is thought to accumulate in the fat (Bois et al. 1991a).

#### **The Bois and Paxman Model.**

**Risk Assessment.** The Bois and Paxman model (Bois and Paxman 1992) provided strong evidence that the exposure rate had a significant effect on the rate of formation of several important metabolites of benzene. The authors used the model to simulate metabolite production in human blood and bone marrow after an 8-hour inhalation exposure at the PEL of 1 ppm, and a 15-minute exposure at 32 ppm. The results of the simulation indicated that the levels of metabolites (hydroquinone, catechol, and muconaldehyde) were 20% higher (i.e., area under the curve) after the short-term exposure at the higher level, than after the long-term exposure at the lower level. These metabolites have been identified as being important in the genesis of bone marrow toxicity after benzene exposure (Eastmond et al. 1987). Since the STEL (Short Term Exposure Limit) is currently set at 5 ppm, or roughly 1/6 of the simulated concentration, the authors concluded that the regulated level afforded an appropriate cushion of safety.

**Description of the Model.** Bois and Paxman (1992) produced a model that they used to explore the effect of exposure rate on the production of benzene metabolites. The model had three components, which described the pharmacokinetics of benzene and the formation of metabolites, using the rat as a model. Distribution and elimination of benzene from a five-compartment model, comprised of liver, bone marrow, fat, poorly perfused tissues, and well perfused tissues, made up the first component of the model. The five-compartment model included two sites for metabolism of benzene, liver and bone marrow. The bone marrow component was included for its relevance to

human leukemia. Parameter values for this component were derived from the literature and from the previously published work of Rickert et al. (1979) and Sabourin et al. (1987). The second component described the metabolic transformations of benzene and its by-products in the liver and bone marrow. The reactions were assumed to follow Michaelis-Menten kinetics, with the exception of the transformation of benzene oxide into phenol, which occurs spontaneously, and may be described by a first order reaction. The third component was the distribution of phenol. In addition to the compartments described for benzene, phenol was also assumed to distribute to the lung and gastrointestinal tract.

**Validation of the Model.** The model was validated against the data of Cassidy and Houston (1984), Sabourin et al. (1987, 1988, 1989a), and Sawahata and Neal (1983). An acceptable fit was observed for the data.

**Target Tissues.** The model was used to predict metabolite production for male rats exposed to benzene by nose-only procedures to three different vapor concentrations and at differing lengths of exposure, and compared to the experimental data of Sabourin et al. (1989a). Actual exposure regimens were 51 ppm for 6 hours, 153 ppm for 2 hours, and 588 ppm for 0.5 hours, which were established such that the product of the exposure level and duration of exposure was constant. Simulation results indicated that the model might over- or underestimate the level of urinary metabolites, although considering the variability inherent in the data, the authors were satisfied with the results.

**Species Extrapolation.** The Bois and Paxman model was used to model human metabolite formation after an 8-hour exposure at the PEL of 1 ppm, and at 32 ppm for 15 minutes, mimicking a high short-term exposure, the limit of which is currently 5 ppm. The model suggests that a higher level (i.e., area under the curve) of metabolites is produced after a high, short-term exposure compared to a low, longer-term exposure. This was true for both blood and bone marrow levels of metabolites.

**High-low Dose Extrapolation.** No high-low dose extrapolation was specifically addressed by the Bois and Paxman model. However, modeling of human occupational exposure (see above) did address both high and low concentrations.

**Interroute Extrapolation.** No interroute extrapolation was specifically addressed by the Bois and Paxman model.

**Strengths and Limitations of the Bois and Paxman Model.** The Bois and Paxman model has many aspects which are desirable in a PBPK model. For instance, it specifically addressed metabolism of benzene in the bone marrow, a primary site of toxicity. Specific limitations of the model were not apparent.

**The Sun Model.**

**Risk Assessment.** The Sun model (Sun et al. 1990) can be used to predict the levels of hemoglobin-benzene-derived adducts in the blood, and may be useful in predicting exposure levels that can be monitored through the hemoglobin adduct biomarker.

**Description of the Model.** The Medinsky model (1989a, 1989b, 1989c) was modified to account for the formation of hemoglobin adducts. It was assumed that hemoglobin adduct formation could proceed by both first-order and saturable pathways, and that benzene oxide would be the substrate for both pathways.

**Validation of the Model.** The model was validated using hemoglobin adduct formation data from rats after oral exposure of rats and mice (Sun et al. 1990). Formation of hemoglobin adduct after oral exposure in rats and mice was effectively simulated over a 6-fold range in exposure concentration. Inhalation exposure was validated against the data of Sabourin et al. (1989a), and was successfully predicted by the model.

**Target Tissues.** The model successfully predicted hemoglobin adduct formation after oral and inhalation exposure.

**Species Extrapolation.** No species extrapolation was specifically addressed by this model.

**High-low Dose Extrapolation.** No high-low dose extrapolation was specifically addressed by this model.

**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 of the Sun Model.** The Sun model has many aspects which are desirable in a PBPK model. It uses more than one route of administration, it uses more than one species, and it specifically addresses the production of hemoglobin adduct formation, a biomarker of effect for benzene toxicity. The limitations of the model were not apparent.

**Other Applications of PBPK Modeling for Benzene.** Other investigators have addressed the subject of PBPK modeling in relation to benzene toxicity. The approaches include comparing and refining the models to exclude some design parameters (Woodruff et al. 1992); modeling compound interactions after co-exposure (Purcell et al. 1990; Travis et al. 1992); kinetics in the presence of soil particles after oral exposure (Travis and Bowers 1990); modeling of metabolism (Schlosser et al. 1993); mathematical optimization of the model (Woodruff and Bois 1993); predictions of toxicity based on the parent and metabolite (Bois et al. 1991a); metabolite production in target tissues (Watanabe et al. 1994); modeling mechanism of action (Medinsky 1995); describing age-related changes in animal metabolism (McMahon et al. 1994) and mathematical analysis of the model itself (Spear et al. 1991). These applications of PBPK modeling are beyond the scope of this profile and will not be addressed.

## 2.4 MECHANISMS OF ACTION

Benzene toxicity has been studied extensively, and the current understanding of benzene toxicity includes its metabolic fate, mechanism of toxicity, pharmacokinetic models for disposition, and impact of exposure on human health. These aspects of benzene toxicity will be addressed in the following sections.

### 2.4.1 Pharmacokinetic Mechanisms

There have been numerous attempts to develop pharmacokinetic models of benzene disposition, in an effort to describe and predict benzene toxicity (see Section 2.3.5.1). Benzene disposition has been described as including three to five areas of distribution in the body: the liver; tissues that have a large

blood supply (brain, kidneys, heart, spleen); tissues that have a poor supply of blood (muscle and skin); and fat (Medinsky et al. 1989a; Sato and Nakajima 1979b). Benzene is absorbed into the blood from the lung, gastrointestinal tract, and skin. Metabolism is assumed to take place primarily in the liver, with some secondary metabolism in the bone marrow, the site of characteristic benzene toxicity. Benzene metabolism has been shown to be dose-dependent in all species studied thus far, including humans. At low doses, more of the benzene is converted to putative toxic metabolites than at high doses. At high doses, benzene inhibits phenol metabolism to hydroquinone; phenol also inhibits benzene metabolism (Schlosser et al. 1993). This appears to occur through competition for a common site on the CYP2E1 enzyme to which catechol and hydroquinone also bond. Benzene is absorbed in the fat, and has been shown to bind to blood and tissue proteins. Excretion takes place from the lung and in the urine and feces. Biliary excretion also occurs.

Benzene metabolism to reactive intermediates follows two routes: ring-hydroxylation and ring opening (Sabourin et al. 1988; Snyder 1987; Snyder and Kocsis 1975; Snyder et al. 1993b). Hydroxylated metabolites form glucuronide or sulfate conjugates, and are excreted in the urine as detoxification products because the conjugate formation leads to elimination and the prevention of the formation of toxic metabolites. Ring opening leads to the formation of toxic intermediates (*trans,trans*-muconaldehyde) that are also detoxified and excreted in the urine (Latriano et al. 1986).

#### **2.4.2 Mechanisms of Toxicity**

The most characteristic toxic effect of benzene in both human and animal models is the depression of the bone marrow, leading ultimately to aplastic anemia (Rozen and Snyder 1985; Snyder and Kocsis 1975; Snyder et al. 1993b). Rozen and Snyder (1985) have noted that abnormalities of humoral and cell-mediated immune responses following benzene exposure of C57BL mice by inhalation are presumably caused by a defect in the lymphoid stem cell precursors of both T- and B-lymphocytes. They also observed that bone marrow cellularity and the number of thymic T-cells increased, presumably as a compensatory response in these cell lines in response to benzene exposure. This compensatory proliferation may play a role in the carcinogenic response of C57BL mice to inhaled benzene.

The work of Longacre et al. (1981b) and Snyder et al. (1989) suggests that benzene and benzene metabolites may interfere with the incorporation of iron into bone marrow precursors. In studies on

the erythroid cell line, Longacre et al. (1981b) demonstrated 60-80% decreases in the incorporation of iron (as  $^{59}\text{Fe}$ ) into bone marrow precursors of mice injected subcutaneously with benzene. When given 48 hours prior to measuring  $^{59}\text{Fe}$  uptake, benzene produced a dose-dependent decrease in erythroid cells (Snyder et al. 1989). Following intraperitoneal administration of benzene, hydroquinone, *p*-benzoquinone, and *trans,trans*-muconaldehyde,  $^{59}\text{Fe}$  uptake was reduced. The combination of metabolites most effective in reducing  $^{59}\text{Fe}$  uptake was hydroquinone plus *trans,trans*-muconaldehyde (Snyder et al. 1989).

Rao (1991) showed that hematin catalyzed the autoxidation of hydroquinone or 1,2,4-benzenetriol *in vitro*, producing reduced oxygen species that may be responsible for protein or DNA binding after benzene exposure. Further work along this line of research has provided evidence that *in vitro*, chelates of iron and hydroquinone or 1,2,4-benzenetriol are potent DNA cleaving agents (Rao 1996; Singh et al. 1994), and that 1,2,4-benzenetriol, but not hydroquinone, causes the release of iron from ferritin (Ahmad et al. 1995). 1,2,4-Benzenetriol or hydroquinone have also been shown to release reactive products from glutamate or DNA in the presence of copper ions (Rao and Pandya 1989).

Benzene also causes chromosomal abnormalities indicative of genetic damage, both in blood cells and the cells of other tissues (Forni et al. 1971a, 1971b; Hite et al. 1980; Tice et al. 1980, 1982). Benzene is carcinogenic in animals and leukemogenic in humans (Aksoy and Erdem 1978; Aksoy et al. 1987; Cronkite 1986; Cronkite et al. 1984, 1985, 1989; Farris et al. 1993; Huff et al. 1989; IARC 1982a; Maltoni et al. 1982a, 1982b, 1983, 1985, 1989; NTP 1986, 1994). Benzene has been shown to decrease the number of stem cells and committed stem cells (Baarson et al. 1984; Gill et al. 1980; Green et al. 1981a, 1981b; Seidel et al. 1989a). However, toxicity appears to occur only under conditions that allow metabolism to reactive intermediates.

The major portion of benzene metabolism takes place in the liver, mediated by cytochrome P-450 monooxygenase to form benzene oxide, a reactive intermediate. Muconaldehyde may also be formed in the liver, in addition to hydroquinone (Latriano et al. 1986). These metabolites are genotoxic, and Snyder et al. (1993b) suggest that, although it is unlikely that these metabolites formed in the liver, could reach the bone marrow to exert toxicity, they may be transported to target tissues by being bound to transport molecules. Evidence exists that benzene metabolites bind to blood proteins (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Irons et al. 1980; McDonald et al. 1994). Hydroquinone and catechol have been shown to accumulate in bone marrow, where they could

be substrates for myeloperoxidase, forming metabolites that could covalently bind to protein (Eastmond et al. 1987; Kalf et al. 1990; Snyder et al. 1993b; Subrahmanyam et al. 1990). This pathway has been shown to be possible using human myeloperoxidase (Eastmond et al. 1986; Smith et al. 1989; Subrahmanyam et al. 1991). Myeloperoxidase activity has been confirmed in both murine and human hematopoietic progenitor cells, further strengthening this mechanism of toxicity (Shattenberg et al. 1994).

In addition, hydroquinone and *p*-benzoquinone have been shown to bind to sulfhydryl groups of tubulin in the mitotic spindle, thereby preventing proper formation of microtubules (Irons 1985). Without adequate mitotic spindle support and function, some chromosomes may not complete segregation, giving rise to aneuploidy (Robertson et al. 1991a).

Carbonnelle et al. (1995) looked at the effect of hydroquinone on IL-1 (IL-1) release from human monocytes *in vitro*. Exposure of human monocytes to micromolar amounts of hydroquinone for 2 hours resulted in significantly decreased secretion of IL-1  $\alpha$  and IL-1  $\beta$  at doses of 5  $\mu$ M and above. RNA and protein synthesis were also inhibited, with a 50% inhibitory concentration at 21  $\mu$ M for IL-1  $\alpha$  and 10  $\mu$ M for IL-1  $\beta$ . These results suggest that hydroquinone may contribute to benzene toxicity by inhibiting the production of cytokines by monocytes, which in turn may adversely affect regulation of hematopoiesis. Miller et al. (1994) observed that hydroquinone decreased the content of calpain, the enzyme responsible for the conversion of the precursor form of IL-1  $\alpha$  to IL-1  $\beta$ , in murine bone marrow macrophages in culture. In addition, the work of Irons et al. (1992; Irons and Stillman 1993), who looked at the effect of pretreatment of murine bone marrow cells with hydroquinone on colony-forming response in the presence of growth stimulating factor, found evidence that hydroquinone alters differentiation in the myeloid progenitor cell population, which may be of relevance in the pathogenesis of leukemia secondary to benzene exposure.

Treatment of mice with 800 mg/kg benzene, intraperitoneally, 2 times daily for 2 days resulted in a significant increase in the production of nitric oxide by bone marrow cells stimulated by lipopolysaccharide and interferon  $\gamma$ , alone or in combination with macrophage colony-stimulating factor or granulocyte-macrophage colony-stimulating factor (Punjabi et al. 1994). Cells from benzene-treated mice showed increased sensitivity to the effects of the stimulating factors. Benzene, hydroquinone, 1,2,4-benzenetriol, and *p*-benzoquinone were evaluated for their effect on nitric oxide production by murine bone marrow leukocytes *in vitro* (Laskin et al. 1995). Treatment of mice with benzene,

hydroquinone, 1,2,4-benzenetriol, or *p*-benzoquinone increased nitric oxide production in bone marrow leukocytes, in response to inflammatory mediators and growth factors (Laskin et al. 1995), with hydroquinone and 1,2,4-benzenetriol more effective than benzene or *p*-benzoquinone. Nitric oxide has been shown to suppress cell growth and, in the presence of molecular oxygen, can form a variety of nitrogen oxide intermediates that can react with primary and secondary amines to form nitrosamines, which are carcinogenic. Thus, nitric oxide may be important to the mechanism of toxicity of benzene and its metabolites.

*In vitro* studies of receptor-mediated phagocytosis and cytoskeletal integrity in mouse peritoneal macrophages indicate that benzene metabolites inhibit phagocytosis through some action on the filamentous action of the cell, thus adversely affecting host defense activities of the macrophages (Manning et al. 1994).

Schoeters et al. (1995) evaluated the hematopoietic and osteogenic toxicity of benzene, phenol, hydroquinone, and catechol *in vitro* using murine bone marrow cultures. Evaluation of toxicity to 3T3-fibroblasts was included to determine specific toxicity to marrow cells. Benzene and phenol showed little effect. However, hydroquinone inhibited proliferation of 3T3 cells and marrow precursor cells, and calcification of bone cells, although it was more specific for marrow cells. Catechol inhibited all cells, and showed no specificity.

Benzene, administered intraperitoneally at 600 mg/kg in corn oil, 2 times per day for 2 days to male C57BL/6J mice, caused a significant depression in the total number of nucleated bone marrow cells per femur, when measured on day 3 (Niculescu and Kalf 1995). Additional experiments with 7-day exposure revealed that there was an initial depression in erythroid cells on day 3 which remained constant; lymphocytes exhibited a progressively depressive effect, with the numbers of intermediate and terminally differentiated granulocytes exhibiting a progressive increase over the 7 days of exposure. Upon cessation of the benzene treatment, the bone marrow appeared to begin recovery, with the number of nucleated cells equal to control animals by day 7 after treatment ended. Concomitant administration of IL-1  $\alpha$  prevented the decrease in nucleated bone marrow cells, whereas IL-1  $\alpha$  administered after 2 days of benzene exposure significantly increased the rate of recovery of bone marrow cellularity.

The co-administration of phenol (75 mg/kg/day) and hydroquinone (25-75 mg/kg/day) twice a day for 12 days to B6C3F<sub>1</sub> mice produced myelotoxicity similar to that induced by benzene (Eastmond et al. 1987). The proposed mechanism suggested that selective accumulation of hydroquinone occurred in the bone marrow after the initial hepatic conversion of benzene to phenol and hydroquinone. Additionally, phenol is thought to stimulate the enzymatic activity of myeloperoxidase, which uses phenol as an electron donor, thus producing phenoxy radicals. These radicals further react with hydroquinone to form 1,4-benzoquinone, a toxic intermediate which inhibits critical cellular processes (Eastmond et al. 1987).

Benzene metabolites have been shown to bind to DNA and RNA, and have been shown to induce hyperphosphorylation of gene material, suggesting a mechanism for chromosomal damage and carcinogenesis (Dees and Travis 1994; Lutz and Schlatter 1977; Snyder et al. 1978b). Copper-mediated oxidation of hydroquinone to semiquinone anion radicals has been implicated in direct DNA damage (Li et al. 1995). In addition, benzene metabolites appear to be able to inhibit enzymes involved with DNA replication and repair, specifically the topoisomerase enzymes (Chen and Eastmond 1995). Singh et al. (1994) have studied the prooxidant and antioxidant properties of iron-hydroquinone and iron-1,2,4-benzenetriol complexes with respect to DNA degradation in rat bone marrow cells. Bleomycin-dependent DNA degradation was increased 1.5-fold in the presence of the iron hydroquinone chelate and 2.5-fold in the presence of the iron-1,2,4-benzenetriol chelate. However, the polyphenol chelates were less effective free radical generators than iron alone. Carcinogenesis may then be the result of aberrations of repair or replicating processes (Snyder et al. 1993).

In an attempt to elucidate the *in vivo* adduct formation by benzene metabolites, Reddy et al. (1989b) developed a highly sensitive nuclease P<sub>1</sub>-enhanced <sup>32</sup>P-postlabeling assay for adduct detection at a frequency of one modification per 10<sup>9-10</sup> DNA nucleotides. When DNA was prepared from Zymbal glands of rats given 200 mg/kg of benzene, orally, 5 days per week for 10 weeks, 3 potential adducts were discovered. However, no adducts were detected in the DNA from the liver, kidney, or mammary gland of rats at any dose (Reddy et al. 1989a). The adduct levels in the Zymbal gland corresponded to 4 lesions per 10<sup>9</sup> DNA nucleotides. The presence of adducts in the Zymbal DNA from benzenetreated rats may be related to the tumor formation in this organ following benzene treatment although this relationship is not clearly defined.

Benzene metabolites have also been shown to damage murine hematopoietic cells *in vitro* (Seidel et al. 1991). In addition, benzene has been shown to decrease mitochondrial respiration and increase superoxide radical production in isolated rat heart mitochondria (Stolze and Nohl 1994). The effects of exposure of HL-60 cells (human promyelocytic leukemic cells) to hydroquinone, *p*-benzoquinone, or 1,2,4-benzenetriol were studied by Rao and Snyder (1995). The cytotoxic effect of the metabolites on HL-60 cells, measured as cell viability, could be ranked as *p*-benzoquinone>hydroquinone>1,2,4-benzenetriol, with viability from 50% to 70% after incubation with concentrations up to 100 pM for 4 hours. Basal levels of superoxide anion or nitric oxide production were not affected by incubation of the cells with the metabolites, but in the presence of TPA, each metabolite increased superoxide anion production; however, nitric oxide production was increased with hydroquinone and *p*-benzoquinone, but not 1,2,4-benzenetriol. HL-60 cells showed increased production of hydrogen peroxide after exposure to the three benzene metabolites. This study suggests that benzene metabolites may predispose the cells to oxidative damage by inhibiting or reducing antioxidant mechanisms within the cell.

Benzene leukemogenesis has been recently reviewed and summarized by Snyder and Kalf (1994). Detailed discussion of the proposed mechanisms of benzene toxicity is beyond the scope of this profile. However, the work of Snyder and Kalf (1994) in reviewing benzene toxicity can be summarized, in part, as follows. Chronic exposure to benzene results in the presence of benzene, hydroquinone, and other metabolites of benzene in the bone marrow. As a result of the toxic reaction of these compounds, lymphocytes are reduced, and granulocytes increased in the bone marrow. Chronic exposure to benzene might also result in leukemogenesis through activation of protein kinase C, involved in proliferation of human myeloid cells. In addition, activation of protein kinase C by chronic benzene exposure may result in overphosphorylation of cellular proteins, leading to an alteration in the activation and expression of some cellular protooncogenes. Benzene and its phenolic metabolites have been shown to induce oxidative DNA damage, which may play a role in leukemogenesis. As noted in Section 2.10.3, Ongoing studies, other possible mechanisms regarding leukemogenesis are currently under investigation.

### **2.4.3 Animal-to-Human Extrapolations**

Recent PBPK models have tried to address benzene metabolism in an effort to derive animal-to-human extrapolations (Bois et al. 1991a; Medinsky 1995; Medinsky et al. 1989a; Spear et al. 1991; Travis et

al. 1990). Each model described a multicompartimental 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 model is not sufficiently refined to allow it to accurately predict human metabolism. Thus, although PBPK modelling has provided a means to improve animal to human extrapolations, the models need to be improved.

## 2.5 RELEVANCE TO PUBLIC HEALTH

### Overview.

Benzene is widely distributed in the environment. The exposure scenario of most concern to the general public is low-level inhalation over long periods. This is because the general population is exposed to benzene mainly through inhalation of contaminated air, particularly in areas of heavy traffic and around gas stations, and through inhalation of tobacco smoke from both active and passive smoking (Adlkofer et al. 1990; Appel et al. 1990; Chan et al. 1993, 1994; Dor et al. 1995; Fleming 1990; Fuselli et al. 1995; Gilli et al. 1994; Guerra et al. 1995; Lumley et al. 1990; Van Wijnen et al. 1995). Smoking has been identified as the single most important source of benzene exposure for the estimated 40 million U.S. smokers (Wallace 1989a, 1989b). Smoking accounts for approximately half of the total benzene exposure of the general population (Wallace 1989a). Individuals employed in industries that make or use benzene, or products containing benzene, are probably exposed to the highest concentrations of atmospheric benzene (Kawai et al. 1990, 1991; Moen et al. 1995a, 1995b; NOES 1990). In addition, benzene is a common combustion product, providing high inhalation exposure potential for firefighters (Burgess and Crutchfield 1995). Of the general population, those residing around certain chemical manufacturing sites or living near waste sites containing benzene may be exposed to concentrations of benzene that are higher than background air concentrations. In private homes, benzene levels in the air have been shown to be higher in homes with attached garages, or where the inhabitants smoke inside the house (Thomas et al. 1993).

Although low levels of benzene have been detected in certain foods, beverages, and tap water, these do not constitute major sources of exposure for most people. However, leakage from underground gasoline storage tanks and seepage from landfills and hazardous waste sites have resulted in significant benzene contamination of well water (ATSDR 1991; CEH 1983; Cline and Viste 1985; Plumb 1987;

Tucker et al. 1986). People with contaminated tap water can be exposed from drinking the water or eating foods prepared with it. In addition, exposure can also occur via inhalation during showering, bathing, or cooking with contaminated tap water (Lansari et al. 1992; Sidhu and Chadzynski 1994). Showering and bathing can also contribute significantly to dermal exposure.

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 (ATSDR 1995). Although no causal relationship has been proposed for health conditions identified in the subregistry, continued follow-up of the population is planned to provide information on health effects of environmental exposure to benzene.

Several *in vivo* and *in vitro* studies conclusively demonstrate that benzene can be absorbed through human skin (Blank and McAuliffe 1985; Franz 1984; Susten et al. 1985; Tsuruta 1989). In general, skin absorption is considered a minor source of concern in the occupational environment as it occurs at a much lower rate and extent compared with benzene absorption through the respiratory system (OSHA 1989). However, benzene absorption through the skin as a result of benzene contamination in rubber solvents is a major route of exposure in tire building operations (Susten et al. 1985). Benzene is an irritant to the skin and, by defatting the keratin layer, may cause erythema, vesiculation, and dry and scaly dermatitis (Sandmeyer 1981). Therefore, it is important to avoid skin contact; training and education should be provided for occupationally exposed workers and protective clothing should be used.

Acute inhalation and oral exposures of humans to high concentrations of benzene have caused death (Cronin 1924; Greenburg 1926; Tauber 1970; Thienes and Haley 1972). These exposures are also associated with central nervous system depression (Flury 1928; Greenburg 1926). Chronic low-level exposures have been associated with peripheral nervous system effects (Baslo and Aksoy 1982). Abnormalities in motor conduction velocity were noted in 4 of 6 pancytopenic individuals occupationally exposed to adhesives containing benzene.

The most noted systemic effect resulting from intermediate and chronic benzene exposure is hematotoxicity. A common clinical finding in benzene hematotoxicity is cytopenia, which is a decrease in various cellular elements of the circulating blood manifested as anemia, leukopenia, or thrombocytopenia in humans (Aksoy 1991; Aksoy et al. 1971, 1972, 1987; Cody et al. 1993; Fishbeck

et al. 1978; Kipen et al. 1989; Midzenski et al. 1992; Townsend et al. 1978; Tsai et al. 1983; Xia et al. 1995; Yin et al. 1982, 1987b, 1987c) and in animals (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite et al. 1982, 1985, 1989; Dempster and Snyder 1991; Dow 1992; Farris 1993; Gill et al. 1980; Green et al. 1981b; Hsieh et al. 1988b, 1991; Huff et al. 1989; Li et al. 1986; Luke et al. 1988b; Maltoni et al. 1983, 1985; NTP 1986; Plappert et al. 1994a, 1994b; Rozen et al. 1984; Seidel et al. 1989b; Shell 1992; Snyder et al. 1978a, 1980, 1984; Toft et al. 1982; Vacha et al. 1990; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956). Benzene-associated cytopenias vary and may involve a reduction in one (unicellular cytopenias) to all three (pancytopenia) cellular elements of the blood. Furthermore, a causal relation exists between benzene exposure and aplastic anemia in humans (Aksoy et al. 1972, 1974; Erf and Rhoads 1939; Yin et al. 1987c) and in animals (Cronkite et al. 1982, 1985, 1989; Luke et al. 1988b; Seidel et al. 1989b; Snyder et al. 1980, 1984; Toft et al. 1982; Ward et al. 1985). This disorder is characterized by reduction of all cellular elements in the peripheral blood and in bone marrow. Aplastic anemia that results from benzene exposure is also associated with an increased risk of developing acute non-lymphocytic leukemia (Aksoy 1980; Aksoy et al. 1974).

Limited information is available on other systemic effects reported in humans and associated with benzene exposure. Respiratory effects have been noted after acute exposure of humans to benzene vapors (Avis and Hutton 1993; Midzenski et al. 1992; Winek and Collum 1971; Winek et al. 1967; Yin et al. 1987b). Cardiovascular effects, particularly ventricular fibrillation, have been suggested as the cause of death in fatal exposures to benzene vapor (Avis and Hutton 1993; Winek and Collum 1971). Gastrointestinal effects have been noted in humans after fatal inhalation exposure (congestive gastritis, Winek and Collum 1978) and ingestion (toxic gastritis and pyloric stenosis, Greenburg 1926) of benzene. Myelofibrosis was reported by a gasoline station attendant who had been exposed to benzene by inhalation, and probably also through dermal contact (Tondel et al. 1995). Myalgia was also reported in steel plant workers exposed to benzene vapors (Ruiz et al. 1994). Reports of renal effects in humans after benzene exposure consist of kidney congestion after fatal inhalation exposure (Winek and Collom 1971). Dermal and ocular effects including skin irritation and burns, and eye irritation have been reported after exposure to benzene vapors (Avis and Hutton 1993; Midzenski et al. 1992; Yin et al. 1987b). Swelling and edema have been reported to occur in a human who swallowed benzene (Greenburg 1926). Studies in animals show systemic effects after inhalation exposure, including cardiovascular (Magos et al. 1990; Nahum and Hoff 1934). Oral administration of benzene to animals has yielded information concerning hepatic effects (Pawar and Mungikar 1975). A study conducted in rabbits lends support to the finding that benzene is irritating and damaging to the skin

and also shows that it is irritating and damaging to the eyes following dermal or ocular application (Wolf et al. 1956).

Neurological effects have been commonly reported in humans after acute inhalation exposure (Cronin 1924; Davies 1929; Flury 1928; Greenburg 1926; Kraut et al. 1988; Midzenski et al. 1992; Tauber 1970; Tondel et al. 1995; Yin et al. 1987b). Fatal inhalation exposure has been associated with vascular congestion in the brain (Avis and Hutton 1993; Flury 1928). Chronic inhalation exposure has been associated with distal neuropathy in humans (Baslo and Aksoy 1982), difficulty in sleeping, and memory loss (Kahn and Muzyka 1973). Oral exposure of humans results in symptoms similar to inhalation exposure (Thienes and Haley 1972). Studies in animals suggest that inhalation exposure to benzene results in depressed electrical activity in the brain (Frantik et al. 1994), loss of involuntary reflexes and narcosis (Carpenter et al. 1944), decrease in hind-limb grip strength and tremors (Dempster et al. 1984), and narcosis (Evans et al. 1981), among other symptoms. Oral exposure to benzene has not been shown to cause significant changes in behavior (Hsieh et al. 1990, 1991). No neurological effects have been reported after dermal exposure to liquid benzene in either humans or animals.

Evidence of an effect of benzene exposure on human reproduction is not sufficient to demonstrate a causal association (Mukhametova and Vozovaya 1972; Stucker et al. 1994; Vara and Kinnunen 1946). Some animal studies provide limited evidence that benzene affects reproductive organs following inhalation exposure (Ungvary and Tatrai 1985; Ward et al. 1985; Wolf et al. 1956). Results from studies of benzene administered orally to rats and mice indicate no adverse effect on male or female reproductive organs at 17 weeks, but at 2 years, endometrial polyps were observed in female rats, preputial gland lesions were observed in male mice, and ovarian lesions were observed in female mice (NTP 1986). Results are conflicting or inconclusive as to whether inhalation of benzene vapors reduces the number of live fetuses (Ungvary and Tatrai 1985) and/or the incidences of pregnancy (Gofmekler 1968). Other studies are negative for effects on reproductive competence (Coate et al. 1984; Exxon 1986; Green et al. 1978; Kuna et al. 1992; Murray et al. 1979).

Epidemiological studies implicating benzene as a developmental toxicant have many limitations, and thus it is not possible to assess the effect of benzene on the human fetus (Budnick et al. 1984; Forni et al. 1971a; Funes-Cravioto et al. 1977; Goldman et al. 1985; Heath 1983; Olsen 1983). Results of inhalation studies conducted in animals are fairly consistent across species and demonstrate that, at

levels of  $\geq 47$  ppm, benzene is fetotoxic as evidenced by decreased fetal weight and/or minor skeletal variants (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). Benzene has also been shown to reduce pup body weight in mice (Seidenberg et al. 1986). A persistent decrease in number of erythroid precursors was found in mice exposed *in utero* (Keller and Snyder 1986, 1988). Benzene has not been shown to be teratogenic, but has been shown to be fetotoxic in animals at high concentrations that are maternally toxic.

Damage to both the humoral and cellular components of the immune system has been known to occur in humans following inhalation exposure. This is manifested by decreased levels of antibodies and decreased levels of leukocytes in workers (Aksoy et al. 1971, 1972, 1987; Cody et al. 1993; Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Lange et al. 1973a, 1973b; Ruiz et al. 1994; Xia et al. 1995; Yin et al. 1987c). Animal data support these findings (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite 1986; Cronkite et al. 1982, 1985, 1989; Dow 1992; Fan 1992; Gill et al. 1980; Green et al. 1981a, 1981b; Hsieh et al. 1988b, 1990, 1991; Huff et al. 1989; Li et al. 1986, 1992; Maltoni et al. 1983, 1985; Neun et al. 1992; NTP 1986; Plappert et al. 1994a, 1994b; Rosenthal and Snyder 1985, 1987; Rozen et al. 1984; Shell 1992; Snyder et al. 1984, 1988; Stoner et al. 1981; Toft et al. 1982; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956; Yin et al. 1982).

*In vivo* and *in vitro* data from both humans and animals indicate that benzene and/or its metabolites are genotoxic. Chromosomal aberrations in peripheral lymphocytes and bone marrow cells are the predominant effects seen in humans (Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hartwich et al. 1969; Hedli et al. 1990; Karacic et al. 1995; Major et al. 1994; Picciano 1979; Popp et al. 1992; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Sellyei and Kelemen 1971; Tompa et al. 1994; Tough and Court-Brown 1965; Tough et al. 1970; Turkel and Egeh 1994; Van den Berghe et al. 1979).

Many epidemiological and case studies correlate benzene exposure with leukemia, and some provide risk assessment of benzene exposure (Aksoy 1980, 1987; Aksoy and Erdem 1978; Bond et al. 1986b; Cody et al. 1983; Crump 1994; Hunting et al. 1995; Hurley et al. 1991; Infante 1978; Infante et al. 1977; Li et al. 1994; Ott et al. 1978; Paxton et al. 1994a, 1994b; Rinsky et al. 1981, 1987; Travis et al. 1994; Vigliani and Forni 1976; Wong 1995; Wong and Raabe 1995; Wong et al. 1983; Yin et al. 1987a, 1987c, 1989, 1994). These studies indicate that benzene is carcinogenic (leukemogenic).

Studies in humans following benzene exposure did not provide sufficient information on exposure levels and their correlation with observed effects. Data from animal studies have, therefore, been used to derive MRLs.

### **Minimal Risk Levels for Benzene.**

#### ***Inhalation MRLs.***

- An MRL of 0.05 ppm has been derived for acute-duration inhalation exposure (14 days or less) to benzene.

The acute-duration inhalation MRL of 0.05 ppm was derived from a LOAEL value of 10 ppm for the reduced lymphocyte proliferation following mitogen stimulation in mice (Rozen et al. 1984). The ratio of the blood/gas partition coefficients was assumed to be 1. The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10 ppm) by 6/24 to correct for less than a full day of exposure. The resulting adjusted LOAEL, 2.5 ppm, was then converted to a human equivalent concentration (HEC) by multiplying by the ratio of the mouse ventilation rate (2.21 L/hour) to its body weight (0.0316 kg) divided by the ratio of the human ventilation rate (833 L/hour) to human body weight (70 kg) (formula 4-11, EPA 1990h; ventilation rate and body weight values taken from EPA 1988e). The resulting LOAEL<sub>(HEC)</sub> of 14.7 ppm was then divided by an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolation from animals to humans after adjusting for the human equivalent concentration, and 10 for human variability) to yield the MRL value of 0.05 ppm (see Appendix A). Increased number of MN-PCEs, decreased numbers of granulopoietic stem cells (Toft et al. 1982), lymphopenia (Cronkite et al. 1985), lymphocyte depression, and increased susceptibility to bacterial infection (Rosenthal and Snyder 1985) are among the adverse hematological and immunological effects observed in several other acute-duration inhalation studies. The study by Rozen et al. (1984) shows benzene immunotoxicity (reduced mitogen-induced lymphocyte proliferation) at a slightly lower exposure level than these other studies. C57BL/6J mice were exposed to 0, 10.2, 31, 100 and 301 ppm benzene for 6 days at 6 hours per day. Control mice were exposed to filtered, conditioned air only. Lymphocyte counts were depressed at all exposure levels; erythrocyte counts were elevated at 10.2 ppm, equal to controls at 31 ppm and depressed at 100 and 301 ppm. Femoral B-lymphocyte and splenic B-lymphocyte numbers were reduced at 100 ppm. Levels of circulating lymphocytes and mitogen-induced blastogenesis of femoral B-lymphocytes were depressed after

exposure to 10.2 ppm benzene for 6 days. Mitogen-induced blastogeneses of splenic T-lymphocytes were depressed after exposure to 31 ppm of benzene for 6 days. In another study, mice exhibited a 50% decrease in the population of CFU-E after exposure to 10 ppm benzene for 5 days, 6 hours per day (Dempster and Snyder 1991). In a study by Wells and Nerland (1991), groups of 4-5 male Swiss-Webster mice were exposed to 3, 25, 55, 105, 199, 303, 527, 1150, or 2290 ppm benzene for 6 hours/day for 5 days. The number of leukocytes in peripheral blood and spleen weights were significantly decreased compared with untreated controls at all concentrations  $\geq 25$  ppm. Therefore, 3 ppm was the NOAEL and 25 ppm was the LOAEL for these effects. These data support the choice of Rozen et al. (1984) as the study from which to derive the MRL.

- An MRL of 0.004 ppm has been derived for intermediate-duration inhalation exposure (15-365 days) to benzene.

The intermediate-duration inhalation MRL of 0.004 ppm was derived from a LOAEL value of 0.78 ppm for neurological effects of intermediate-duration inhalation exposure of mice to benzene (Li et al. 1992). The ratio of the blood/gas partition coefficients was assumed to be 1. The dose was adjusted for intermittent exposure by multiplying the LOAEL (0.78 ppm) by 2 hours/24 hours and by 6 days/7 days to adjust to continuous exposure. The resulting adjusted LOAEL 0.056 ppm was then converted to a human equivalent concentration (HEC) by multiplying by the ratio of the mouse ventilation rate (2.21 L/hour) to its body weight (0.0316 kg) divided by the ratio of the human ventilation rate (833 L/hour) to human body weight (70 kg) (formula 4-11, EPA 1990h; ventilation rate and body weight values taken from EPA 1988e). The resulting LOAEL<sub>(HEC)</sub> of 0.33 ppm was then divided by an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 3 for extrapolation from animals to humans after adjusting for the human equivalent concentration, and 10 for human variability), yielding the MRL of 0.004 ppm (see Appendix A). Forty adult Kunming male mice were divided into 4 groups (5 per group) and exposed to 0, 0.78, 3.13, and 12.52 ppm benzene for 2 hours per day, 6 days per week for 30 days. Control mice were exposed to air. Benzene level was monitored by gas chromatography every 30 minutes for 3 days at the beginning of the experiment. Locomotor activity was measured in 15-minute sessions. A Y-maze, designed with a starting point and safety area, was used to measure rapid response. Motivation of the mice was accomplished by electrical shock. Fore-limb grip strength of mice was determined by persistent time (in seconds) while grasping a vertical iron stick. Body weight, food consumption, relative organ weight, blood and brain acetylcholinesterase activity, and bone marrow panel were determined. Body weight and food

consumption were not affected. Significant differences in organ-to-body weight ratios occurred only for the liver and spleen, but not for the kidney. A 12% increase in liver weight and a 26% decrease in spleen weight were observed at 12.52 ppm. The increase in liver weight was not considered to be adverse. There was decreased grip strength at 12.52 ppm, but increased grip strength occurred at the lower doses. Low level exposure also affected frequency of rapid response; at 0.78 ppm, the response was increased, whereas at the higher doses, the response was depressed. All of the changes were significantly different from the control values. Locomotor activity increased at the low dose, was similar to control values at the mid dose, and decreased at the high dose. However, these changes were not significantly different from the control value. A significant decrease of AChE activity in the brain was observed at 12.52 ppm, but the decrease was not large enough to be considered an adverse effect. Bone marrow profile showed decreased percentages of precursors of leukocytes and erythrocytes with increased concentrations of inhaled benzene; this effect was most consistent at the high dose. Myelocytes, premyelocytes, myeloblasts, and metamyelocytes were among the cell types most affected. Li et al. (1992) observed neurological changes at doses that were lower than those that caused the expected changes in bone marrow cells. Based on the observation of neurological changes in a study that also identified classic indicators of benzene toxicity, it seems reasonable to use the neurological effects observed by Li et al. (1992) as a basis for an intermediate-duration MRL.

Benzene is known to cause adverse neurological effects in both humans and animals. Following acute inhalation of benzene, humans exhibited 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, included 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 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 transferred from the problem area (Kraut et al. 1988; Yin et al. 1987b). In reports of cases of benzene poisoning, subjects exhibited headaches, nausea, tremor, convulsions, and unconsciousness, among other neurological effects (Cronin 1924; Davies 1929; Greenburg 1926; Midzenski et al. 1992; Tauber 1970).

Chronic exposure to benzene has been reported to produce neurological abnormalities in humans. Of 8 patients (6 with aplastic anemia and 2 with preleukemia) with previous occupational exposure to

adhesives and solutions containing 9-88% benzene, 4 of the 6 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 or higher. These findings suggest that benzene may induce toxic effects on the nervous system involving peripheral nerves and/or spinal cord. However, 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/m<sup>3</sup>), while the toluene vapors did not exceed the 5 mg/m<sup>3</sup> level. Subsequently (the authors do not specify when), the air levels of both benzene and toluene have not exceeded the 5 mg/m<sup>3</sup> level. Of the examined workers, 74 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.

The neurotoxicity of benzene has not been studied extensively in animals. 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 (Carpenter et al. 1944). As the time after exposure progressed so did the symptoms to include excitation and tremors (after 5 minutes), loss of pupillary reflex to strong light (after 6.5 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 per 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).

No chronic-duration MRLs were derived for benzene because of a lack of appropriate data on effects on chronic-duration inhalation exposure. The study by Tsai et al. (1983) was considered. In this study, a benzene cohort of 454 male workers employed at a large Texas refinery between September 15, 1952 and January 1978 was studied. Participants were included regardless of length of employment. Data of exposure to benzene for the years 1973-82 were examined since data prior to 1973 were inadequate for assessing worker exposure. Benzene exposures were <1 ppm, with a median exposure of 0.14 ppm for refinery workers, and 0.53 ppm for those in the benzene-related units. The relative risk for all causes of death decreased. There was no increased risk compared with either the U.S. general population or the general refinery workers. Evaluation of data for a 21-year period showed no significant changes in blood indices of selected employees. The study was not accepted for derivation of a chronic-duration inhalation MRL because there was no attempt to adjust for TWA exposure levels; most of the study was concerned with retrospective mortality results compared with the U.S. national rates; and in the medical surveillance study for hematological effects, benzene workers were defined by the supervisor of each unit, with no indication of how the subjects were selected, no controls, and no information regarding follow-up. Additional limitations included that the study was not originally designed to detect chronic health effects in the selected populations and focused more on mortality and cancer end points. It is unclear how the study subjects described in the medical testing portion were selected and followed. The study was judged inappropriate for deriving an MRL. In other chronic studies, the lowest LOAEL of 3 ppm was considered a serious LOAEL because pancytopenia was observed in humans exposed occupationally (Doskin 1971). The LOAEL is also higher than the LOAEL<sub>(HEC)</sub> of 0.33 ppm used as the basis for the intermediate-duration MRL.

#### ***Oral MRLs.***

No acute-duration oral MRL was derived due to a lack of appropriate data on the effects of acute oral exposure to benzene.

In a study by Thienes and Haley (1972), central nervous system symptoms of giddiness, vertigo, muscular incoordination, unconsciousness, and death of humans have been reported when doses of 125 mg/kg/body weight benzene or higher were ingested. The only lower doses in the acute oral data base are 50 mg/kg/day, at which pregnant rats experienced alopecia of hindlimbs and trunk (Exxon 1986), an endpoint not suitable for MRL derivation for benzene because the toxicological significance is not clear; and 88 mg/kg/day, at which slight non-dose-related central nervous system depression occurred in rats (Cornish and Ryan 1965).

No intermediate-duration oral MRL was derived. Examination of the literature indicated that studies by Hsieh et al. (1988b, 1991) and Wolf et al. (1956) might yield an intermediate-duration oral MRL. Adult male CD-1 mice were exposed to 0, 8, 40, or 180 mg/kg/day benzene in the drinking water for 4 weeks (Hsieh et al. 1988b, 1991). Serious hematological and immunological effects occurred at the lowest dose, precluding its use for MRL derivation. In the study by Wolf et al. (1956), a NOAEL of 1 mg/kg/day and a LOAEL of 50 mg/kg/day for leukopenia were identified in rats given benzene in oil by gavage for 6 months. However, closer examination of the study by Wolf et al. (1956) revealed that the results were not adequately supported by the data and analysis presented in the paper. Therefore, Wolf et al. (1956) was not selected for derivation of the MRL.

No chronic-duration oral MRLs were derived for benzene because of lack of appropriate data on effects of oral exposure to benzene. The NTP (1986) carcinogenesis bioassay was considered. In the NTP (1986) bioassay, male rats were given 50, 100, or 200 mg/kg/day and female rats and mice of both sexes were given 0, 25, 50, and 100 mg/kg/day benzene in corn oil for 2 years. The dose of 25 mg/kg/day was a LOAEL for hematotoxicity and immunotoxicity in rats and mice, and is higher than the serious LOAEL of 8 mg/kg/day in the intermediate-duration data base. Therefore, the threshold for hematological and immunological effects of benzene has not been identified for oral exposure, precluding the derivation of a chronic MRL.

**Death.** Acute inhalation exposure to high concentrations of benzene has caused death. Lethality in humans following inhalation exposure has been attributed to asphyxiation, respiratory arrest, central nervous system depression, or cardiac collapse (Avis and Hutton 1993; Hamilton 1922; Winek and Collom 1971). Many of the fatalities resulted from asphyxiation due to inhalation of high concentrations of benzene in enclosed places (Avis and Hutton 1993; Cronin 1924; Greenburg 1926; Tauber 1970). Based on case studies, it has been estimated that exposure to a benzene concentration

of 20,000 ppm for 5-10 minutes is likely to be rapidly fatal (Flury 1928). Animal studies lend support to human data that indicate that benzene has a relatively low toxicity following acute inhalation exposures. An  $LC_{50}$  of 13,700 ppm has been reported for rats after a 4-hour exposure (Drew and Fouts 1974). Similarly, exposure to 16,000 ppm for 4 hours was lethal for 4 of 6 rats (Smyth et al. 1962).

Concentrations of benzene in the workplace above 10 ppm are associated with hematological abnormalities, including aplastic anemia and leukemia, which have resulted in death (Aksoy and Erdem 1978; Ott et al. 1978; Rinsky et al. 1981, 1987). Cancer studies conducted in animals support the findings in humans that benzene is capable of shortening lifespan (Farris et al. 1993; Maltoni et al. 1982a, 1983; Paxton et al. 1994a; Snyder et al. 1978a).

Lethality in humans following oral exposure to benzene has been attributed to respiratory arrest, central nervous system depression, or cardiac collapse (Greenburg 1926). Oral lethal doses for humans have been estimated to be about 125 mg/kg (Thienes and Haley 1972). Accidental ingestion and/or attempted suicide with lethal oral doses 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). Animal studies lend support to human data indicating that there is a rapid progression of toxicity following high acute oral exposures to benzene. An  $LD_{50}$  of 930 mg/kg/day has been reported for rats after a 1-day exposure (Cornish and Ryan 1965). No data are available on the effects of benzene on lifespan following intermediate and chronic oral exposures in humans. Increased mortality was observed in rats and mice after chronic-duration oral exposure to benzene at 250 mg/kg/day and 100 mg/kg/day, respectively (NTP 1986).

No data are available on death in animals following dermal exposure to benzene. Increased risk of death was associated with dermal exposure to gasoline by vehicle mechanics (Hunting et al. 1995).

Inhalation exposure to high concentrations of benzene in the occupational setting may lead to death from pancytopenia and leukemia. However, environmental exposure to benzene in the air, drinking water, soil, or food is unlikely to be fatal. People living near hazardous waste sites who are chronically exposed to contaminated air, water, or soil may be at a higher risk of death due to adverse health conditions, including leukemia and other types of cancer.

**Systemic Effects.**

**Respiratory Effects.** Respiratory effects have been reported after acute exposure to benzene vapors (Avis and Hutton 1993; Winek and Collum 1971). 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). Similar results were noted after intentional inhalation resulting in death (Winek and Collum 1971). Mucous membrane irritation and dyspnea have been noted at occupational exposures of greater than 60 ppm for up to 3 weeks (Midzenski et al. 1992). Thus, benzene has been shown to have irritative properties; contact with benzene vapor is capable of damaging respiratory tissues. A dose of 500 mg/kg of benzene administered subcutaneously once daily, 5 days a week for 26 weeks to Wistar rats caused atrophy of the peribronchial lymphoidal tissue with blurring of the structure of the lymphoidal nodules in the lungs after 12 weeks of exposure (Bloch et al. 1990). After 26 weeks, a progression of these changes was noticed. Thus, exposure to benzene by routes other than inhalation could also cause respiratory effects.

Inhalation exposure benzene is likely to cause respiratory irritation. However, environmental exposure to benzene in the air, drinking water, soil, or food is unlikely to cause significant adverse respiratory effects. People living near hazardous waste sites who are chronically exposed to contaminated air, water, or soil may be at a higher risk for respiratory effects from exposure to the vapor, or through accumulation of benzene in the body after ingestion of contaminated food or water.

**Cardiovascular Effects.** In humans, ventricular fibrillation has been proposed as a contributing factor to death after acute inhalation exposure to benzene (Avis and Hutton 1993; Winek and Collom 1971). Electrocardiograms indicated that exposure of cats and monkeys to high but unspecified concentrations of benzene vapor caused extra systoles and ventricular tachycardia of the prefibrillation type in cats and monkeys (Nahum and Hoff 1934). Similar results have been observed for rats exposed to very high concentrations (3,526-8,224 ppm) for 15 minutes (Magos et al. 1990). A dose of 500 mg/kg of benzene administered subcutaneously once daily, 5 days a week for 26 weeks to Wistar rats resulted in muscle fiber necrosis in the left ventricle (Bloch et al. 1990).

The effect of benzene on the respiration of isolated heart mitochondria was studied by Stolze and Nohl (1994). Respiratory control and ATP/oxygen values decreased in the presence of benzene at 100  $\mu$ M.

A concomitant increase in superoxide radical formation was observed. This suggests a mechanism for benzene-mediated damage to cardiovascular tissue and other tissues as well.

Based on this limited information, it is possible that inhalation of high concentrations of benzene vapors, such as those encountered in an occupational accident, could induce similar changes in humans in the industrial setting. Environmental exposure to low levels of benzene found in contaminated air, water, food, and soil is unlikely to cause these cardiovascular changes.

***Gastrointestinal Effects.*** Few studies are available on the gastrointestinal effects of exposure to benzene. After intentional inhalation of benzene resulting in death, congestive gastritis was noted at the autopsy of an 18-year-old boy (Winek and Collum 1971). In the case report of a human who swallowed benzene, intense toxic gastritis and later pyloric stenosis were reported (Greenburg 1926). In long-term studies (2 years) in rats and mice, hyperkeratosis, acanthosis, and hyperplasia were observed in the forestomach after oral exposure by gavage to relatively high doses (200 mg/kg/day) of benzene (NTP 1986). Therefore, it is possible that accidental or intentional inhalation or ingestion of very high doses of benzene will cause these effects in humans. Environmental exposure to levels found in drinking water, soil, food, or air in or around hazardous waste sites is unlikely to cause adverse gastrointestinal effects in exposed populations.

***Hematological Effects.*** Both human and animal studies have shown that benzene exerts toxic effects on various parts of the hematological system. All the major types of blood cells are susceptible (erythrocytes, leukocytes, and platelets). In the less severe cases of toxicity, specific deficiencies occur in individual types of blood elements. A more severe effect occurs when there is hypoplasia of the bone marrow, or hypercellular marrow exhibiting ineffective hematopoiesis so that all types of blood cells are found in reduced numbers. This is known as pancytopenia. A biphasic response (i.e., a hyperplastic effect in addition to destruction of the bone marrow cells) has been observed (Aksoy et al. 1972, 1974; Doskin 1971; Rozen et al. 1984). Severe damage to the bone marrow involving cellular aplasia is known as aplastic anemia and can occur with prolonged exposure to benzene. -This condition can lead to leukemia.

Studies on occupationally exposed populations demonstrate that these effects do indeed occur, but these studies do not provide sufficient data on exposure to determine a threshold level for effects. For these epidemiology studies, it was assumed that the major route of benzene exposure was through the

lungs. However, it is also likely that the people involved had skin contact with the substance, and it is possible that this also contributed to the observed toxicity. The human studies that did provide some estimate of levels of exposure show that adverse hematological effects occurred at levels greater than 10 ppm but generally not at levels less than 1 ppm (Aksoy 1991; Aksoy et al. 1971, 1972, 1987; Cody et al. 1993; Fishbeck et al. 1978; Kipen et al. 1989; Midzenski et al. 1992; Tsai et al. 1983; Xia et al. 1995; Yin et al. 1982, 1987b, 1987c). The animal studies support the findings in humans and can be used to provide more accurate data on the relationship between specific effects and exposure levels in air. Adverse hematological effects begin to appear in animals at concentrations of 10-100 ppm and above (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite et al. 1982, 1985, 1989; Dempster and Snyder 1991; Dow 1992; Farris 1993; Gill et al. 1980; Green et al. 1981b; Hsieh et al. 1988b, 1991; Huff et al. 1989; Li et al. 1986; Luke et al. 1988b; Maltoni et al. 1983, 1985; NTP 1986; Plappert et al. 1994a, 1994b; Rozen et al. 1984; Seidel et al. 1989b; Shell 1992; Snyder et al. 1980, 1984; Toft et al. 1982; Vacha et al. 1990; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956).

Only one study was found that described hematological effects in humans after oral exposure to benzene (Selling 1916). No reports describing hematological effects in humans following direct dermal exposure to benzene were found. However, intermediate- and chronic-duration animal studies show that loss of blood elements occurs in animals exposed to benzene in drinking water or by gavage at doses as low as 8-25 mg/kg/day (Hsieh et al. 1988b, 1990; Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986; Shell 1992; Wolf et al. 1956).

Based on information found in the literature, it is reasonable to expect that adverse hematological effects might occur in humans after inhalation, oral, or dermal exposure, since absorption of benzene through any route of exposure would increase the risk of damage to blood elements. Studies show that the hematological system is susceptible to chronic exposure at low levels, so people living in and around hazardous waste sites that may be exposed to contaminated air, drinking water, soil, or food may be at an increased risk for adverse hematological effects. Deficiencies in various types of blood cells lead to other disorders, such as hemorrhagic conditions from a lack of platelets, susceptibility to infection from the lack of leukocytes, and increased cardiac output from the lack of erythrocytes.

Several mechanisms to explain how benzene affects the bone marrow have been proposed. These include suppression of RNA and DNA synthesis (Moeschlin and Speck 1967; Post et al. 1985),

alkylation of cellular sulfhydryl groups (Irons 1985), disruption of the cell cycle (Irons et al. 1979; Snyder et al. 1981c), oxygen activation or free radical formation (Irons 1985), covalent binding of benzene metabolites to cellular macromolecules (Gill and Ahmed 1981; Longacre et al. 1981a; Snyder et al. 1978b), damage to stromal cells (Frash et al. 1976; Kalf et al. 1987), and loss of specific protein growth factors (Clark and Kamen 1987).

The effect of administration of benzene on iron metabolism was investigated by giving 0.5 mL/kg intraperitoneally or 1 ml/kg subcutaneously to female rats for 10 consecutive days (Ahmad et al. 1994). Serum from benzene-treated female rats offered less protection against iron-catalyzed lipid peroxidation of brain tissue than the serum of control female rats. Serum iron concentration and total iron binding capacity did not exhibit any significant change after benzene administration. Iron saturation of transferrin was not affected by benzene.

Benzene, administered intraperitoneally at 600 mg/kg in corn oil, 2 times per day for 2 days to male C57BL/6J mice, caused a significant depression in the total number of nucleated bone marrow cells per femur, when measured on day 3 (Niculescu and Kalf 1995). Additional experiments with 7-day exposure revealed that there was an initial depression in erythroid cells on day 3 which remained constant; lymphocytes exhibited a progressively depressive effect, and the numbers of intermediate and terminally differentiated granulocytes exhibited a progressive increase over the 7 days of exposure. Upon cessation of the benzene treatment, the bone marrow appeared to begin recovery, with the number of nucleated cells equal to control animals by day 7 after treatment ended. Concomitant administration of IL-1  $\alpha$  prevented the decrease in nucleated bone marrow cells, whereas IL-1  $\alpha$  administered after 2 days of benzene exposure significantly increased the rate of recovery of bone marrow cellularity.

There is general agreement among various investigators in the field of benzene toxicity that benzene metabolites, rather than benzene, are the primary toxic agents in the induction of hematotoxicity. This agreement has evolved as a result of studies in which agents known to alter benzene metabolism (toluene, phenobarbital, and ethanol) have also altered benzene toxicity (Andrews et al. 1977; Sammett et al. 1979). Toluene, Aroclor 1254, and phenobarbital appear to alleviate benzene toxicity, while ethanol generally increases benzene toxicity. Therefore, 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 or activated elsewhere and transported to the marrow. When several of the

metabolites of benzene were compared with the parent compound in hematotoxicity studies, the metabolites were less toxic than benzene. Tunek et al. (1981) suggested that perhaps the metabolites tested conjugate strongly in the liver and other organs and do not reach the bone marrow in amounts sufficient to produce the expected effect. On the other hand, one metabolite of benzene, *trans,trans*-muconaldehyde (LD<sub>50</sub>, 6.7-7.1 mg/kg), was highly toxic to the bone marrow of mice administered 2 mg/kg, intraperitoneally, for 10-16 days (Witz et al. 1985). Similarly, 6-hydroxy-*trans,trans*-2,4-hexadienal, a reactive intermediate of *trans,trans*-muconaldehyde, was administered to male CD-1 mice intraperitoneally; leukocyte count increased in mice dosed at 5 mg/kg/day for 16 consecutive days, but bone marrow cellularity and red blood cell parameters were not affected (Zhang et al. 1995c). At 10 mg/kg/day for 16 consecutive days, a decrease in nonprotein sulfhydryl groups of the bone marrow, but no change in peripheral blood parameters, was noted. At 25 mg/kg/day for 3 consecutive days followed by 20 mg/kg for one day and sacrifice 24 hours later, a decrease in nucleated bone marrow cells and leukocyte count was noted. Iron incorporation in erythrocytes was inhibited by 3 consecutive doses of 20 or 30 mg/kg 6-hydroxy-*trans,trans*-2,4-hexadienal; 15 mg/kg had no effect on iron incorporation. The results of this study further support the concept that metabolites of benzene are hemotoxic, and can reach the bone marrow. Hedli et al. (1990) administered benzene metabolites phenol and hydroquinone intraperitoneally to rats and examined bone marrow cellularity and DNA adducts in the bone marrow. Data indicated that the metabolites were able to cause a decrease in bone marrow cellularity, and form DNA adducts in the bone marrow when administered *in vivo*. DNA adducts were observed at doses that did not affect bone marrow cellularity.

Carbonnelle et al. (1995) looked at the effect of hydroquinone on IL-1 release from human monocytes *in vitro*. Exposure of human monocytes to micromolar amounts of hydroquinone for 2 hours resulted in significantly decreased secretion of IL-1  $\alpha$  and IL-1  $\beta$  at doses of 5  $\mu$ M and above. RNA and protein synthesis were also inhibited, with a 50% inhibitory concentration at 21  $\mu$ M for IL-1  $\alpha$  and 10  $\mu$ M for IL-1  $\beta$ . Bone marrow mononuclear cells or purified hematopoietic progenitor cells were incubated with 30  $\mu$ M hydroquinone alone or in the presence of IL-1  $\beta$  or tumor necrosis factor, and the colony-forming ability of the cells (CFU-C) was evaluated (Colinas et al. 1995). Hydroquinone alone reduced CFU-C frequencies by approximately 60% for both bone marrow mononuclear cells and purified hematopoietic progenitor cells. Neither IL-1  $\beta$  or tumor necrosis factor protected the bone marrow mononuclear cells from the inhibitory activity of hydroquinone. For purified hematopoietic progenitor cells, however, treatment with tumor necrosis factor, but not IL-1  $\beta$ , provided protection against

inhibition of formation of progenitor cell colonies. Irons et al. (1992) looked at the effect of pretreatment of murine bone marrow cells with hydroquinone, phenol, catechol, and *trans,trans*-muconaldehyde on colony-forming response. Cells were stimulated with recombinant granulocyte/macrophage colony stimulating factor after exposure to the benzene metabolites. Pretreatment with hydroquinone for 30 minutes in the picomolar to micromolar range caused a 1.5-4.6-fold enhancement in colonies formed, with optimal enhancement at 1  $\mu$ M hydroquinone. Synergism between hydroquinone and the growth factor was observed in cells not normally responsive to the growth factor, suggesting an effect of hydroquinone on cells not normally responsive to growth factors. Pretreatment with the other metabolites did not enhance growth factor response.

Equal numbers of male Swiss Webster and C57BL/6J mice were exposed to air or to 300 ppm benzene 6 hours per day, 4 days per week for 2 weeks (Neun et al. 1994). Bone marrow was isolated from the mice on the morning following final inhalation. Bone marrow cells from benzene-exposed and control mice were cultured with one of five benzene metabolites: 1,4-benzoquinone (BQ), catechol (C), hydroquinone (HQ), muconic acid (MA) or phenol (P); the abilities of these cultured bone marrow cells to produce erythroid (CFU-E) or granulocyte/macrophage colonies (GM-CFU-C) were assessed. Bone marrow cells isolated from both strains of mice exposed to benzene showed higher percentages of plated erythroid colony forming cells surviving culture with BQ, HQ, or muconic acid than marrow cells isolated from control mice. In contrast, marrow cells isolated from both strains of exposed mice showed decreased percentages of plated CFU-E surviving culture with catechol than cells isolated from control mice. *In vitro* treatment with phenol induced no differential effects on CFU-e isolated from exposed or control mice of either strain. Cells isolated from exposed Swiss Webster mice showed increased percentages of plated GM-CFU-c surviving culture with HQ than cells isolated from controls. In contrast, marrow cells isolated from exposed C57BL/6J mice showed decreased percentages of GM-CFU-C surviving culture with HQ than cells isolated from controls. Benzene exposure did not affect the total hepatic sulfhydryl (SH) concentrations in either mouse strain but the SH content of the bone marrow was more than doubled after benzene exposure in both strains.

Treatment of mice with 800 mg/kg benzene, intraperitoneally, 2 times daily for 2 days resulted in a significant increase in the production of nitric oxide by bone marrow cells stimulated by lipopolysaccharide and interferon  $\gamma$ , alone or in combination with macrophage colony-stimulating factor or granulocyte-macrophage colony-stimulating factor (Punjabi et al. 1994). Cells from benzene-treated mice showed increased sensitivity to the effects of the stimulating factors. Benzene,

hydroquinone, 1,2,4-benzenetriol, and *p*-benzoquinone were evaluated for their effect on nitric oxide production by murine bone marrow leukocytes *in vitro* (Laskin et al. 1995). Treatment of mice with benzene (800 mg/kg, intraperitoneally), hydroquinone (100 mg/kg), 1,2,4-benzenetriol (25 mg/kg), or *p*-benzoquinone (2 mg/kg) sensitized bone marrow leukocytes to produce more nitric oxide in response to stimulation from lipopolysaccharide, interferon- $\gamma$ , and increased the sensitivity of the cells to granulocyte/macrophage colony-stimulating factor and macrophage colony-stimulating factor. Cells from hydroquinone and 1,2,4-benzenetriol-treated animals were significantly more responsive than those from benzene or *p*-benzoquinone-treated animals. Nitric oxide has been shown to suppress cell growth and, in the presence of molecular oxygen, can form a variety of nitrogen oxide intermediates that can react with primary and secondary amines to form nitrosamines, which are carcinogenic. Thus, nitric oxide may be important to the mechanism of toxicity of benzene and its metabolites. Rao (1991) showed that hematin catalyzed the autoxidation of hydroquinone or 1,2,4-benzenetriol *in vitro*, producing reduced oxygen species that may be responsible for protein or DNA binding after benzene exposure. Further work along this line of research has provided evidence that *in vitro*, chelates of iron and hydroquinone or 1,2,4-benzenetriol are potent DNA cleaving agents (Rao 1996; Singh et al. 1994), and that 1,2,4-benzenetriol, but not hydroquinone, causes the release of iron from ferritin (Ahmad et al. 1995). 1,2,4-Benzenetriol or hydroquinone have also been shown to release reactive products from glutamate or DNA in the presence of copper ions (Rao and Pandya 1989). For a further discussion on biotransformation of benzene, see Section 2.3.3.

Scheding et al. (1992) have analyzed hematotoxic effects observed in mice in *in vivo* studies, using a mathematical model. Their results indicate that erythropoietic cells were the most sensitive, granulopoietic cells were about half as sensitive as erythropoietic cells, and hematopoietic stem cells exhibited a sensitivity somewhere between the two. A dose-response relationship between benzene exposure and hematotoxic effects was derived for doses up to 300 ppm; effects plateaued at higher doses. This study indicates that mathematical modeling represents a useful approach to investigate the action of hematotoxic agents.

***Musculoskeletal Effects.*** There is little data describing musculoskeletal effects of benzene exposure. Two studies provide information describing musculoskeletal effects in humans after inhalation and possibly dermal exposure. Myelofibrosis was diagnosed in a 46-year-old man who had worked as a gasoline station attendant for 17 years (Tondel et al. 1995). The TWA concentration for gasoline station attendants was estimated to be ~0.2 ppm. Exposure was probably also dermal. Ruiz et al.

(1994) reported myalgia in employees from a steel plant of Cubatao, S. Paulo, Brazil, who presented with neutropenia due to benzene exposure. No histopathological lesions were found in bone tissues of rats or mice exposed to benzene orally for up to 2 years (NTP 1986). Although musculoskeletal effects may occur after chronic exposure to benzene, these effects are not likely to be of primary concern to people exposed occupationally or to the low levels that are likely to be found in ambient air, water, and soil, or in or around hazardous waste sites.

**Hepatic Effects.** No reliable studies were located regarding the hepatic effects of benzene in humans following inhalation, oral, or dermal exposures. Aksoy et al. (1972) reported enlarged livers in workers chronically exposed to benzene, but no additional data were given.

In animals, increased liver weight is the most common hepatic effect observed after inhalation or oral exposure to benzene (Pawar and Mungikar 1975; Tatrai et al. 1980a, 1980b), and histopathological examination of the liver of rats and mice exposed by gavage to doses up to 600 or 200 mg/kg/day, respectively, for up to two years revealed no treatment-related effects (Huff et al. 1989; NTP 1986).

Benzene is metabolized primarily in the liver by the cytochrome P-450 system (Parke 1989). It appears that the metabolism of benzene by the hepatic cytochrome P-450 system plays an important role in its bioactivation and toxicity. Sammett et al. (1979) provided corroborative evidence of this by showing that partial hepatectomy of rats diminished both the rate of metabolism of benzene and its toxicity. An increase in altered hepatic foci has been shown in male rats after benzene exposure in conjunction with initiator and promotor administration (Dragan et al. 1993). A dose of 500 mg/kg of benzene administered subcutaneously once daily, 5 days a week for 26 weeks to Wistar rats resulted in focal fine droplet fatty metamorphosis with accompanying lymphoidal infiltration in the liver after 12 weeks (Bloch et al. 1990). In some cases a proliferated histocyte-like cells formed clusters in the vicinity of the periportal fields. After 26 weeks, more diffuse steatosis, feathery degeneration of hepatocytes, single necrotic cells were seen.

The effect of administration of benzene, 0.5 mL/kg intraperitoneally or 1 mL/kg subcutaneously, to male and female rats for 10 consecutive days on free sulfhydryl content and lipid peroxidation in the liver was investigated (Ahmad et al. 1994). The free sulfhydryl content of the liver showed a significant decrease in groups dosed intraperitoneally, but not subcutaneously. The rate of lipid

peroxidation was increased in all benzene-treated animals.  $\alpha$ -Tocopherol levels in the liver were not affected.

Based on the data found in the literature, hepatic effects do not appear to be of primary concern with regard to human health after exposure to benzene occupationally, in the environment, or at hazardous waste sites.

**Renal Effects.** Renal congestion has been noted at autopsy after a fatal human exposure by inhalation (Winek and Cullom 1971). No adverse renal effects were observed in animals administered benzene orally for acute, intermediate, or chronic durations (Exxon 1986; Hsieh et al. 1990; Huff et al. 1989; NTP 1986). Administration of benzene, 438 mg/kg intraperitoneally or 877 mg/kg subcutaneously, to male and female rats for 10 consecutive days resulted in a decrease in serum uric acid (Ahmad et al. 1994). Based on these limited data, adverse renal effects do not appear to be of primary concern with regard to human health after exposure to benzene occupationally, in the environment, or at hazardous waste sites.

**Dermal Effects.** Limited information is available on the dermal effects of benzene. Benzene was slightly irritating to the skin of rabbits following application one time per day for 4 weeks (Wolf et al. 1956). The skin showed moderate erythema, edema, and moderate necrosis. No dose information was provided in this study. Exposure of humans to benzene vapor in air has resulted in dermal effects that include erythema, edema, frank burns, and necrosis (Avis and Hutton 1993; Midzenski et al. 1992). Limited information also suggests that oral ingestion of high doses (near lethal) may cause swelling and edema (Greenburg 1926).

These data indicate that exposure to high levels of benzene in air, direct contact of the skin with benzene, and ingestion of high doses may result in dermal effects, but it is unlikely that levels of benzene in ambient air, in contaminated drinking water, or at hazardous waste sites are high enough to be of concern.

**Ocular Effects.** Eye irritation has been reported in workers exposed occupationally to benzene (Yin et al. 1987b), and lacrimation occurred in rats exposed to benzene in air (Shell 1980). Moderate conjunctival irritation and transient corneal damage were observed in rabbits subsequent to the placement of 2 drops of benzene into the eyeball (Wolf et al. 1956). Thus, eye irritation is a concern

for people exposed to high concentrations of benzene in air or if benzene is splashed in the eyes, but levels of benzene in the ambient air, in contaminated drinking water, or at hazardous waste sites are probably not high enough to be of concern.

**Body Weight Effects.** Relatively few studies report body weight effects after benzene exposure. Tondel et al. (1995) reported unspecified weight loss in a gasoline station attendant who had been exposed to benzene by inhalation and probably through dermal contact for 17 years. Decreases in body weight (15%) were seen in DBA/2 mice after exposure to 300 ppm benzene for 6 hours per day, 5 days per week for 2 weeks (Chertkov et al. 1992). Decreased body weight (16%) has also been noted in mice exposed to 50 or 200 ppm of benzene for 6 hours per day for 7 or 14 days (Aoyama 1986). C57BL mice exhibited a 50% decrease in body weight gain after exposure to 300 ppm benzene, 6 hours per day, 5 days per week for 70 weeks (Snyder et al. 1980). Other studies have shown decreased body weight in animals exposed by inhalation or orally (Exxon 1986; Green et al. 1978; Kuna and Kapp 1981; Maltoni et al. 1983, 1985; NTP 1986; Snyder et al. 1978a; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). However, in other animal studies, no change in body weight was seen after inhalation or oral exposure Green et al. 1981b; Hsieh et al. 1990; Kuna et al. 1992; Li et al. 1992; Shell 1992; Snyder et al. 1984; Tanningher et al. 1995; Ward et al. 1985). Thus, body weight changes may not be primary indicators of benzene toxicity in humans.

**Immunological and Lymphoreticular Effects.** Benzene has been shown to have adverse immunological effects in humans following inhalation exposure for intermediate and chronic durations. Adverse immunological effects in animals occur following both inhalation and oral exposure for acute, intermediate, and chronic durations. The effects include damage to both humoral (antibody) and cellular (leukocyte) responses. Human studies of intermediate and chronic duration have shown that benzene causes decreases in the levels of circulating leukocytes in workers at low levels (30 ppm) of exposure (Aksoy et al. 1971, 1972, 1974) and decreases in levels of circulating antibodies in workers exposed to benzene at 3-7 ppm in a mixture with xylene and toluene (Lange et al. 1973b). Other studies have shown decreases in human lymphocytes and other blood elements after exposure (Aksoy et al. 1987; Cody et al. 1993; Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Lange et al. 1973a; Ruiz et al. 1994; Xia et al. 1995; Yin et al. 1987c). Animal data support these findings (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite 1986; Cronkite et al. 1982, 1985, 1989; Dow 1992; Fan 1992; Gill et al. 1980; Green et al. 1981a, 1981b; Hsieh et al. 1988b, 1990, 1991; Huff et al. 1989; Li et al. 1986, 1992; Maltoni et al. 1983, 1985; Neun et al. 1992; NTP 1986;

Plappert et al. 1994a, 1994b; Rosenthal and Snyder 1985, 1987; Rozen et al. 1984; Shell 1992; Stoner et al. 1981; Snyder et al. 1984, 1988; Toft et al. 1982; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956; Yin et al. 1982). Both humans (Yin et al. 1982) and rats (Li et al. 1986; Yin et al. 1982) have shown increases in leukocyte alkaline phosphatase activity. No studies regarding effects from oral or dermal exposure in humans were located. However, exposure to benzene through ingestion or dermal contact could cause immunological effects similar to those seen after inhalation exposure in humans and inhalation and oral exposure in animals.

Animal studies have also shown that benzene decreases circulating leukocytes and decreases the ability of lymphoid tissue to produce the mature lymphocytes necessary to form antibodies. This has been demonstrated in animals exposed for acute (Cronkite et al. 1982, Gill et al. 1980; Green et al. 1981a, 1981b; Li et al. 1986; Rozen et al. 1984), intermediate (Aoyama 1986; Cronkite et al. 1982, 1985, 1989; Green et al. 1981a, 1981b; Ward et al. 1985; Wolf et al. 1956), or chronic periods (Snyder et al. 1980, 1984) via the inhalation route. This decrease in lymphocyte numbers is reflected in impaired cell-mediated immune functions in mice following intermediate inhalation exposure to 100 ppm of benzene (Rosenthal and Snyder 1987). The impaired cellular immunity after benzene treatment was observed both *in vivo* and *in vitro*. Mice exposed to 100 ppm for a total of 100 days were challenged with 104 polyoma virus-induced tumor cells (PYB6). Nine of 10 mice had reduced tumor resistance resulting in the development of lethal tumors. In the same study lymphocytes were obtained from spleens of benzene-treated mice and tested for their immune capacity *in vitro*. The results showed that two other immune functions, alloantigen response (capacity to respond to foreign antigens) and cytotoxicity, were also impaired (Rosenthal and Snyder 1987). Similar effects were noted in mice exposed to benzene via the oral route for intermediate time periods (Fan 1992; Hsieh et al. 1988b) and in rats and mice exposed for chronic time periods (Huff et al. 1989; NTP 1986). A decrease in spleen weight was observed in mice after acute-duration exposure to benzene at 25 ppm, the same dose levels at which a decrease in circulating leukocytes was observed (Wells and Nerland 1991). Similar effects on spleen weight and circulating leukocytes were observed in mice exposed to 12 ppm benzene 2 hours per day for 30 days (Li et al. 1992). A dose of 500 mg/kg of benzene administered subcutaneously once daily, 5 days a week for 26 weeks to Wistar rats resulted changes in the immunological and lymphoreticular system after 12 weeks (Bloch et al. 1990). The microscopic structure of most follicles became blurred, with damage to the germinal centers and an increase in volume of red pulp. After 26 weeks, a progression of the above changes were seen with atrophy of the white pulp, further expansion of the red pulp, and the appearance of an increased number of

fibroblasts. In the spleen, the lymphatic system showed signs of damage which became more pronounced in joint exposure to benzene and ethanol. The acute inhalation MRL was based on the study showing decreased mitogen-induced blastogenesis of B-lymphocytes following exposure to 10 ppm in mice (Rozen et al. 1984).

Selected measures of cellular immunity were evaluated in wild juvenile cotton rats after dietary protein restriction and intraperitoneal exposure benzene (McMurry et al. 1994a). Forty-seven-day-old juvenile cotton rats were maintained on one of two isocaloric diets containing either 4 or 16% crude protein for a 26-day experimental period. Animals were treated intraperitoneally with either 0 (corn oil), 100, 500, or 1,000 mg/kg benzene in corn oil for 3 consecutive days. The first dose was administered on day 15-17 of the experimental period. Animals were terminated on day 27. Relative weight of the spleen, but not the thymus, was greater in rats on the low protein diet and exposed to benzene, compared to the low protein control animals; no difference in spleen or thymus weight was noted between benzene-treated 16% protein diet rats and the 16% protein controls. With regard to peripheral leukocyte counts, control animals receiving 16% protein in their diet had more neutrophils and fewer lymphocytes than their 4% protein counterparts. Total neutrophils were higher in rats receiving benzene compared to controls. Splenocyte yield, although higher in control rats receiving 16% protein compared to the low protein diet group, were not affected by benzene treatment. Cellular immunity, measured *in vivo*, and splenocyte proliferation, measured *in vitro*, were not affected by benzene treatment. In a companion study, treatment of 21-day-old rats on a regular diet with 0, 100, or 1,000 mg/kg benzene intraperitoneally in corn oil for 3 consecutive days caused a depression in relative thymus weight, but no effect on relative spleen weight was observed (McMurry et al. 1994b). Benzene treatment had no effect on leukocyte counts, or viable splenocyte yields, cellular immunity, or splenocyte proliferation.

Benzene, administered intraperitoneally at 600 mg/kg in corn oil, 2 times per day for 2 days to male C57BL/6J mice, caused a significant depression in the total number of nucleated bone marrow cells per femur, when measured on day 3 (Niculescu and Kalf 1995). Additional experiments with 7-day exposure revealed that there was an initial depression in erythroid cells on day 3 which remained constant; lymphocytes exhibited a progressively depressive effect, with the numbers of intermediate and terminally differentiated granulocytes exhibited a progressive increase over the 7 days of exposure. Upon cessation of the benzene treatment, the bone marrow appeared to begin recovery, with the number of nucleated cells equal to control animals by day 7 after treatment ended. Concomitant

administration of IL-1  $\alpha$  prevented the decrease in nucleated bone marrow cells, whereas IL-1  $\alpha$  administered after 2 days of benzene exposure significantly increased the rate of recovery of bone marrow cellularity.

*In vitro* studies of the effect of benzene metabolites on receptor-mediated phagocytosis and cytoskeletal integrity in mouse peritoneal macrophages provides information on the immunotoxicity of benzene (Manning et al. 1994). In mouse peritoneal macrophage cultures, benzoquinone, 1,2,4-benzenetriol, and hydroquinone inhibited receptor mediated phagocytosis; catechol had no effect. Cells treated with benzoquinone or benzenetriol did not recover, even after overnight incubation in the absence of treatment. Additional observations suggest that for benzoquinone, at least, the inhibition of receptor-mediated phagocytosis is caused by a disruption of the filamentous action in the cell, although the specific effect is not known.

Based on information found in the literature, it is reasonable to expect that adverse immunological effects might occur in humans after inhalation, oral, or dermal exposure, since absorption of benzene through any route of exposure would increase the risk of damage to the immunological system. Studies show that the immunological system is susceptible to chronic exposure at low levels, so people living in and around hazardous waste sites that may be exposed to contaminated air, drinking water, soil, or food may be at an increased risk for adverse immunological effects

**Neurological Effects.** In humans, results of occupational studies indicate that there is a cause-and-effect relationship between acute inhalation of benzene and symptoms indicative of central nervous system toxicity (Cronin 1924; Davies 1929; Flury 1928; Greenburg 1926; Midzenski et al. 1992; Tauber 1970). These symptoms, observed following both acute nonlethal and lethal exposures, include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. These symptoms are reversible when symptomatic workers are transferred from the problem area (Kraut et al. 1988; Yin et al. 1987b). Comparable toxicity in humans has been reported following ingestion of benzene at doses of 125 mg/kg and above (Thienes and Haley 1972). No studies were located regarding neurological effects in humans after dermal exposure to benzene. Occupational exposure to benzene has also been reported to produce neurological abnormalities in humans. Electromyographical and motor conduction velocity examinations were conducted on six patients with aplastic anemia, all of whom worked in environments where adhesives containing benzene were used (in one case, air concentrations bracketed around 210 ppm) (Baslo and Aksoy 1982). Abnormalities in motor

conduction velocity were noted in four of the six pancytopenic individuals and were thought to result from a direct effect of benzene on the peripheral nerves and/or spinal cord.

In its acute stages, benzene toxicity appears to be due primarily to the direct effects of benzene on the central nervous system, whereas the peripheral nervous system appears to be the target following chronic low-level exposures. In addition, because benzene may induce an increase in brain catecholamines, it may also have a secondary effect on the immune system via the hypothalamuspituitary-adrenal axis (Hsieh et al. 1988b). Increased metabolism of catecholamines can result in increased adrenal corticosteroid levels, which are immunosuppressive (Hsieh et al. 1988b).

Animal studies provide additional support that benzene affects the nervous system following acute inhalation and oral exposures. Effects reported include narcosis, nervous system depression, tremors, and convulsions (Carpenter et al. 1944; Cornish and Ryan 1965). Acute and intermediate inhalation exposures have also been reported to produce adverse neurological effects in animals including a reduction in hind-limb grip strength and evoked electrical activity in the brain, and behavioral disturbances (Dempster et al. 1984; Frantik et al. 1994). Effects of benzene on learning were investigated in male hooded rats of the Sprague-Dawley strain given 550 mg/kg of benzene in corn oil or corn oil without benzene, intraperitoneally, on days 9, 11, and 13 postpartum (Geist et al. 1983). The rats exhibited a significantly impaired learning ability when tested on problems of the closed-field, maze-learning task. This sign of neurotoxicity following benzene exposure was not observed in control animals. In another study, 47-day-old juvenile cotton rats were maintained on one of two isocaloric diets containing either 4% or 16% crude protein for a 26-day experimental period (McMurry et al. 1994a). Animals were treated intraperitoneally with either 0 (corn oil), 100, 500, or 1,000 mg/kg benzene in corn oil for 3 consecutive days. The first dose was administered on day 15-17 of the experimental period. Animals were terminated on day 27. During the experimental period, severe loss of coordination was observed in some rats on the low protein diet immediately after exposure to benzene, but this subsided.

Intermediate oral exposures resulted in changes in the levels of monoamine transmitters in the brain without treatment-related behavioral changes (Hsieh et al. 1988a). Mice exposed to 3 ppm for 2 hours per day for 30 days exhibited increased levels of acetylcholinesterase in the brain (Li et al. 1992). In the same experiment, grip strength was decreased at 12 ppm and rapid response was increased at

0.78 ppm. The intermediate inhalation MRL was based on this study, showing increased rapid response following exposure to 0.78 ppm in mice (Li et al. 1992).

*In vitro* studies suggest that benzene may have a direct effect on brain cells (Vaalavirta and Tahti 1995). Primary astrocyte cultures prepared from neonatal rat cerebella were treated with 3, 6, or 9 mmol/L benzene for 1 hour. ATPase and Mg<sup>2+</sup>-ATPase activity were inhibited in a dose-related manner, and were detected at 78-92% of control values for ATPase, and 60-74% of control values for Mg<sup>2+</sup>-ATPase.

These data suggest that humans exposed to benzene in the occupational setting for acute, intermediate, or chronic durations via the inhalation and oral routes are at risk of developing neurological effects. However, benzene levels in ambient air, drinking water, and at hazardous waste sites are not likely to be of concern.

**Reproductive Effects.** Menstrual disorders characterized by profuse or scanty blood flow and dysmenorrhea occurred in 12 of 30 women who worked in an environment containing benzene (Vara and Kinnunen 1946). Although air measurements of benzene were not reported, it is assumed that benzene levels at the work site may have been much higher than those in today's work areas. Ten of the 12 women were married. Of these 10 women, 2 had spontaneous abortions and no births occurred. However, the study failed to provide verification that the absence of birth was due to infertility. Gynecological exams revealed that scanty menstruations of 5 of the patients were due to ovarian atrophy. In a study of 360 female gluing operators exposed to petroleum (a major source of benzene) and chlorinated hydrocarbons, both dermally and by inhalation, no significant difference in fertility between exposed workers and unexposed controls was found (Mukhametova and Vozovaya 1972). However, incidences of spontaneous abortion and premature birth increased. Because of study limitations, no conclusions can be drawn concerning adverse reproductive effects of benzene in humans following inhalation exposure. The study limitations included limited follow-up, lack of appropriate control populations, and/or simultaneous exposure to other substances. No adverse effects on reproduction were detected in a study of paternal exposure to benzene in the chemical industry in France (Stucker et al. 1994).

Some effects on testes (atrophy/degeneration, decrease in spermatozoa, moderate increases in abnormal sperm forms) have been noted in experimental animals exposed by inhalation (Ward et al. 1985; Wolf

et al. 1956). Thus, results in humans are inconclusive, and results in animals indicate that inhalation of benzene vapors reduces the number of live fetuses (Murray et al. 1979; Ungvary and Tatrai 1985) and/or the incidences of pregnancy (Gofmekler 1968) only at high doses or not at all.

Evidence of an effect of benzene exposure on human reproduction is not sufficient to demonstrate a causal association. In addition, animal studies only provide suggestive evidence (i.e., effects on testes) that benzene affects reproduction following inhalation exposure, with most studies indicating no effect on reproductive indices even after high doses.

**Developmental Effects.** Epidemiological studies implicating benzene as a developmental toxicant have many limitations, so it is not possible to assess the effect of benzene on the human fetus. 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 concurrent 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 a study conducted in the Love Canal area by Heath (1983), the outcome of pregnancy was evaluated in populations living in the proximity of waste sites where benzene and other hazardous substances had been identified. No clear increase in occurrence of spontaneous abortion, birth defects, or low infant birth weight was observed in women living next to the canal. The study limitations of inadequate sample size and lack of exposure history preclude an assessment of the significance of these findings. In another study conducted by Goldman et al. (1985) on the Love Canal area, birth weight was assessed in 239 infants exposed during gestation life. There was an association between low birth weight and hazardous waste exposure. However, there were inherent problems in the study design and methodology. One of the study groups was comprised of low-income renters and predominantly black individuals. Blacks have a high incidence of low birth weights. Information was retrieved by telephone survey, and some of the low-income renters may have lacked telephones. The methods also failed to include 235 families that were evacuated from the Love Canal area. Finally, no statistically significant clusters of birth defects were found in populations living around the Drake Superfund site in Pennsylvania, an area contaminated with benzene and other carcinogens (Budnick et al. 1984). However, the significance of this finding cannot be determined because of design methodology inadequacies including inadequate sample size and lack of quantification of exposure levels. One toxicokinetic study showed that benzene crosses the human placenta and is present in cord blood in amounts equal to or greater than those in maternal blood (Dowty et al. 1976).

Most of the animal data are from inhalation experiments because this has been the principal route of concern (Coate et al. 1984; Keller and Snyder 1986, 1988; Ungvary and Tatrai 1985). Results of inhalation exposure have been fairly consistent across species; they demonstrate that at high levels benzene is fetotoxic as evidenced by decreased fetal weight and/or minor skeletal variants. It has been suggested that benzene fetotoxicity is a function of maternal toxicity because the occurrence of a decrease in fetal weight and an increase in skeletal variants usually occurs when there is a decrease in maternal weight. However, the mechanisms of this toxicity have not been fully elucidated, and there are few data on the effects of benzene on maternal food consumption and on blood levels of benzene and its metabolites in the dams and their fetuses. None of the animal studies demonstrate that benzene is teratogenic at levels that induce maternal and fetal toxicity. Fetotoxic effects in rodents occurred at benzene levels  $\geq 47$  ppm (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). However, there was evidence of transient hematopoietic anomalies in mice exposed *in utero* to benzene at 5 ppm and 10 ppm (Keller and Snyder 1986, 1988). *In utero* exposure of mice to 20 ppm benzene produced similar hematopoietic anomalies that persisted in 6-week-old animals (Keller and Snyder 1988). Thus, changes in the murine hematopoietic system can result from maternal exposure to low concentrations of benzene. This may have relevance for the human fetus after maternal exposure to benzene in that the human fetus is capable of metabolizing xenobiotics to a greater extent than the mouse fetus, thus possibly forming greater amounts of toxic metabolites responsible for the hematopoietic toxicity of benzene.

There are no data available on the developmental effects of benzene in humans or animals following dermal exposures. Oral data are limited to 2 animal studies. Benzene was shown to reduce pup body weight when mice were administered a high oral dose of 1,300 mg/kg/day during gestation (Seidenberg et al. 1986), indicating that benzene may induce fetotoxic effects in humans at high doses. However, no fetotoxicity was reported when rats were dosed orally with 1,000 mg/kg/day during gestation (Exxon 1986).

Benzene has been shown to be embryotoxic to rat embryos *in vitro* (Brown-Woodman et al. 1994). Day 10 Sprague-Dawley rat embryos were exposed to benzene in culture for 2 days, then evaluated. Benzene at 3.14  $\mu\text{mole/mL}$  caused a significant decrease in yolk sac diameter and protein content of the embryo; crown-rump length and somite number were decreased at both 1.56 and 3.14  $\mu\text{mole/mL}$  benzene. A no-effect level of 0.82  $\mu\text{mole/mL}$  was determined. In another study of cultured rat embryos, Chapman et al. (1994) evaluated the embryotoxic effects of benzene and several of its

metabolites. Benzene at 1.6 mM produced little embryotoxicity, with or without hepatic activating enzymes, but phenol showed significant embryotoxicity in the presence of hepatic activation at concentrations as low as 0.01 nM. *Trans,trans*-muconaldehyde was embryotoxic at 0.01 mM and embryolethal at 0.05 nM; hydroquinone, catechol, and benzoquinone were all 100% embryolethal at 0.1 mM.

Based on the available data, other than the possibility of hematological effects in the offspring, it is unlikely that persons living near hazardous waste sites are exposed to levels of benzene in the air, water, or soil high enough to cause fetotoxic effects.

**Genotoxic Effects.** The genotoxic effects of benzene have been studied extensively. The results are summarized in Tables 2-5 and 2-6. Benzene and/or its metabolites seem to be genotoxic to humans, causing primarily chromosomal aberrations (Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hartwich et al. 1969; Hedli et al. 1990; Karacic et al. 1995; Major et al. 1994; Picciano 1979; Popp et al. 1992; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Selley and Kelemen 1971; Tompa et al. 1994; Tough and Court-Brown 1965; Tough et al. 1970; Turkel and Egeh 1994; Van den Berghe et al. 1979). Peripheral lymphocytes and bone marrow cells appear to be the major targets. Inhalation, oral, and dermal routes are all potential pathways of exposure relevant to humans. However, available case-control studies reflect only inhalation and/or dermal exposure. This is because these studies 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. The limitations of the occupational studies include lack of accurate exposure data, possible coexposure to other chemicals, and lack of appropriate control groups. The majority of these studies suggest that benzene is genotoxic, specifically clastogenic (i.e., capable of breaking the DNA molecule in a manner observable at the chromosome level).

Case studies involving individuals who were previously chronically exposed to benzene report significant chromosomal aberrations in bone marrow and lymphocytes (Forni and Moreo 1967, 1969; Hartwich et al. 1969; Major et al. 1992; Picciano 1979; Selley and Kelemen 1971; Tough and Court-Brown 1965; Tough et al. 1970; Van den Berghe et al. 1979; Yardley-Jones et al. 1990). Other mutagens in addition to benzene may have been involved since most of the workers were occupationally exposed to more than one chemical.

Table 2-5. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Results	Reference
Prokaryotic cells:			
<i>Escherichia coli</i> (host mediated DNA repair)	DNA synthesis	-	Hellmer and Bolcsfoldi 1992a
Invertebrate animal cells:			
<i>Drosophila melanogaster</i>	Sex-linked recessive lethal	-	Kale and Baum 1983
<i>D. melanogaster</i> (spermatocytes)	Recombination	-	Kale and Baum 1983
<i>D. melanogaster</i> (spermatogonia)	Recombination	+	Kale and Baum 1983
<i>D. melanogaster</i> (spermatocytes)	Heritable translocation	-	Kale and Baum 1983
Mammalian cells:			
Mouse (spleen lymphocytes)	Chromosomal aberrations	+	Rithidech et al. 1987
Mouse (spleen lymphocytes)	Chromosomal aberrations	+	Au et al. 1991a
Mouse (spleen lymphocytes)	Mutations	+	Ward et al. 1992
Mouse (peripheral erythrocytes)	Micronucleus increase	+	Hayashi et al. 1992
Mouse (bone marrow)	Chromosomal aberrations	(+)	Tice et al. 1982
Mouse (bone marrow)	Chromosomal aberrations	(+)	Tice et al. 1980
Mouse (bone marrow)	Chromosomal aberrations	+	Siou et al. 1981
Mouse (bone marrow)	Chromosomal aberrations	+ <sup>a</sup>	Meyne and Legator 1980
Mouse (bone marrow)	Chromosomal aberrations	+	Shelby and Witt 1995
Mouse (bone marrow)	Micronucleus increase	+	Shelby et al. 1993
Mouse (bone marrow)	Micronucleus increase	+	Shelby and Witt 1995
Mouse (bone marrow)	DNA adducts	-	Reddy et al. 1994
Mouse (bone marrow)	DNA adducts	+	Pathak et al. 1995
Mouse (liver)	DNA adducts	-	Reddy et al. 1994
Mouse (mammary gland)	DNA adducts	-	Reddy et al. 1994
Rat (bone marrow)	DNA oxidative damage	+	Kolachana et al. 1993
Rat (bone marrow)	Chromosomal aberrations	+	Styles and Richardson 1984

Table 2-5. Genotoxicity of Benzene *In Vivo* (continued)

Species (test system)	End point	Results	Reference
Rat (bone marrow)	Chromosomal aberrations	+ <sup>b</sup>	Anderson and Richardson 1981
Rat (bone marrow)	Chromosomal aberrations	+	Philip and Jensen 1970
Rat (bone marrow)	Chromosomal aberrations	+	Fujie et al. 1990
Rat (bone marrow)	Chromosomal aberrations	+	Hoechst 1977
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
Rabbit (bone marrow)	Chromosomal aberrations	+	Kissling and Speck 1973
Human (lymphocytes)	Micronucleus increase	+	Robertson et al. 1991a
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	(+)	Yardley-Jones et al. 1990
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Sasiadek et al. 1989
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	-	Jablonická et al. 1987
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Forni et al. 1971a
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Ding et al. 1983
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Tough and Court Brown 1965
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Picciano 1979
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	+	Popp et al. 1992
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Tompa et al. 1994
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Sasiadek 1992
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Sasiadek and Jagielski 1990
Mouse (spleen lymphocytes)	Increased polyploidy	+	Rithidech et al. 1987
Mouse (peripheral blood PCEs)	Micronuclei increase	+ <sup>c,d</sup>	Luke et al. 1988a
Mouse (peripheral blood NCEs)	Micronuclei increase	+ <sup>d,e</sup>	Luke et al. 1988a
Mouse (peripheral blood NCEs)	Micronuclei increase	+	Rithidech et al. 1988
Mouse <sup>f</sup> (bone marrow PCEs)	Micronuclei increase	+ <sup>g</sup>	Suzuki et al. 1989 <sup>h</sup>

Table 2-5. Genotoxicity of Benzene *In Vivo* (continued)

Species (test system)	End point	Results	Reference
Mouse (bone marrow PCEs)	Micronuclei increase	+ <sup>d</sup>	Ciranni et al. 1988
Mouse (pregnant/bone marrow PCEs)	Micronuclei increase	(+)	Ciranni et al. 1988
Mouse (fetus/liver cells)	Micronuclei increase	+	Ciranni et al. 1988
Mouse (bone marrow PCEs)	Micronuclei increase	+	Erexson et al. 1986
Mouse (bone marrow PCEs)	Micronuclei increase	+	Toft et al. 1982
Mouse (bone marrow PCEs)	Micronuclei increase	+	Harper et al. 1984
Mouse (bone marrow PCEs)	Micronuclei increase	+	Siou et al. 1981
Mouse (bone marrow PCEs)	Micronuclei increase	+ <sup>a</sup>	Meyne and Legator 1980
Mouse (bone marrow PCEs)	Micronuclei increase	+	Hite et al. 1980
Mouse (bone marrow PCEs)	Micronuclei increase	+	Barale et al. 1985
Mouse (bone marrow PCEs)	Micronuclei increase	+	Au et al. 1990
Mouse (peripheral blood NCEs)	Micronuclei increase	+	Barale et al. 1985
Mouse (peripheral blood NCEs)	Micronuclei increase	+	Choy et al. 1985
Mouse (bone marrow PCEs)	Micronuclei increase	+	Diaz et al. 1980
Chinese hamster (bone marrow)	Micronuclei increase	+	Siou et al. 1981
Rat (lymphocytes)	Micronuclei increase	+	Erexson et al. 1986
Mouse (bone marrow)	Sister chromatid exchange	+	Tice et al. 1982
Mouse (bone marrow)	Sister chromatid exchange	+	Tice et al. 1980
Mouse (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
Mouse (pregnant/bone marrow)	Sister chromatid exchange	+	Sharma et al. 1985
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	-	Yardley-Jones et al. 1988
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	-	Seiji et al. 1990
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

Table 2-5. Genotoxicity of Benzene *In Vivo* (continued)

Species (test system)	End point	Results	Reference
Rat (liver mitochondria)	RNA synthesis inhibition	+	Kalf et al. 1982
Rat (liver cells)	DNA adducts	+	Lutz and Schlatter 1977
Mouse (spermatogonia)	Sperm head abnormality	+	Topham 1980

<sup>a</sup>This result was observed following both oral and intraperitoneal exposure.

<sup>b</sup>This result was observed following both inhalation and intraperitoneal exposure.

<sup>c</sup>Increase in micronuclei was exposure duration-independent.

<sup>d</sup>Males affected to a significantly greater degree than females.

<sup>e</sup>Increase in micronuclei was exposure duration-dependent.

<sup>f</sup>Two strains of mouse were tested; Ms/Ae and CD-1. The result applies to both strains.

<sup>g</sup>This result was observed following both oral and intraperitoneal exposure; however, oral exposure produced the greater effect.

<sup>h</sup>The authors of this study did not report statistical comparisons of their results.

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

Table 2-6. 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	Seixas et al. 1982
<i>S. typhimurium</i> (azaquanine reversion)	Gene mutation	+	No data	Kaden et al. 1979
<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	Hellmer and Bolcsfoldi 1992b
Plasmid DNA $\phi$ 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	+	Morimoto 1976
Human (lymphoblastoid culture)	Intrachromosomal recombination	No data	+	Aubrecht et al. 1995
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	+	Eastmond et al. 1994
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	-	Gerner-Smidt and Friedrich 1978
Human (leukemia cells)	DNA oxidative damage	No data	+	Kolachana et al. 1993
Chinese hamster (ovary cell culture)	Sister chromatid exchange	-	-	Gulati et al. 1989
Chinese hamster (ovary cell culture)	Sister chromatid exchange	-	-	Douglas et al. 1985
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
Chinese hamster (ovary cell culture)	Micronuclei increase	-	-	Douglas et al. 1985
Human (bone marrow)	DNA adducts	No data	+	Bodell et al. 1993
Human (leukemia cells)	DNA adducts	No data	+	Bodell et al. 1993
Human (bone marrow)	DNA adducts	No data	+	Levay and Bodell 1992
Human (leukemia cells)	DNA adducts	No data	+	Levay and Bodell 1992

Table 2-6. Genotoxicity of Benzene *In Vitro* (continued)

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Calf thymus DNA	DNA adducts	No data	+	Chenna et al. 1995
Rabbit (bone marrow mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984
Rat (liver mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984
Rat liver epithelial cells	DNA hyperphosphorylation	No data	+	Dees and Travis 1994
Rat (hepatocytes)	DNA breaks	No data	-	Bradley 1985
Chinese hamster (V79 cell culture)	DNA breaks	-	-	Swenburg et al. 1976
Chinese hamster (ovary cell culture)	DNA breaks	+	+	Douglas et al. 1985
Chinese hamster (ovary cell culture)	DNA breaks	+	+ <sup>a</sup>	Lakhanisky and Hendricks 1985
Mouse (L5178Y cell culture)	DNA breaks	No data	-	Pellack-Walker and Blumer 1986
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	-	Probst and Hill 1985
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	-	Williams et al. 1985
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	(+)	Glauert 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 $\alpha$ /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
Rat (liver mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982
Rabbit (bone marrow mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982
Cat (bone marrow mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982

<sup>a</sup>Benzene'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

In one epidemiological study testing benzene's effects on chromosomal aberrations, benzene appeared to exert a nonrandom effect on chromosomes one, two, four, and nine (Sasiadek et al. 1989). However, this study is limited because relatively few controls were used and all participants (control and exposed) were smokers. The effects of smoking may have confounded the results. In a later paper, extra break points were confirmed on chromosomes 2 and 4, only (Sasiadek and Jagielski 1990). Sister chromatid exchange was not found to be a significant effect of benzene exposure in humans (Seiji et al. 1990; Yardley-Jones et al. 1988); however, poor control selection was used in both studies. Refer to Table 2-5 for a further summary of these results.

*In vivo* animal studies provide convincing evidence of benzene's genotoxicity (Table 2-5). Furthermore, the finding that male mice are more sensitive than females to benzene-induced chromosomal damage is consistent among reports (Choy et al. 1985). Consistently positive findings for chromosomal aberrations in bone marrow and lymphocytes support the human case reports and epidemiological studies in which chromosomal damage was linked to benzene exposure. Micronuclei tests are popular methods for crudely analyzing DNA damage in animals. Positive results were observed in all studies testing for increased micronuclei frequencies. One of these micronuclei studies investigated the effects of different inhalation exposure durations on PCEs and NCEs in peripheral blood. 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. 1990). 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). However, one study of intraperitoneal treatment of B6C3F<sub>1</sub> mice for 4 days with 500 mg/kg benzene in olive oil showed no DNA adduct formation in the liver, mammary gland, and bone marrow (Reddy et al. 1994). 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 2-5.

*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 mitochondria (Rushmore et al. 1984), and RNA synthesis in rat liver mitochondria and in rabbit and cat bone marrow mitochondria (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. Another study discovered a possible connection between certain benzene metabolites and DNA damage via the formation of oxygen radicals (Lewis et al. 1988a). Researchers found that both 1,2,4-benzenetriol (BT) and hydroquinone (HQ) produce DNA strand breaks, BT to a greater degree than HQ. Consistent with these findings was the observation that BT generates a greater concentration of oxygen radicals than HQ. The study concluded that BT damages DNA by producing oxygen radicals, while HQ probably exerts its genotoxic effects by some other mechanism (Lewis et al. 1988a); Li et al. (1995) has proposed that hydroquinone exerts its genotoxic effect through the copper-mediated generation of semiquinone radicals that are responsible for DNA damage.

Chromosomal breaks and hyperdiploidy were also observed in human lymphocytes after exposure to hydroquinone *in vitro* (Eastmond et al. 1994). Benzene metabolites have been shown to form DNA

adducts in human bone marrow and HL-60 cells (Bode11 et al. 1993; Levay and Bode11 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. The increase was dose-related. 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).

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  $\mu\text{M}$ . 1,4-Benzoquinone and 1,2,4-benzenetriol inhibited human topoisomerase II *in vitro*, at 500 and 250  $\mu\text{M}$  without bioactivation. However, following bioactivation, phenol and 2,2'-biphenol showed inhibitory effects at doses as low as 50  $\mu\text{M}$ , whereas 4,4'-biphenol inhibited topoisomerase II at concentrations of 10  $\mu\text{M}$ .

Data also exist that 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; Lakhanisky 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 2-6 for the results of these and other *in vitro* studies.

In summary, both structural and numerical 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 (see Sections 2.2.1.7, 2.2.2.7, and 2.2.3.7). 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 2.3) 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.

**Cancer.** It has been established that exposure to commercial benzene or benzene-containing mixtures can cause damage to the hematopoietic system including pancytopenia with subsequent manifestation of acute myelogenous leukemia (AML) (Aksoy et al. 1971, 1972, 1974). Numerous review articles can be found in the open literature that discuss benzene carcinogenesis (e.g., Aksoy 1991; Cox 1991; Gold et al. 1993; Snyder and Kalf 1994; Snyder et al. 1993b). In AML, there is diminished production of normal erythrocytes, granulocytes, and platelets, which leads to death by anemia, infection, or hemorrhage. The hallmark of AML is the appearance in the peripheral blood of cells morphologically indistinguishable from myeloblasts. These events can be rapid. Case reports and epidemiological studies of workers have established a causal relationship between benzene exposure and AML (Aksoy and Erdem 1978; Crump 1994; Infante 1978; Infante et al. 1977; Ott et al. 1978; Paxton et al. 1994a, 1994b; Rinsky et al. 1987; Travis et al. 1994; Wong 1987a, 1987b, 1995; Wong and Raabe 1995). A series of studies (Infante 1978; Infante et al. 1977; Rinsky et al. 1981, 1987) analyzing the mortality of workers exposed to benzene at two rubber hydrochloride manufacturing locations demonstrated excess risk of leukemia. The most recent study demonstrated SMRs of 337 for leukemia and 409 for multiple myeloma. (An SMR of 100 is the normal value if an excess is not observed. An SMR of 200 represents 100% excess risk over normal.) The Rinsky et al. (1987) study included excellent follow-up; 96.6% of the employees in this cohort were traced to determine whether they were alive or dead and, if dead, the cause of death. The study also carefully analyzed extensive past-exposure data and was able to assign qualitative exposure levels to individuals exposed 10-40 years in the past. The Rinsky et al. (1987) study also demonstrated a qualitative (i.e., ppm-years) dose-response relationship. The SMRs for exposed workers ranged from 105 to 6,637. The dose-response relationship increases the confidence in the results, provides a stronger basis for risk assessment, and provides measured (as opposed to extrapolated) evidence that lowering

exposure substantially reduces risk. Recent updates of this study support the original conclusions (Paxton et al. 1994a, 1994b).

Patients with MDS (disorders of stem cells characterized by maturation defects) may die of infection or hemorrhage (due to platelet/clotting abnormalities), or their condition may progress to acute leukemia. In a review by Rinsky et al. (1989), it was pointed out that the majority of benzene workers who died from hematologic disorders, including aplastic anemia, acute myelocytic anemia, and blood dyscrasia with secondary anemia and leukemoid reaction, may actually have been MDS patients, (i.e., patients whose stem cells had undergone malignant transformation, but who died of anemia plus complications before frank acute leukemia became manifest). MDS patients should be included in the population at risk of developing leukemia if a significant time interval (years) has elapsed between the last benzene exposure and death.

While some studies have implicated other types of leukemia or even lymphomas, only AML and its variants have consistently been in excess in groups of workers with high benzene exposure (Goldstein 1988).

Benzene has also been shown to cause leukemia and lymphoma in laboratory animals. Snyder and coworkers (Snyder et al. 1980, 1988) administered a continuous lifetime inhalation concentration of 300 ppm benzene to C57BL/6 male mice and observed a significant increase in hematopoietic neoplasms that included 6 cases (15%) of thymic lymphomas. Myelogenous leukemia was found in Sprague-Dawley rats exposed to 100 ppm for life (Snyder et al. 1984). A highly significant increase in thymic and nonthymic lymphomas was found in C57BL/6 mice exposed to 300 ppm (Cronkite et al. 1984, 1985), and leukemia was found in CBA/Ca mice exposed to  $\geq 100$  ppm (Cronkite et al. 1986, 1989) of benzene by inhalation for 16 weeks. Lymphoma was also observed in CBA/Ca mice exposed to 300 ppm benzene for 16 weeks (Farris et al. 1993) and in B6C3F<sub>1</sub> mice treated by gavage with benzene at  $\geq 25$  mg/kg/day for 2 years (NTP 1986).

Experimental data in animals and studies of human cases of benzene intoxication indicate a link between the decrease in bone marrow cellularity and the development of leukemia. Many cases of benzene-induced leukemia appear to have been preceded by aplastic anemia (Toft et al. 1982). The compensatory response (regenerative hyperplasia) observed in the bone marrow, thymus, and spleen of

exposed animals may play a role in the carcinogenic response (Rozen and Snyder 1985; Snyder 1987; Snyder and Koscis 1975; Snyder et al. 1984).

One of the favored mechanisms for benzene toxicity or carcinogenicity is related to the covalent binding of benzene metabolites to cellular macromolecules. In mice administered radiolabeled benzene for relatively short durations, metabolites have been found covalently bound to liver, bone marrow, kidney, spleen, blood, and fat (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Gill and Ahmed 1981; Irons et al. 1980; Longacre et al. 1981a; McDonald et al. 1994; Snyder et al. 1978b). The label was bound to the nucleic acids and other macromolecules of the mitochondria (Gill and Ahmed 1981), and as the levels of covalently bound and water-soluble metabolites increased, so did benzene toxicity (Longacre et al. 1981a). Hydroquinone and catechol may accumulate in bone marrow to act as substrates for myeloperoxidase, forming metabolites that could bind to protein (Eastmond et al. 1987; Kalf et al. 1990; Snyder et al. 1993b; Subrahmanyam et al. 1991). Since myeloperoxidase activity has been confirmed in both murine and human hematopoietic progenitor cells (Shattenberg et al. 1994), this mechanism of toxicity is of more interest.

Phenol, hydroquinone, catechol, benzoquinone, and 1,2,4-trihydroxybenzene form adducts in bone marrow mitochondria, resulting in the inhibition of the synthesis of mitochondrial proteins that are necessary for mitochondrial function (Kalf et al. 1982).

Recent research has focused on the molecular mechanism by which benzene metabolites adversely affect cells. Production of reactive oxygen species that may bind to DNA (Rao 1991), formation of chelates with iron that may act as DNA cleaving agents (Rao 1996; Singh et al. 1994), causing the release of iron from ferritin (Ahmad et al. 1995), and producing reactive products from glutamate or DNA in the presence of copper (Rao and Pandya 1989) are only a few of the areas of investigation into the mechanism of toxicity of benzene metabolites.

Hydroquinone has been extensively studied. Carbonnelle et al. (1995) have investigated the effect of hydroquinone on IL-1 release from human monocytes, in an effort to show that reduced interleukin release may inhibit cytokine release by monocytes, thereby adversely affecting the regulation of hematopoiesis. Support for this mechanism comes from the work of Miller et al. (1994) who showed that hydroquinone decreased the enzyme responsible for the conversion of interleukin precursors in murine bone marrow macrophages. In addition, hydroquinone has been shown to alter differentiation

of myeloid progenitor cells which may be important to benzene leukemogenesis (Irons and Stillman 1993; Irons et al. 1992).

Nitric oxide, which has been shown to suppress cell growth and form reactive intermediates that lead to carcinogenic products, has been detected in bone marrow cells treated with benzene (Laskin et al. 1995; Punjabi et al. 1994) and may be important to the mechanism of benzene toxicity.

The ultimate mechanism for benzene-induced suppression of cellular growth and the development of leukemia is not known at this time; it could be one of the mechanisms discussed above (see also Section 2.4.2) but most likely the mechanism involves a combination of factors. Identification of the mechanism would be facilitated by clear identification of the specific target cell and intracellular target of benzene and its metabolites (Andrews and Snyder 1986).

Although only acute myelogenous leukemia and its variants have consistently been in excess in groups of workers exposed to benzene, other studies in humans also suggest increased risks of kidney, brain, and bladder cancer and soft-tissue sarcoma (Greenland et al. 1994; Hunting et al. 1995; Lagoreost et al. 1994b; Serraino et al. 1992; Steineck et al. 1990). Furthermore, benzene is a multipotential carcinogen in animals, as evidenced by the array of treatment-related increased incidences of neoplasms in rats and mice exposed by inhalation or orally (Cronkite et al. 1989; Huff et al. 1989; Maltoni et al. 1982a, 1982b, 1983, 1985; NTP 1986; Snyder et al. 1984). These include cancers of the liver, Zymbal gland, harderian gland, skin, mammary gland, lungs, oral cavity, nasal cavity, forestomach, ovary, and preputial gland.

Benzene is considered to be a human carcinogen by the Department of Health and Human Services (DHHS), International Agency for Research on Cancer (IARC), EPA, American Conference of Governmental and Industrial Hygienists (ACGIH) and OSHA. The DHHS has determined that benzene is a known human carcinogen (NTP 1994). EPA (IRIS 1996) has verified the weight-of-evidence classification for carcinogenicity of benzene as EPA Group A, based on a sufficient level of human evidence supported by a sufficient level of animal evidence. The American Conference of Governmental Industrial Hygienists (ACGIH) classified benzene as an A1-substance-a confirmed human carcinogen (ACGIH 1996a). There are still questions regarding both the mechanisms of benzene carcinogenesis and the most appropriate models for developing human risk estimates. EPA (IRIS 1996) has reviewed the human and animal carcinogenicity data on benzene. The following

paragraphs are a summary of the EPA calculations of unit risk values for leukemia based on human epidemiological studies. It should be noted that these values are estimates of human risk since the true human risk at low doses cannot be accurately identified.

The cancer potency of benzene has been estimated based on three separate epidemiological studies (Ott et al. 1978; Rinsky et al. 1981; Wong et al. 1983). Equal weight was given to cumulative dose and weighted cumulative dose, as well as relative and absolute model forms. EPA (IRIS 1996) estimated a unit risk value of  $2.7 \times 10^{-4}$  for leukemia from a lifetime inhalation exposure to 1 ppm benzene. Based on this value, the exposure levels associated with individual lifetime upper-bound risks of  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$  have been calculated to be  $4 \times 10^{-3}$ ,  $4 \times 10^{-4}$ ,  $4 \times 10^{-5}$ , and  $4 \times 10^{-6}$  ppm, respectively. The unit risk should not be used if the air concentration exceeds  $100 \mu\text{g}/\text{m}^3$  (31 ppb) since above this the unit risk may not be appropriate. One ppm of benzene ( $3,190 \mu\text{g}/\text{m}^3$ ) exceeds the upper limit of 31 ppb. A peer review workshop conducted in 1989 to review the EPA's risk assessment of benzene, recommended that the epidemiological studies, including their updates, be reviewed, with consideration given to appropriate disease end points, dose-response model, the use of animal as opposed to human data, relative as opposed to absolute risk, and other factors (Bond et al. 1986b; Rinsky et al. 1987; Voytek and Thorslund 1991; Wong 1987a). Additional independent reviews of the epidemiological data and risk assessment can also be found in the open literature (Brett et al. 1989; Cox and Ricci 1992; Crump 1994; Crump et al. 1993; Hallenbeck and Flowers 1992; Hawkins 1991; Johnson and Lucier 1992; Paxman and Rappaport 1990).

EPA has used cancer risk data from human epidemiological studies to derive risk factors associated with oral exposure to benzene. The oral dose levels associated with specific carcinogenic risks have been extrapolated by converting the risk value of  $2.7 \times 10^{-2}$  for a lifetime inhalation exposure to 1 ppm to a slope factor of  $2.9 \times 10^{-2}$  for oral exposure of 1 mg/kg/day and assuming identical levels of absorption of benzene into the body. The slope factor corresponds to a drinking water unit risk of  $8.3 \times 10^{-3}$  per mg/L (ppm). Using the method described in the EPA report (IRIS 1996), the dose levels associated with individual upper-bound estimates of  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$  have been-calculated to be  $1 \times 10^{-1}$ ,  $1 \times 10^{-2}$ ,  $1 \times 10^{-3}$ , and  $1 \times 10^{-4}$  mg/L (ppm), respectively, which are equivalent to dose levels of  $3 \times 10^{-3}$ ,  $3 \times 10^{-4}$ ,  $3 \times 10^{-5}$  and  $3 \times 10^{-6}$  mg/kg/day, respectively. The unit risk should not be used if the water concentration exceeds  $10,000 \mu\text{g}/\text{L}$  (ppb) since above this concentration the slope factor may no longer be accurate.

Based on the above information, it is reasonable to assume that benzene could cause cancer in humans if inhaled or ingested in sufficient quantities. The increased risk that individuals may have by living near waste sites through contaminated air, drinking water, or soil cannot be ascertained until exposure estimates of benzene at these locations become better defined.

## 2.6 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 (NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to benzene are discussed in Section 2.6.1.

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 often not 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 2.6.2.

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 2.8, Populations That Are Unusually Susceptible.

### **2.6.1 Biomarkers Used to Identify or Quantify Exposure to Benzene**

Biomarkers of exposure are available for benzene, and have been reviewed (Ong and Lee 1994). Adequate monitoring of benzene exposure may depend on the use of a series of biomarkers with correlation of the results. Endpoints specific for benzene and endpoints not specific for benzene have been used. One non-specific test is the urinary sulfate ratio test, which is based on the premise that with increasing exposure there will be an increase in benzene metabolites conjugated with sulfate moieties (Hammond and Herman 1960). Estimates of benzene exposure can be made by comparing the ratio of inorganic to organic sulfates in the urine. Inorganic sulfate levels amounting to 80-95% of total urinary sulfates are considered normal background, 70-80% levels indicate some exposure to benzene, 60-70% levels indicate a dangerous level of exposure, and 0-60% levels indicate an extremely hazardous exposure. Urinary sulfate levels are, however, quite variable, and they have not been used to identify exposure levels of benzene associated with minimal toxic effects. Additionally, the test for urinary sulfates is not specific for benzene.

Urinary phenol measurements have routinely been used for monitoring occupational exposure to benzene (OSHA 1987b), and there is some evidence that urinary phenol levels can be correlated with exposure level (Astier 1992; Inoue et al. 1986, 1988b; Karacic et al. 1987; Pagnotto et al. 1961; Pekari et al. 1992). However, correlating urinary phenol with benzene exposure is complicated-by potentially high and variable background levels of phenol that 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 also been found in persons with no known exposure to benzene (NIOSH 1974). In addition, coexposure to toluene, a common solvent, has been shown to inhibit transformation of benzene to phenol (Inoue et al. 1988b).

Changes in urinary phenol levels (pre- and post-shift sample results) were observed in examinations carried out on 33 female workers (23-54 years old) employed at a shoe factory for 5-30 years (Karacic et al. 1987). Their exposure was to solvents, glues, and paints known to contain benzene. Workers were known to be coexposed to toluene, which was listed as an ingredient of several of the glues. Urine samples were collected on Monday mornings before work ( $M_1$ ) and on Wednesdays before work ( $W_1$ ) and after work ( $W_{11}$ ). Additionally, venous blood was taken on  $W_{11}$  only. All samples were coupled with controls from 29 females (19-58 years old) who were not occupationally exposed to solvents. Results indicated there was no difference in the urinary phenol concentrations measured on  $M_1$  and  $W_1$ , but  $W_{11}$  was about 2 times that of  $W_1$ . Both benzene and toluene were found in the blood samples of exposed workers but were not detected in control blood samples. The difference in pre- and post-shift phenol concentrations in the urine was found to be correlated (regression coefficient,  $r=0.465$ ;  $p<0.01$ ) with benzene concentrations in the blood. The study did not assess the impact that concomitant exposure to toluene had on the data. In another study, urinary phenol levels were found to correlate well with benzene exposure, especially when these levels were expressed in terms of creatinine excretion (Inoue et al. 1986, 1988b). The calculated mean correlation coefficient was approximately 0.858-0.891.

In a small group of chemical workers whose TWA exposure to benzene was 0.4 ppm, no correlation was found between the TWA exposure and urinary phenol excretion (Perbellini et al. 1988). Additionally, analysis of data collected from 49 chemical workers in the coke oven industry led the examiners to conclude that urinary phenol could not be used as an indicator of benzene uptake for exposures of about 1 ppm or less (Drummond et al. 1988). Concomitant exposure to other chemicals (e.g., toluene) may have affected the metabolism of benzene.

The data suggest that variations in urinary phenol due to other factors interfere with determination of phenol formed from low levels of benzene. Therefore, benzene exposures of 10 ppm or less would be difficult to monitor by measuring urinary phenol levels, and consequently, correlations between phenol levels and LOAELs would not be likely for effects occurring in this dose range. Additionally, for exposures of  $\leq 1$  ppm, workplace standard, urinary phenol is an inadequate assay to determine extent of benzene exposure. Nevertheless, the measurement of urinary phenol is still useful in determining whether or not an individual has been exposed to substantial levels of benzene. In such situations, biological exposure indices (BEI) are directly correlated with threshold limit values. The ACGIH has established 50 mg phenol/g creatinine in the urine as the BEI for benzene exposure in the workplace

(ACGIH 1996b). The BE1 is primarily an index of exposure and not a level at which health effects might occur from exposure to benzene. ACGIH has indicated that the BE1 for benzene will be changed to 25  $\mu\text{g PhAC/g creatinine}$  in the urine (ACGIH 1996b).

In addition to phenol, other phenolic metabolites have been investigated as biomarkers of benzene exposure (Inoue et al. 1988a). 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. 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. Coexposure to toluene suppressed hydroquinone formation but had little effect on formation of catechol (Inoue et al. 1988b). However, there was more overlap in measured values of urinary catechol between subjects in the exposed and unexposed groups than occurred in the hydroquinone measurements (Inoue et al. 1988a).

Urinary muconic acid has also been investigated as an indicator of acute exposure to benzene (Ducos et al. 1990, 1992; Inoue et al. 1989; Lee et al. 1993; Melikian et al. 1993, 1994). A study of 152 shoe and paint manufacturers exposed to benzene reported that urinary muconic acid correlated linearly with the TWAs of benzene (Inoue et al. 1989). Measurements of muconic acid were able to distinguish between workers exposed to benzene at concentrations of 6-7 ppm and nonexposed workers. The high sensitivity of muconic acid measurement is due to the very low urinary background levels of muconic acid in the general population, in contrast to the high background levels found for phenol, catechol, and hydroquinone. However, the authors concluded that, while muconic acid measurements were useful for determining group exposures to benzene, they could not be used to monitor individual exposure because of the large variations in individual urinary muconic acid values. In addition, urinary muconic acid excretion was suppressed by coexposure to toluene. 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 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, and female nonpregnant and pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher, respectively, 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 ( $p=0.001$ ), nonpregnant ( $p=0.001$ ) and pregnant smokers ( $p=0.002$ ). Mean concentrations of muconic acid levels in female nonpregnant smokers are similar to those of male smokers. Mean concentrations of muconic acid in groups of 42 male smokers and 53 female nonpregnant smokers were  $0.22\pm 0.03$  and  $0.24\pm 0.02$  mg/g creatinine, or  $0.13\pm 0.06$  and  $0.13\pm 0.07$  mg/mg cotinine, respectively. Mean concentrations of muconic acid in groups of 63 pregnant smokers and 53 nonpregnant female smokers were  $0.27\pm 0.04$  and  $0.24\pm 0.02$  mg/g creatinine, or  $0.24\pm 0.06$  and  $0.13\pm 0.07$  mg/mg cotinine, respectively. Mean concentrations of urinary cotinine in pregnant smokers were significantly lower than in the group of nonpregnant smokers ( $1.13\pm 0.12$  mg/g creatinine compared to  $1.82\pm 0.14$  mg/g creatinine).

Recently, a sensitive gas chromatography/mass spectrometry (GC/MS) assay was developed to detect and quantitate muconic acid in the urine after benzene exposure (Bechtold and Henderson 1993; Bechtold et al. 1991). The detection limit of the assay is also greater, and muconic acid at levels greater than 100 ng/mL can be detected. A high performance liquid chromatography method also exists, and has been used to detect muconic acid in urine (Schad et al. 1992). Although muconic acid is used as a marker for benzene exposures, muconic acid in the urine can also result from ingestion of sorbic acid, a common food preservative (Ducos et al. 1990).

Another urinary metabolite of benzene is *S*-phenyl-*N*-acetyl cysteine (PhAC) (Jongeneelen et al. 1987). No background excretion of PhAC was found in rats or in humans. However, the authors concluded that biological monitoring of industrial exposure to benzene by the determination of PhAC in the urine is not better than the determination of phenol in urine. The work by van Sittert et al. (1993) in refinery workers indicates a strong correlation between 8-hour exposure to airborne benzene at concentrations of 0.3 ppm and higher) and urinary PhAC concentrations in end of shift samples. A strong correlation was also found between urinary phenol and PhAC concentrations. Similar results with regard to benzene concentrations in air and blood, and urinary concentrations of muconic acid and PhAC were observed by Popp et al. (1994) in car mechanics. PhAC has also been suggested as a useful biomarker in both humans and animals by Stommel et al. (1989). As noted above, ACGIH intends to change the BE1 to 25  $\mu\text{g}$  PHAC/g creatinine in urine (ACGIH 1996b). In a recent study, a very sensitive gas chromatography/mass spectroscopy assay was developed to quantify the binding of the benzene metabolite benzene-oxide to cysteine groups in hemoglobin (Bechtold and Henderson 1993; Bechtold et al. 1992a). Using this assay, the hemoglobin adduct *S*-phenylcysteine was detected in the blood of mice and rats exposed by inhalation or by gavage to benzene. Further work necessary

for the application of this method to humans is in progress. *S*-phenylcysteine was not detected in the hemoglobin of women exposed to up to 28 ppm benzene in a rubber factory (Bechtold et al. 1992a). However, albumin adducts of *S*-phenylcysteine were detected in these women, and levels were linearly correlated with exposure, although the data showed great variability (Bechtold et al. 1992b). An immunoassay has also been developed to detect hemoglobin adducts formed after benzene exposure (Grassman and Haas 1993). Hemoglobin adducts formed with hydroquinone, catechol, and trihydroxybenzene were detectable in mice treated with radiolabeled compound. Further development of this assay is needed to prove its usefulness as a tool to detect exposure to benzene. Hemoglobin adducts may be useful in evaluating cumulative exposure to benzene.

Ong et al. (1995) evaluated the 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).

Benzene is partially excreted in expired air, and breath levels have been used as a measure of exposure. An early study on humans found that benzene could be detected in expired air 24 hours after a 100 ppm exposure (Hunter 1468). Additionally, this study was among the first to suggest that it may be possible to extrapolate back to the concentration in the inspired air. However, the amount of benzene in expired air varies, and benzene is not exhaled in large quantities until the capacity of the body to metabolize benzene is exceeded. ACGIH has established BEIs for benzene in exhaled air as 0.12 ppm for end-exhaled air and 0.08 ppm for mixed-exhaled air prior to next workshift (ACGIH 1996b). Monitoring of expired benzene as a marker of exposure is still being explored (Pekari et al. 1992).

Comparative studies of residents in urban and rural areas revealed higher levels of benzene in the expired air of urban dwellers (Wester et al. 1986). For nonsmokers, benzene breath levels were  $2.5 \pm 0.8$  ppb in the urban area and  $1.8 \pm 0.2$  ppb in the rural area. However, in both cases breath levels were higher than ambient air levels ( $1.4 \pm 1.1$  and  $1.0 \pm 0.1$  ppb, respectively), suggesting that there were other sources of exposure to benzene.

In another study, benzene levels measured in the breath of occupationally exposed workers 16 hours after exposure ended were elevated as compared to benzene levels in the breath of people not occupationally exposed to benzene (Brugnone et al. 1989). A group of coal tar distillation workers with TWA exposure to benzene between 0.2 and 4.1 ppm had measurable benzene vapor in their breath 16 hours after exposure and showed a progressive build-up of benzene in their expired air during the working week (Money and Gray 1989).

Benzene levels in blood and expired air were determined in a population of 168 men divided into 4 groups: blood donors, hospital staff, and chemical workers with and without benzene exposure (Brugnone et al. 1989). Workers with a TWA benzene exposure of 0.4 ppm had measurable blood and alveolar concentrations of benzene that were significantly different from the other 3 groups. However, blood levels would be expected to provide a more accurate assessment of internal dose and, thus, a more accurate prediction of target organ effects than other monitoring end points. In addition, baseline levels of benzene in the blood of environmentally sensitive individuals not known to have exposure to benzene have been estimated. These could provide a baseline for monitoring exposure to benzene (DeLeon and Antoine 1985). Similar determinations have been made with smokers and nonsmokers (Angerer et al. 1991). Determination of benzene in postmortem blood has been used to determine glue-sniffing as the cause of death (Chao et al. 1993).

As discussed in Chapter 6, the primary method of analyzing benzene in body fluids and tissues is gas chromatography (GC) in conjunction with either mass spectrometry (MS), photoionization detection (PID), or flame ionization detection (FID). For detection of benzene metabolites, both GC/FID and high-performance liquid chromatography (HPLC) with ultraviolet detection (UV) have been used. A recent article describes the development of simple and sensitive methods for determination of blood and urinary benzene levels using gas chromatography headspace method (Kok and Ong 1994).

The presence of benzene metabolite-DNA adducts may be useful as a biomarker of exposure. DNA adducts with benzene metabolites have been found after benzene exposure (Hedli et al. 1990; Lutz and Schlatter 1977; Reddy et al. 1989a). However, the feasibility of using DNA adducts themselves as biomarkers of exposure is not clear at this time. Norpoth et al. (1988) found that degradation products of DNA adducts could be detected in the urine of rats after high-dose exposure. Assay of these degradation products may prove to be a more useful method of biomonitoring benzene exposure.

### 2.6.2 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  $4,000/\text{mm}^3$  or erythrocyte counts fell below  $4,000,000/\text{mm}^3$  (ITII 1975; OSHA 1987b). 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. Additional studies planned by Hayes seek to define the role of benzene-related leukopenia in the disease process initiated by benzene exposure (Hayes 1992). Leukocyte alkaline phosphatase (LAP) activity was increased in benzene workers exposed to about 31 ppm for a chronic time period (Yin et al. 1982). 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. 1990; 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 cell are being used more often but are not yet diagnostic. 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. (1990) 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 (Bode 11 et al. 1993; Chen et al. 1994; Lagorio et al. 1994a; Reddy et al. 1989a).

## 2.7 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.

Agents that alter benzene metabolism may also modify benzene toxicity. Ethanol and benzene induce the formation of the hepatic cytochrome P-450 isoenzyme, CYP2E1, in rabbits and rats (Gut et al. 1993; Johansson and Ingleman-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). When ethanol was administered to benzene-treated mice, it increased the severity of benzene-induced anemia, lymphocytopenia, and bone marrow aplasia and produced transient increases in normoblasts and peripheral blood atypia (Baarson et al. 1982). The modulating effects of benzene were dose dependent. 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). 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 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 per 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  $\mu\text{mol}$ ) 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 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).

## 2.8 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 may result in reduced detoxification or excretion of benzene, or compromised function of target organs affected by benzene. Populations who are at greater risk due to

their unusually high exposure to benzene are discussed in Section 5.6, Populations With Potentially High Exposure.

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.

Individual susceptibility is one of the most important variables in the development of chronic benzene toxicity. Aksoy et al. (1976) suggested familial susceptibility as one of the main factors in the frequency of chronic benzene toxicity. Two patients with pancytopenia associated with chronic exposure to benzene were cousins. One of these two patients died of aplastic anemia; the son of this patient presented with leukopenia a short time after exposure to this chemical. Genetic polymorphism with regard to the P-450 enzymes that metabolize benzene to its reactive metabolites may render some individuals at higher risk to the toxic effects of benzene (Kato et al. 1995). Although there are possible indications of genetic susceptibility to benzene, it is believed that all humans are susceptible to the pancytopenic effects of this agent.

There is a possible relationship between thalassemia (abnormal hemoglobins) and exposure to benzene. Aksoy (1989) presented a series of 44 pancytopenic patients, 4 of which had  $\beta$ -thalassemia heterozygotes. Two of these four patients had high levels of fetal hemoglobin, while a third patient had pseudo-Pelger-Huet anomaly. Thus, some forms of  $\beta$ -thalassemia may increase the deleterious effects of benzene on the hematopoietic system.

The occurrence of aplastic anemia in chronic benzene toxicity may be accelerated in individuals with viral hepatitis (Aksoy 1989). Furthermore, children and fetuses may be at increased risk-because their hematopoietic cell populations are expanding and dividing cells are at a greater risk than quiescent cells.

*In vitro* studies of benzene-caused chromosomal aberrations in human lymphocytes taken from people with different health practices suggests that “unhealthy lifestyles,” including smoking, excessive

alcohol consumption, inadequate physical exercise and sleep, excessive stress, and inadequate nutritional balance, could make cells more sensitive to the production of chromosomal aberrations after exposure (Morimoto et al. 1983).

## 2.9 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 references provide specific information about treatment following exposures to benzene: "Benzene" in *Occupational Medicine*, 3rd edition, 1994, Mosby, St. Louis, Carl Zenz et al., eds.; "Benzene" in *Goldfrank's Toxicologic Emergencies*, 5th edition, 1994, Norwalk, C.K. Aaron and M.A. Howland; "Benzene and the Aromatic Hydrocarbons" in *Clinical Management of Poisoning and Drug Overdose*, 2nd edition, 1990, W.B. Saunders, Philadelphia, L.M. Haddad and J.F. Winchester, eds.; *Benzene Contamination in Well Water*, Perdido, Alabama, CEH, 1983.

### 2.9.1 Reducing Peak Absorption Following Exposure

Human exposure to benzene can occur by inhalation, oral, or dermal contact. 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 and Winchester 1990; HSDB 1996; 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 and Winchester 1990; HSDB 1996; 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 and Winchester 1990; HSDB 1996; 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; Ellenhom and Barceloux 1988; Haddad and Winchester 1990; 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 unless the patient is or

could rapidly become obtunded, comatose, or convulsive (Ellenhorn and Barceloux 1988; HSDB 1996); however, other sources do not recommend the use of emetics because of the risk of aspiration pneumonitis (Bronstein and Currence 1988; Haddad and Winchester 1990). Some sources recommend gastric lavage if indicated (Haddad and Winchester 1990; Kunisaki and Augenstein 1994;). While lavage may be useful, induction of emesis should be regarded with great caution because of the rapid onset of central nervous system depression. 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 1996; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). Diazepam and phenytoin may be helpful in controlling seizures (Bronstein and Currence 1988; HSDB 1996; Stutz and Janusz 1988). Administration of epinephrine or other catecholamines has not been recommended because of the possibility of myocardial sensitization (Haddad and Winchester 1990; HSDB 1996; Nahum and Hoff 1934).

### **2.9.2 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, 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.

### 2.9.3 Interfering with the Mechanism of Action for Toxic Effects

Since ethanol can increase the severity of benzene-induced hematotoxicity (Baarson et al. 1982; Nakajima et al. 1985) and ethanol and benzene are both considered strong nervous system depressants (Carpenter et al. 1944; Cornish and Ryan 1965) avoidance of ethanol following benzene exposure may help to reduce the toxic effects of benzene.

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. 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  $\alpha$  and IL-1  $\beta$  at concentrations of 5  $\mu$ 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 6 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 (6 per group) was administered intravenously with 60  $\mu$ 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.

The covalent binding of benzene metabolites to nucleic acids and proteins is thought to play a major role in the toxicity and carcinogenicity of benzene (Gill and Ahmed 1981; Irons et al. 1980; Longacre et al. 1981a, 1981b; Lutz and Schlatter 1977; Norpoth et al. 1988; Rushmore et al. 1984; Snyder et al. 1978b; Sun et al. 1990). In addition to the covalent binding to cellular macromolecules, several other mechanisms have been proposed to explain how benzene affects the bone marrow. These include suppression of RNA and DNA synthesis (Moeschlin and Speck 1967; Post et al. 1983, alkylation of cellular sulfhydryl groups (Irons 1985), disruption of the cell cycle (Irons et al. 1979; Snyder et al. 1981c), oxygen activation or free radical formation (Irons 1985), damage to stromal cells (Frash et al. 1976; Kalf et al. 1987), and loss of specific protein growth factors (Clark and Kamen 1987). Further studies to determine the significance and details of these possible mechanisms could lead to additional approaches for reducing the toxic effects of benzene. These approaches could include ways to reduce binding as well as a more direct interference with the toxic effects caused by benzene metabolites/macromolecule complexes.

Other mechanisms may also be involved in benzene toxicity and carcinogenicity. Such mechanisms may include synergism between metabolites (Eastmond et al. 1987; Snyder et al. 1989) or synergism between glutathione-depleting metabolites of benzene and oxygen radicals (Parke 1989). It may be possible to develop methods to interfere with these mechanisms as well. Work by Zhu et al. (1995) indicated that in mice and rats, both glutathione and quinone reductase were important in modulating the hematotoxicity of hydroquinone. Mice, which exhibit a greater susceptibility to benzene-induced hematotoxicity, had less glutathione in bone marrow stromal cells, compared to rats. Induction of endogenous glutathione and quinone reductase, or pretreatment of mice with glutathione ethyl ester provided dramatic protection against hydroquinone toxicity.

Benzene also affects the central nervous system. Effects noted include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness (Flury 1928; Greenburg 1926; Kraut et al. 1988; Yin et al. 1987b). Since benzene may induce an increase in brain catecholamines, it may also have a secondary effect on the immune system via the hypothalamus-pituitary-adrenal axis (Hsieh et al. 1988b). Increased metabolism of catecholamines can result in increased adrenal corticosteroid levels, which are immunosuppressive (Hsieh et al. 1988b). Further studies to determine the molecular mechanism of these effects could lead to additional approaches for reducing the toxic effects of benzene.

## **2.10 ADEQUACY OF THE DATABASE**

Section 104(i)(5) of CERCLA 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 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.

### **2.10.1 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 2-6. 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* (ATSDR 1989), is substance-specific

2. HEALTH EFFECTS

Figure 2-6. Existing Information on Health Effects of Benzene

	Systemic									
	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
Inhalation	●	●	●	●	●	●	●	●	●	●
Oral	●	●	●			●				●
Dermal	●	●								●

**Human**

	Systemic									
	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
Inhalation	●	●	●	●	●	●	●	●	●	●
Oral	●	●	●	●	●	●	●	●	●	●
Dermal		●	●							●

**Animal**

● Existing Studies

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 2-6, 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 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.

### 2.10.2 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 occupational exposure to more than 60 ppm benzene (Midzenski et al. 1992). Data for hematological effects in animals after acute-duration inhalation oral 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; 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 inhalation MRL of 0.05 ppm was determined based on the LOAEL for immunologic effects in the mouse (Rozen et al. 1984) (discussed below under Immunotoxicity). No acute oral studies were suitable for deriving MRLs. Additional animal or human data on hematological effects following acute oral or dermal exposure would be useful to provide threshold levels for populations living near hazardous waste sites. However, 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 intermediate 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- and chronic-duration exposures to benzene in the workplace (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1987; Townsend et al. 1978; Yin et al. 1987c). 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 effects (e.g., Baarson et al. 1984; Cronkite et al. 1982, 1985, 1989; Dow 1992; Farris et al. 1993; Green et al. 1981b; Luke et al. 1988b; Plappert et al. 1994a, 1994b; Seidel et

al. 1989b; Snyder et al. 1978a, 1980; Toft et al. 1982; Vacha et al. 1990; Ward et al. 1985; Wolf et al. 1956), but none were considered appropriate for calculating an MRL. An intermediate-duration inhalation MRL of 0.004 ppm was derived based on the LOAEL for neurological effects in mice (Li et al. 1992), which are discussed below under Neurotoxicity. 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 again, no intermediate-duration oral MRL could be derived because the threshold for hematological (and immunological) effects could not be identified. Additional animal or human data on hematological effects following intermediate oral or dermal exposure would be useful to provide threshold levels for populations living near hazardous waste sites. However, intermediate 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. Hematological toxicity was reported in studies of humans chronically exposed to benzene in the air in the workplace (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974, 1987; Cody et al. 1993; Doskin 1971; Erf and Rhoads 1939; Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Li et al. 1994; Townsend et al. 1978; Yin et al. 1987c). No human data are available to evaluate hematological effects following oral exposure. Chronic animal studies are available for hematological effects following inhalation exposure (Snyder et al. 1978a, 1980, 1984) and oral exposure (Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986). No chronic inhalation or oral MRLs were derived. Although the study of occupational inhalation exposure to benzene by Tsai et al. (1983) defines a NOAEL for hematological effects, no MRL could be derived because the study did not address TWA exposure levels, there were no controls, and no follow-up was done, in addition to other deficiencies in the study design. In other chronic studies, the lowest LOAEL value was serious (pancytopenia) (Doskin 1971). No chronic-duration oral MRL was derived since the most extensive study found (NTP 1986) did not conclusively define a NOAEL or less serious LOAEL for end points that can be used to derive an MRL. Since the hematological system is a target organ, further human and animal data regarding the effects of benzene in the air and drinking water are important for the purposes of defining threshold levels for populations living near hazardous waste sites, and to derive MRLs. Dermal data for humans and animals were not available. However, chronic 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.

Benzene can induce leukemia. The leukemogenic potential of benzene for humans has been estimated from data from three separate occupational epidemiological studies and their analyses (Bond et al. 1986b; Infante 1977; Ott et al. 1978; Paxton et al. 1994a, 1994b; Rinsky et al. 1981, 1987; Wong et al. 1983). Additional studies of workers in China may be found in the literature (Li et al. 1994; Travis et al. 1994; Yin et al. 1987a, 1987b, 1989). The older data are limited because of the lack of complete records documenting benzene levels in workplace air, individual worker exposure histories, and other confounding factors. Newer data are more informative. Animal data show that benzene is leukemogenic following inhalation exposure (Cronkite et al. 1984; Snyder et al. 1984). AML is the most common leukemia associated with benzene exposure in humans (animals develop leukemia and lymphomas and other neoplasms in numerous tissues that have not been associated with benzene exposure in humans) (Crump 1994; Wong 1995; Wong and Raabe 1995). However, there is a need for more information on the mechanism of benzene induction of AML. For this, an appropriate animal model needs to be identified. Development of a suitable animal model to conduct mechanistic studies of benzene carcinogenesis has been suggested as a data need (Goldstein and Warren 1993). Additionally, there is a possibility that some of the metabolites of benzene may interact with each other to produce effects leading to toxicity or to cancer. More research is needed to define what these interactions are and how they enhance the toxicity and carcinogenicity of benzene. Data are sufficient to validate the carcinogenic potential of benzene in animals following inhalation exposure (Cronkite 1986; Cronkite et al. 1989; Maltoni et al. 1982a, 1982b, 1983; Snyder et al. 1980, 1984, 1988) and oral exposure (Huff et al. 1989; Maltoni et al. 1989; NTP 1986). Newer literature is addressing these issues (see Section 2.10.3, Ongoing Studies). Although the exact mechanism of benzene carcinogenicity is not known, 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. 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 do benzene metabolites produce greater-than-additive effects, what are the critical target genes, is aplastic anemia essential to the development of leukemia, and what is the role of cytokines and growth factor pathways in benzene toxicity. Bioassays using oral or dermal exposures in animals should provide quantitative data to make the establishment of the dose-response relationship more precise for each route. However, dermal exposure is not likely to be the most relevant route of exposure for humans at hazardous waste sites.

**Genotoxicity.** Evidence for the genotoxicity of benzene in humans comes from studies of occupationally exposed populations (Ding et al. 1983; Eastmond 1993; Forni et al. 1971a; Major et al. 1992; Popp et al. 1992; Sasiadek 1992; Sasiadek and Jagielski 1990; Sasiadek et al. 1989; Tompa et al. 1994; Tough and Court-Brown 1965; Tough et al. 1970; Yager et al. 1990; Yardley-Jones et al. 1990). 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. 1991a; Erexson et al. 1986; Fujie et al. 1992; Kolachana et al. 1993; Siou et al. 1981; Toft et al. 1982; Ward et al. 1992). 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; Lakhanisky 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).

There are currently no reliable data on the dose-response relationship of benzene exposure and chromosomal effects. In light of the data of Ward et al. (1992), further investigation of mutational effects at low doses seems appropriate. Additional data on the quantitative relationship between measured exposures and clastogenic effects might provide an alternative biomarker of effect.

**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). 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 weight and/or minor skeletal variations at concentrations  $\geq 47$  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. 1982; 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. 1984b; 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; Yin et al. 1982), and chronic (Snyder et al. 1980, 1984, 1988) periods. An acute 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 inhalation MRL has been derived based on this study. No human data on the oral route were available, but animal data showed immunological 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; Davies 1929; 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). Acute oral exposures of 950 mg/kg benzene altered neurotransmitter levels in the brains of rats (Kanada et al. 1994). Intermediate oral exposures of  $\geq 8$  mg/kg/day resulted in changes in the levels of monoamine transmitters in the brain without treatment-related behavioral changes (Hsieh et al. 1988a). An intermediate-duration inhalation study found increased rapid response in mice at 0.78 ppm (Li et al. 1992), and an intermediate-duration MRL has been derived from this LOAEL.

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 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 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 case studies on humans are occupational studies and associated analysis (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974; Baslo and Aksoy 1982; Bond et al. 1986a; Ciccone et al. 1993; Cody et al. 1993; Dosemeci et al. 1994; Doskin 1971; Crump 1994; Infante 1978; Infante et al. 1977; Kipen et al. 1989; Ott et al. 1978; Paustenbach et al. 1992; Paxton et al. 1994a, 1994b; Rinsky et al. 1981, 1987; Travis et al. 1994; Utterback and Rinsky 1995; Wong 1987b; Wong

et al. 1983; Yin et al. 1989, 1994). 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 (ATSDR 1995). Although no causal relationship has been proposed for health conditions identified in the subregistry, continued follow-up of the population is planned to provide information on health effects of environmental exposure to benzene. Hematopoietic and immune system end points are fairly easy to measure and could provide information on the possible progression of the disease to leukemia and lymphoma in humans.

Few well conducted epidemiology studies of exposed populations exist. A recently published epidemiology 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 dumping history, 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 recent studies include the series describing workers in China (Dosemeci et al. 1994; Yin-et al. 1987c, 1994), and reanalysis of data from the Pliofilm cohort (Crump 1994; Paxton et al. 1994a, 1994b; Utterback and Rinsky 1995). A recent publication by Liroy and Pellizzari (1995) outlines an appropriate framework for the National Human Exposure Assessment Survey, using benzene as a candidate compound. Other recent 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.

Most, but not all studies of chromosomal damage have been done on workers with preexisting hematological effects and cancer (Ding et al. 1983; Forni et al. 1971a; Picciano 1979; Yardley-Jones et al. 1988). Chromosomal aberration data could be useful in monitoring possible adverse effects in individuals living near hazardous waste sites if more quantitative data were available regarding the relationship of dose to clastogenic effect.

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, but they are not unique to benzene exposure. Estimates of benzene exposure can be made by comparing the ratio of inorganic to organic sulfates in the urine (Hammond and Herman 1960). However, urinary sulfate levels are variable, and they have not been used to identify exposure levels of benzene associated with minimal toxic effect. Other more specific biomarkers, including adducts with hemoglobin and albumin, in addition to benzene levels, themselves, may be useful as biomarkers (Bechtold et al. 1992a, 1992b; Bechtold and Henderson 1993; Pekari et al. 1992; Schad et al. 1992).

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 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-phenyl-N-acetyl cysteine (PhAC), the proposed urinary BE1 (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. 1989; Lee et al. 1993; Melikian et al. 1993; Ong and Lee 1994; Witz et al. 1990). 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. 1989). 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; Nomiyama and Nomiyama 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). Ongoing epidemiological studies are currently focusing on this data need (see Section 2.10.3).

**Effect.** Monitoring of benzene workers has included monthly blood counts. Toxic effects occur in the bone marrow and are 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). Ongoing studies are currently focusing on this data need (see Section 2.10.3).

**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; Nomiyama and Nomiyama 1974a; Sabourin et al. 1987; Schrenk et al. 1941; Srbova et al. 1950). 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 1953; 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 classified as a substrate of both cytochrome P-448 and cytochrome P-450 (Parke 1989). Within the cytochrome P-450 family, benzene is a preferential substrate of cytochrome P-450ALc (P450IIE). This same family of cytochromes also

metabolizes alcohol. The induction of cytochrome P450IIE by benzene with subsequent generation of oxygen radicals, circulating lipid peroxides, and hydroxyl radicals could be associated with hematopoietic toxicity and carcinogenicity of benzene (Parke 1989). Metabolites have been identified consequent to cytochrome P-450 induction by benzene (Henderson et al. 1989). However, it would be of value to know if reconstituted cytochromes P-450I, P-450IIB, and P-450IIE metabolize benzene to different products. Cytochromes P-450 are not confined to the liver, but are ubiquitous to all tissue. Benzene metabolism in bone marrow is not well understood; additional data regarding the initial oxidation step and the comparatively low levels of cytochrome P-450 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. Ongoing studies are currently focusing on problems in toxicokinetics (see Section 2.10.3).

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. More information from animal model systems is needed on excretion following oral and dermal exposure to benzene.

**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 2.10.3).

**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. Current lines of investigation in other areas include the use of non-steroidal anti-inflammatory drugs to block prostaglandin and prostaglandin synthetase-mediated activity after benzene exposure, and the support of IL-1 cytokine activity to prevent depression of hematopoiesis.

### 2.10.3 Ongoing Studies

Ongoing studies were found in abstract form in the open literature. These studies include the investigation of hemopoietic effects in adult mice at low exposures (Fart-is et al. 1995), strain differences in metabolism (Grotz and Witz 1995), analysis of benzene metabolites and glutathione in the bone marrow of mice (Henderson et al. 1995), comparison of pulmonary and hepatic-microsomal metabolism of benzene in rats (Chaney and Carlson 1995), chromosomal aberrations or DNA repair in exposed humans (Anderson et al. 1995; Hallberg et al. 1995; Mills et al. 1994; Smith et al. 1995), DNA adduct formation (Hedli et al. 1995), effects of chronic, low dose benzene on liver enzymes in mice (Daiker et al. 1995), effects of hydroquinone on T-cell proliferation (Li et al. 1995), and influence of variations in metabolism on benzene toxicity (Seaton et al. 1995). Weisel and Yu (1995)

are studying exhaled breath of benzene and urinary muconic acid after environmental benzene exposure in humans. Lox (1995) is investigating effects in coagulant activity in male rats after inhalation exposure to benzene, as a marker of pathogenesis. Additional work is being conducted on the PBPK model for benzene and its metabolites (Kenyon and Medinsky 1995). Current research aimed at substituting glucose for benzene as a starting material in many industrial applications may eventually limit worker exposure to benzene (Black 1995). The most recent ongoing research spans many different approaches, and includes deriving potency factors for benzene and some of its metabolites (Davidson and Faust 1996); cell-specific metabolism of benzene in progenitor cells in human bone marrow (Ross et al. 1996); hematotoxicity of 1,2,4-benzenetriol in mice (Hedli et al. 1996); use of protein adduct in rat blood to study reactive intermediates of benzene (Yeowell-O'Connell et al. 1996); inhibition of human topoisomerase II by the benzene metabolite diphenoquinone (Frantz and Eastmond 1996); benzene metabolism and toxicity in transgenic mice (Valentine et al. 1996); effects of benzene and unleaded gasoline coexposures on benzene metabolism and toxicity (Seaton et al. 1996); mechanistic studies of benzene toxicity related to DNA-protein crosslinking (Schoenfeld et al. 1996); effects of benzoquinone, a benzene metabolite, on human T-cell function (Geiselhart and Freed 1996); the role of iron in hydroquinone and catechol-induced inhibition of lymphoblast proliferation (Li and Freed 1996); and molecular cytogenetics of humans exposed to benzene (Smith et al. 1996).

Recent symposia have addressed the subject of benzene toxicity. "Benzene '95," an international conference on the toxicity, carcinogenesis, and epidemiology of benzene, was held June 17-20, 1995, at Rutgers University, New Brunswick, New Jersey. Proceedings from the symposium, to be published in *Environmental Health Perspectives*, were in press during the development of this profile. Examination of the symposium program indicates that the field of benzene toxicity is moving rapidly. Specific areas of interest include toxicity and leukemogenesis, chromosomal and genetic damage, metabolism, reactive intermediates, exposure assessment, results of human exposure studies in China and Europe, pharmacokinetics and modelling, and risk assessment. Areas of investigation highlighted in the symposium may be summarized in part below.

Epidemiological studies included surveys of workers in China (Hayes 1996; Linet et al. 1996; Rothman et al. 1996; Yin et al. 1996), and in other areas (Raabe and Wong 1996; Rushton 1996). Leukemia mortality and biomarkers were the focus of these investigations. Snyder and Hedli (1996) presented an overview of benzene metabolism, and benzene metabolism in different species was

discussed by Henderson (1996). Additional work on benzene metabolism addressed cell-specific activation and detoxification in mouse and human bone marrow (Ross et al. 1996), phase II metabolism of benzene (phenylsulfate and phenylglucuronide) (Schrenk et al. 1996), reactive ringopened aldehyde metabolites in benzene toxicity (Witz et al. 1996), and the role of cytochromes P-450 (CYP2E1 and CYP2B1) in benzene metabolism and toxicity (Gut et al. 1996). Kinetic modeling was a focus of several presentations, including incorporation of mechanistic information into a PBPK model (Medinsky et al. 1996), and model fitting to human data (Bois et al. 1996). DNA adduct formation after benzene exposure was studied in B6C3F<sub>1</sub> mice (Bode11 et al. 1996; Li et al. 1996), and *in vitro* (Bleasdale et al. 1996). Work defining the mechanism of toxicity of benzene includes the impact of metabolites on bone marrow progenitor cells (Irons et al. 1996), inhibition of human topoisomerase II by hydroquinone and other metabolites (Frantz et al. 1996; Hutt and Kalf 1996), and the effect of benzene and its metabolites on the hemopoietic system (Abraham 1996; Farris et al. 1996; Hazel et al. 1996; Laskin and Laskin 1996). Effects of benzene exposure on DNA and other cytogenetic effects were also an important area of discussion (Angelosanto et al. 1996; Forni 1996; Smith et al. 1996; Tunca and Egeli 1996; Zhang et al. 1996); Weisel et al. (1996) are looking at the practical application of using benzene in exhaled breath and urinary muconic acid as biomarkers of environmental exposure to benzene in tobacco smoke. Exhaled breath concentrations of benzene were used to validate a PBPK model. Similar research is ongoing at DOE's Pacific Northwest Laboratories, where a mass spectrometric procedure with nearly instantaneous results has been developed (Thrall 1997). Additional work on biomarkers includes monitoring S-phenylcysteine in albumin (Bechtold et al. 1996), a comparison of S-phenyl mercapturic acid muconic acid in the urine (Boogaard et al. 1996), and formation of phenyl guanine found in the urine (Norpoth et al. 1996). Risk estimation was addressed by Cox (1996), Ward et al. (1996), Paxton (1996), and Crump (1996).

Ongoing studies regarding the health effects of benzene were also reported in the FEDRIP (1996) and the CRISP Data Base of National Institutes of Health. These studies may further elucidate the mechanism of action of benzene and further elaborate on the toxicokinetics and intermediate metabolism of benzene. These research efforts may add to our ability to perform more accurate risk assessments for benzene. These research grant studies are listed in Table 2-7. In addition, 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; continued follow-up of the population is planned to provide information on health effects of environmental exposure to benzene (ATSDR 1995).

## 2. HEALTH EFFECTS

**Table 2-7. Ongoing Studies on the Health Effects of Benzene**

Investigator	Affiliation	Research description	Sponsor
Smith, M.T.	Univ of California Warren Hall, Berkeley, CA	Biomarkers of benzene exposure and genotoxicity	NIEHS
Freed, B.M.	Albany Medical College Albany, NY	Immunotoxicology of benzene metabolites	NIEHS
Lawrence, D.A.	Wadsworth Center for Labs and Research Albany, NY	Hematotoxic effects of benzene and doxorubicin	NIEHS
Ross, D.	University of Colorado Health Sciences Center Denver, CO	Metabolic basis of benzene-induced myelotoxicity	NIEHS
Witz, G.	University of Medicine and Dentistry of New Jersey Piscataway, NY	Muconaldehyde in relation to benzene hematotoxicity	NIEHS
Smith, M.T.	University of California Berkeley, CA	Biomarkers of genetic damage in human cells	NIEHS
Wiencke, J.K.	University of California Berkeley, CA	Molecular biomarkers of occupational benzene exposure	NIEHS
Bodell, W.J.	University of California Berkeley, CA	DNA adducts as molecular dosimeters of genotoxins	NIEHS
Melikian, A.A.	American Health Foundation Valhalla, NY	Biological markers for tobacco smoke exposure to benzene	NCI
Hoffmann, D.	American Health Foundation	Experimental tobacco carcinogenesis	NCI
Christiani, D.	Harvard University Boston, MA	Petrochemical exposure and reproductive outcomes	NIOSH
Naughton, G.	Marrow Tech Inc.	<i>In vitro</i> models for hematotoxicity and hepatotoxicity	DHHS
Fischer, L.	Michigan State Univeristy East Lansing, MI	Health hazards from groundwater contamination	NIEHS
Turteltaub, K.	University of California Berkeley, CA	Protein and DNA adducts following low dose exposure by accelerator mass spectrometry	NIEHS
Kalman, D.	University of Washington Seattle, WA	Dosimetry assessment of exposure to volatile compounds	NIEHS

## 2. HEALTH EFFECTS

**Table 2-7. Ongoing Studies on the Health Effects of Benzene (continued)**

Investigator	Affiliation	Research description	Sponsor
Anthony, D.C.	Brigham & Women's Hospital	Hemoglobin adducts—dosimeter of internal exposure	NIEHS
Burlingame, A.L.	University of California Berkeley	Detection and characterization of protein and DNA adducts by mass spectrometry	NIEHS
Omiecinski, C. J.	University of Washington	Fingerprinting of cytochrome P-450 profiles as biomarkers of chemical exposure	NIEHS
Kavanaugh, T. J.	University of Washington	Glutathione biosynthesis as a biomarker of toxic exposure	NIEHS
Carlson, G.P.	Purdue University	Ethanol and xenobiotic metabolism and toxicity in lung	NIEHS
Smith, A.	University of California Berkeley	Mutagenesis and carcinogenesis	NIEHS
Adams, D. O.	Duke University	Immunotoxicology—environmental agents and macrophages	NIEHS
King, M.	University of California Berkeley	Human genetics, environmental interactions	NIEHS
Sehnert, S.S.	Johns Hopkins University	Pollutant-particle-lung surfactant interactions	NIEHS
Ehrlich, M.	Tulane University of Louisiana	Sensitive assays to assess various types of genotoxicity	NIEHS
Rappaport, S.M.	University of North Carolina	Development and application of biomarkers of exposure	NIEHS
Hertz-Picciotto, I.	University of North Carolina	Core—epidemiology core	NIEHS
Benjamin, S.A.	Colorado State University	Chemical mixtures as promoters of hepatocarcinogenesis	NIEHS
Yang, R.S.H.	Colorado State University	Toxicological interaction studies in chemical mixtures—pharmacokinetics	NIEHS

## 2. HEALTH EFFECTS

**Table 2-7. Ongoing Studies on the Health Effects of Benzene (continued)**

Investigator	Affiliation	Research description	Sponsor
Billings, R.E.	Colorado State University	Mechanisms of toxic chemical interaction in the liver—hepatotoxicity	NIEHS
Lowndes, H. E.	Rutgers State University	Glutathione conjugation and its role in neurotoxicity	NIEHS
Barrett, J.	NIEHS	Role of mutagenesis in carcinogenesis	NIEHS
French, J. E.	NIEHS, NIH	Karyotype of malignant skin tumors of tg.ac (zeta-globin promoted V-ha-ras) mice	NIEHS
Singer, B.	University of California-Lawrence Berkeley Laboratory	Chemical basis of carcinogenic risk from benzene	NIEHS
Spalding, J. W.	NIEHS, NIH	A transgenic mouse model for dermal carcinogenesis	NIEHS
Blot, W.	NCI	Analytical cancer studies, including field studies in high-risk areas	Division of Cancer Etiology
Irons, R.	University of Colorado Denver, CO	Mechanism(s) of chemical leukemogenesis	NIEHS
Henderson, R.	Inhalation Toxicology Research Institute Albuquerque, NM	Evaluation of bone marrow metabolites for dosimetry and biomarkers	American Petroleum Institute
Irons, R.	University of Colorado Denver, CO	Biological mechanism of benzene leukemogenesis	American Petroleum Institute
Kalf, G.	Jefferson Medical College Philadelphia, PA	Influence of hydroquinone on benzene leukemogenesis	American Petroleum Institute
Bechtold, W.F.	Inhalation Toxicology Research Institute Albuquerque, NM	Use of benzene metabolite adducts with (S-phenylcysteine) as a marker of exposure	Health Effects Institute
Melikian, A.A.	American Health Foundation	Quantification of urinary metabolites; metabolic activation pathways	Health Effects Institute
Turteltaub, K.W.	Livermore National Laboratory and University of California at San Francisco San Francisco, CA	<i>In vivo</i> metabolic studies; characterization of DNA and protein adducts in bone marrow	Health Effects Institute

## 2. HEALTH EFFECTS

**Table 2-7. Ongoing Studies on the Health Effects of Benzene (continued)**

Investigator	Affiliation	Research description	Sponsor
Eastmond, D.A.	University of California Riverside, CA	Chromosomal alterations induced in mice and humans	Health Effects Institute
Eastmond, D.A.	University of California	Chromosomal mechanisms in environmental carcinogenesis	U.S. Department of Agriculture
Cross, T.A.	Florida State University	Protein stability—catalytic and noncatalytic solvents	National Institute of General Medical Sciences
Trager, W.F.	University of Washington	Isotope Effects— cytochrome P-450 catalyzed oxidations	National Institute of General Medical Sciences
Hanzlik, R.P.	University of Kansas	Mechanisms of P-450 catalyzed C oxidations	National Institute of General Medical Sciences
Grimes, P.	Charles R. Drew University	Phenolic chemicals and viral agents in the pathogenesis of immune abberations	National Institute of General Medical Sciences
Gonzalez, F. J.	NCI, NIH	Transgenic mice, gene knockout mice, and cytochrome P-450 function	Division of Cancer Etiology
Shields, P. G.	NCI, NIH	Molecular epidemiology of human lung cancer	Division of Cancer Etiology
Nims, R. W.	NCI, NIH	Mechanisms and interspecies differences in tumor promotion	Division of Cancer Etiology
Poirier, M. C.	NCI, NIH	Use of immunological techniques to study the interaction of carcinogens with DNA	Division of Cancer Etiology
Gail, M. H.	NCI, NIH	Consulting on epidemiologic methods	Division of Cancer Etiology

DHHS = Department of Health and Human Services; ORD = Office of Research and Development; NCI = National Cancer Institute; NIEHS = National Institute of Environmental Health; NIH = National Institutes of Health; NIOSH = National Institute for Occupational Safety and Health