CHAPTER 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 on the toxicology of benzene. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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

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 by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to benzene, but may not be inclusive of the entire body of literature. A systematic review of the scientific evidence of the health effects associated with exposure to benzene was also conducted; the results of this review are presented in Appendix C.

Animal inhalation studies are presented in Table 2-1 and Figure 2-2, animal oral studies are presented in Table 2-2 and Figure 2-3; and animal dermal studies are presented in Table 2-3.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The alpha-numeric identifier for each point in the LSE figures identifies the specific study number in the corresponding LSE table and test species (e.g., 2R refers to study number 2 conducted in rats). The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. Effects have been classified into “less serious LOAELs” or “serious LOAELs (SLOAELs).” “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 endpoint 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 endpoints. 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. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of benzene are indicated in Table 2-1 and Figure 2-2 (inhalation) and Table 2-2 and Figure 2-3 (oral).

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

The health effects of benzene have been extensively studied in human and laboratory animals. These studies provide a preponderance of evidence that the primary target for benzene toxicity is hemopoietic tissues (bone marrow, spleen, thymus). Benzene disrupts hematopoiesis, leading to decreased numbers of peripheral lymphocytes and suppressed immune function of lymphocytes. Benzene also produces genotoxicity in hematopoietic stem cells and progenitor cells that leads to bone marrow failure, myelodysplastic syndromes, and AML. Toxicity and genotoxicity of benzene results from reactive metabolites of benzene formed in hematopoietic tissues, as well as in liver and other tissues. The primary enzymes involved in generating reactive metabolites of benzene include cytochrome P450 2E1 (CYP2E1), myeloperoxidase (MPO), and NAD(P)H:quinone oxidoreductase (NQO1), although other enzymes are also involved. The major systems affected by exposure to benzene include the following:

- ***Hematological:*** The primary effect of benzene on the hematological system is disruption of hematopoiesis. The following hematological effects have been observed in humans and laboratory animals in association with exposure to benzene: (1) decreased numbers of peripheral blood cells (erythrocytes, thrombocytes, leukocytes); (2) decreased numbers of hematopoietic stem cells and progenitor cells in hematopoietic tissues (bone marrow, spleen); (3) decreased

cellularity of hematologic tissues (bone marrow, spleen, thymus); and (4) histopathological changes to hematopoietic tissues (bone marrow, spleen, thymus).

- ***Immunological:*** Benzene decreases the number of peripheral lymphocytes through the disruption of hemopoiesis, which contributes to immunosuppression. Studies conducted in laboratory animals show that that exposure benzene can alter immune responses to antigens, function of peripheral lymphocytes, and levels of circulating antibodies.

- ***Developmental:*** Results of developmental studies in laboratory animals have reported decreased fetal weight, increased skeletal variations, alterations in hematological parameters, neurodevelopmental effects, and altered glucose homeostasis. However, human data are inadequate verify or refute findings in animals. Note that developmental effects were not considered for systematic review as the LOAEL values for developmental effects were higher than those for hematological effects.

- ***Cancer:*** Studies conducted in workers have shown that exposure to benzene is associated with increased risk of myelodysplastic syndromes and AML. Studies in laboratory animals exposed to benzene induced tumors at multiple sites in rats and mice, with a tendency towards induction of lymphomas in mice.

The HHS has determined that benzene is a known human carcinogen (NTP 2021), IARC (2018) has placed benzene in Group 1 (carcinogenic to humans), and the EPA (IRIS 2003) has classified benzene as a Group A carcinogen (known human carcinogen).

The bulk of the epidemiological evidence for health effects of benzene derives from studies of workers. Numerous studies of worker populations (e.g., shoe manufacture, petrochemical, fuel handling and storage) have reported associations between benzene exposure and adverse health outcomes, primarily hematologic and hematologic cancer. Many of the worker studies have limitations that preclude their use in estimating exposure-outcome relationships. These limitations include lack of accurate exposure data, co-exposure to other chemicals, and lack of appropriate reference populations. In this profile, studies that provide quantitative estimates of associations between exposures to benzene and health effects are summarized in tables that identify the type of epidemiological design, the estimated exposure levels, the outcomes, and the direction of the association (e.g., decreasing peripheral leukocytes with increasing benzene exposure). Criteria for inclusion in these tables are: (1) reliable estimates of benzene exposure (measured levels in air or biomarker); (2) analysis of potential confounders of the measures of association; and (3) appropriate statistical analysis or measures of variance. Not included in the tables are numerous studies that provide qualitative evidence for associations; for example, studies that compare outcomes in exposed workers and a reference population with ambient level exposures and studies where the actual exposures to the workers were not reported or were highly uncertain (e.g., years worked). Studies of general populations exposed to ambient levels of benzene were reviewed and excluded from discussion in this profile for the following reasons. At ambient levels, concentrations of benzene tend to

be correlated with concentrations of other chemicals in emissions from fuels and fuel combustion (e.g., benzene, toluene, ethylbenzene, and xylenes [BTEX]; nitrogen dioxide [NO_2]; particulate matter <10 μm [PM_{10}]). These correlations introduce a major uncertainty into the interpretation of these studies because benzene exposures (measured as air concentrations or biomarkers) may be a surrogate variable for exposure to combustion-derived "air pollution" in general.

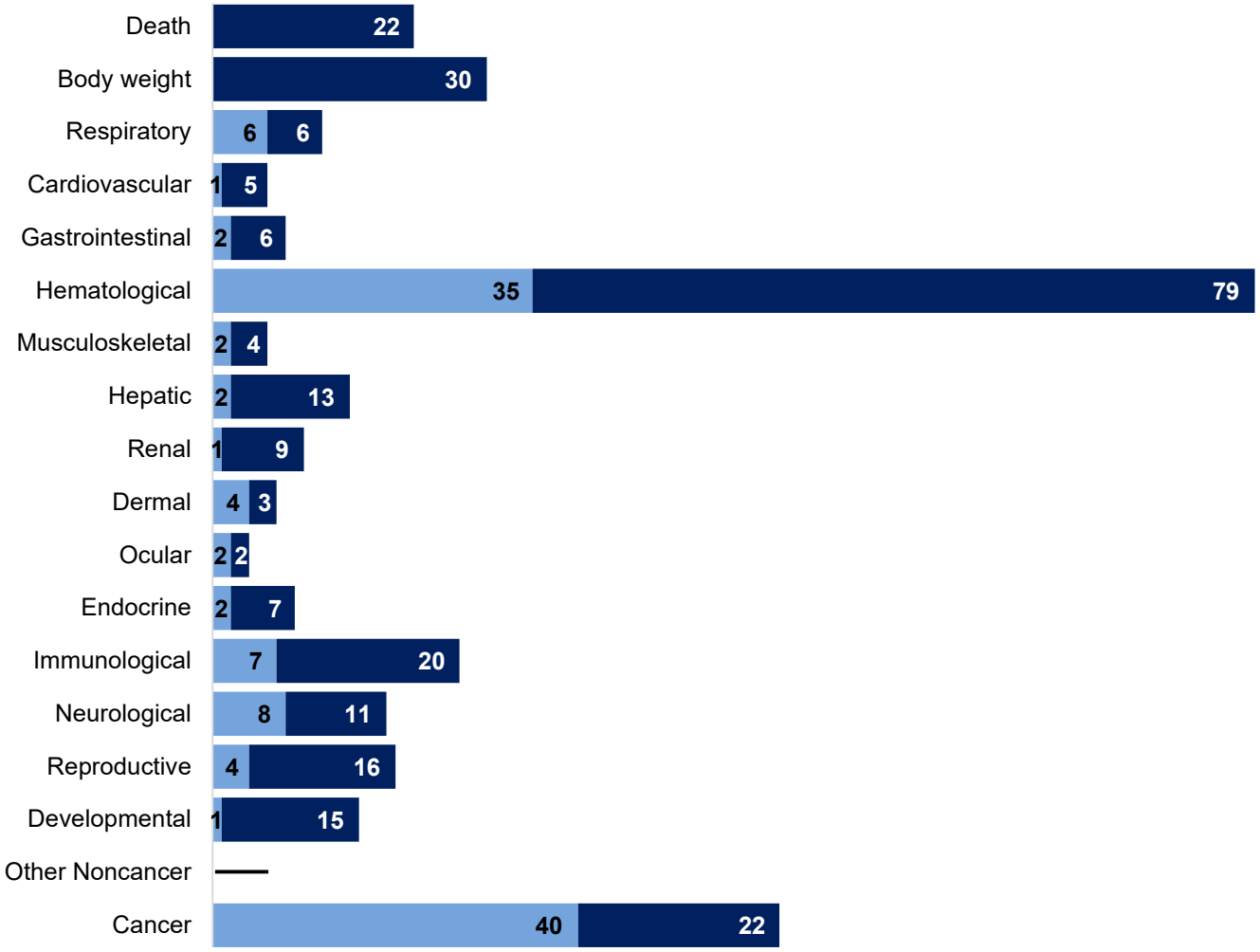
The toxicology of inhaled and oral benzene has also been studied extensively in mice and, to a lesser extent, in rats. These studies have confirmed the toxicity of benzene to hematopoietic tissues. Outcomes observed in animal studies include decreases in peripheral leukocytes and erythrocytes, decreases in hematopoietic stem and progenitor cells in hematopoietic tissues (e.g., marrow, spleen), hematopoietic tissue cytotoxicity, impaired lymphocyte function, impaired humoral and cellular immunity, and tumors of the hematopoietic and lymphoid tissues.

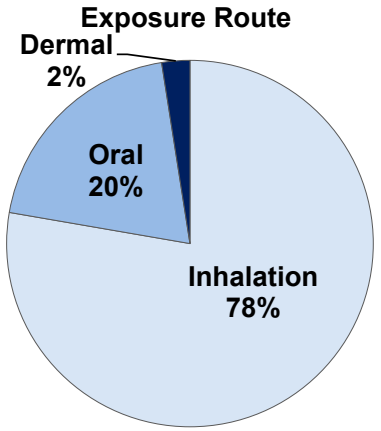
As illustrated in Figure 2-1, numerous human and animal studies evaluating adverse effects of benzene exposure were reviewed and included in this document. Most studies evaluated the effects of inhalation exposure, followed by oral exposure. The most studied endpoints include the hematological and immunological systems and cancer. Hematological and immunological effects are the most sensitive (i.e., occurred at the lowest exposures).

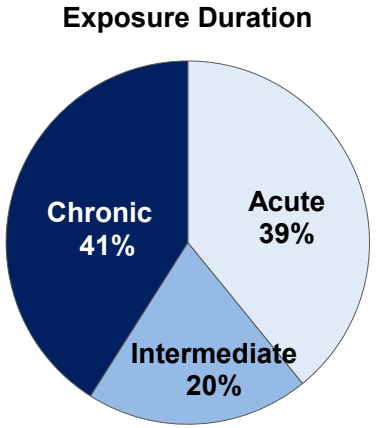
Figure 2-1. Overview of the Number of Studies Examining Benzene Health Effects*

Most studies examined the potential hematological, cancer, and body weight effects of benzene

Fewer studies evaluated health effects in humans than animals (counts represent studies examining endpoint)







*Health effect displayed only at the most sensitive dose; most studies examined multiple endpoints.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Armenta-Reséndiz et al. 2019									
1	Rat (Wistar) 10 M	30 minutes (WB)	0, 2,000, 4,000, 8,000	NX	Neuro			2,000	Decreased learning and memory in passive avoidance test; anxiety-like behavior, and altered motor coordination and social interaction
Coate et al. 1984									
2	Rat (Sprague-Dawley) 40 F	GDs 6–15 6 hours/day	0, 1, 10, 40, 100	DX, LE	Repro	100			
					Develop	40	100		Fetal weight decreased by 6%
Drew and Fouts 1974									
3	Rat (Sprague-Dawley) NS F	4 hours	0, 11,500-15,500	GN, CS, LE, OW, BW	Death			13,700	LC_{50}
Green et al. 1978									
4	Rat (Sprague-Dawley) 14–18 F	GDs 6–15 6 hours/day	0, 100, 300, 2,200	DX	Repro	2,200			
					Develop		100		Increased incidence of missing sternebrae
Kuna and Kapp 1981									
5	Rat (Sprague-Dawley) 17–20 F	GDs 6–15 7 hours/day	0, 10, 50, 500	HE, RX, DX	Bd wt	10		50	34% decreased maternal weight gain on GDs 5–15
					Hemato	500			
					Repro	500			
					Develop	10		50	Fetal weight decreased by 14%; fetuses with lagging ossification in rib cage and extremities; increased incidence of fetuses with variants (visceral and skeletal)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Li et al. 1986									
6	Rat (Wistar) 5–7 F	7 days 8 hours/day	0, 20, 50, 100, 300, 1,000, 3,000	BC, BI	Hemato	50	100		Decreased peripheral WBCs
					Immuno	50	100		Increased leukocyte alkaline phosphatase
Robinson et al. 1997									
7	Rat (Sprague-Dawley) 16 M	2 weeks 5 days/week 6 hours/day	0, 30, 200, 400	HE, IX, OW, BW	Hemato	200	400		Decreased absolute thymus weight
Smyth et al. 1962									
8	Rat (NS) 6 B	4 hours	16,000	CS, LE	Death			16,000	4/6 died
Tatrai et al. 1980a									
9	Rat (CFY) 20 F	GDs 7–14 24 hours/day	0, 125	BW, OW, LE, DX	Bd wt			125	Maternal weight decreased by 32%
					Hepatic	125			
					Repro	125			
					Develop			125	Fetal weight decreased by 20%
Tatrai et al. 1980b									
10	Rat (CFY) 20–48 F	GDs 7–14 24 hours/day	0, 47, 141, 470, 939	DX	Bd wt			47	Maternal body weight decreased by 27%
					Develop		47	141	LOAEL: Fetal weight decreased by 5% SLOAEL: Increased resorptions, 28% decreased fetal weight
Ward et al. 1985									
11	Rat (Sprague-Dawley) 50 M, 50 F	2 weeks 5 days/week 6 hours/day	0, 1, 10, 30, 300	HP, BC, BI, BW, OW, CS	Hemato	30	300		Decreased peripheral WBCs; decreased peripheral lymphocytes

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Aoyama 1986									
12	Mouse (BALB/c) 5–8 M	7 days 6 hours/day	0, 50, 200	LE, BI, HE, OW, BW	Bd wt	47	211		Terminal body weight decreased by 16%
					Hemato	47	211		Decreased peripheral WBCs, decreased relative spleen weight
					Immuno		47		Decreased splenic lymphocyte antibody production
Aoyama 1986									
13	Mouse (BALB/c) 5–8 M	14 days 6 hours/day	0, 50, 200	LE, BI, HE, OW, BW	Bd wt	48	208		Terminal body weight decreased by 18%
					Hemato		48		Decreased peripheral WBCs, decreased relative spleen and thymus weights
					Immuno		48		Decreased splenic lymphocyte antibody production
Chertkov et al. 1992									
14	Mouse (DBA/2) 20 B	2 weeks 6 hours/day 5 days/week	0, 300	HE	Bd wt		300 M		Terminal body weight decreased by 15%
					Hemato		300 M		Decreased peripheral WBCs; decreased marrow cellularity
Cronkite 1986									
15	Mouse (CBA/Ca) NS B	2 weeks 5 days/week 6 hours/day	0, 10, 25, 100, 300, 400	HP, BC, CS	Hemato	10	25		Decreased peripheral lymphocytes
Cronkite et al. 1982									
16	Mouse (Hale-Stoner) 2–4 M	11 days 5 days/week 6 hours/day	0, 400	CS, HE, HP	Hemato		400		Decreased peripheral RBCs and WBCs; decreased marrow cellularity

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Cronkite et al. 1985									
17	Mouse (C57BL/6 BNL) 5–10 B	2 weeks 5 days/week 6 hours/day	0, 10, 25, 100, 300, 400	LE, HP, BC, HE	Hemato	10	25	100	LOAEL: Decreased peripheral lymphocytes SLOAEL: Decreased hematocrit, hemolytic anemia
Cronkite et al. 1989									
18	Mouse (C57BL/6BNL) NS F	8 days 6 hours/day	0, 3,000	HE	Hemato		3,000		Decreased marrow cellularity
Cronkite et al. 1989									
19	Mouse (Hale-Stoner) NS M	2 days 5 days/week 6 hours/day	0, 400	HE, BC	Hemato		400		Decreased marrow CFU-E cells
Dempster and Snyder 1991									
20	Mouse (DBA/2J) 4–5 M	5 days 6 hours/day	0, 10.3	HE	Hemato		10.3		Decrease in marrow CFU-E cells
					Immuno		10.3		Decreased response of marrow CFU-E to erythropoietin
Dempster et al. 1984									
21	Mouse (C57BL) 30 M	1–14 days 5 days/week 6 hours/day	0, 100, 300, 1,000, 3,000	CS, NX, BC, BW	Neuro		300	3,000	LOAEL: Increased licking of sweetened milk behavior SLOAEL: Tremors, marked decrease in hind limb grip strength
Evans et al. 1981									
22	Mouse (CD1, C57BL/6J) 60 M	5 days 6 hours/day	0, 300, 900	CS, NX	Neuro		300		Hyperactivity (increased eating and grooming; reduced sleeping and resting)
Gill et al. 1980									
23	Mouse (C57B1/6) 4–12 M	2-8 days 24 hours/day	0, 100, 500, 1,000	BC	Hemato		100		Decreased peripheral WBCs; decreased marrow cellularity

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Green et al. 1981a									
24	Mouse (CD-1) 11–47 M	5 days 6 hours/day	0, 1.1, 9.9, 103, 306, 603, 1,276, 2,416, 4,862	HP	Hemato	9.9	103		Decreased marrow and splenic cellularity; decreased splenic granulocytes
Green et al. 1981b									
25	Mouse (CD-1) 3–23 M	5 days 6 hours/day	0, 1.1, 9.9, 103, 306, 603, 1,276, 2,416, 4,862	LE, HE, BW, OW	Bd wt	4862			
					Hemato	9.9	103		Decreased peripheral lymphocytes; decreased marrow cellularity
Keller and Snyder 1988									
26	Mouse (Swiss-Webster) 5–10 F	GDs 6–15 6 hours/day	0, 5, 10, 20	BC, CS, BI	Develop	10	20		Decreased circulating erythroid precursors, elevation of granulocytic precursor cells in neonates and 6-week-old offspring
Mukhopadhyay and Nath 2014									
27	Mouse (Swiss Albino) 5 M	2 weeks 5 days/week 6 hours/day (WB)	0, 100, 300	LE, BC, HE, HP	Death			100	Decreased cumulative survival time by 5.3 weeks (during the post-exposure follow-up period) compared to control
					Hemato	100	300		Decreased peripheral lymphocytes, HCT percent and MCV, increased abnormal cells in bone marrow
					Hepatic		300		Extended sinusoids in hepatocytic cell cords, increased AST and ALT
Mukhopadhyay and Nath 2014									
28	Mouse (Swiss Albino) 5 M	2 weeks 6 days/week 10 hours/day (WB)	0, 150	LE, HE	Death			150	Decreased cumulative survival time by 15.5 weeks (during the post-exposure follow-up period) compared to control
					Hemato		150		Decreased peripheral lymphocytes and HCT

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Mukhopadhyay and Nath 2014									
29	Mouse (Swiss Albino) 5 M	2 weeks 5 days/week 12 hours/day (WB)	0, 150	LE, HE	Death			150	Decreased cumulative survival time by 17.2 weeks (during the post-exposure follow-up period) compared to control
					Hemato		150		Decreased peripheral lymphocytes and HCT
Murray et al. 1979									
30	Mouse (CF-1) 37 F	GDs 6–15 7 hours/day	0, 500	HE, RX, DX	Hemato	500			
					Repro	500			
					Develop	500			
Neun et al. 1992									
31	Mouse (Swiss Webster, C57B1/6J) 31–32 M	2 weeks 4 days/week 6 hours/day	0, 300	HE	Hemato		300		Decreased marrow cellularity and marrow CFU-Es
Plappert et al. 1994a									
32	Mouse (Hybrid) 7 F	5 days 5 days/week 6 hours/day	0, 100, 300, 900	HE	Hemato	100	300		Decreased marrow CFU-Es, increased peripheral helper lymphocytes
Rosenthal and Snyder 1985									
33	Mouse (C57BL/6) 5–7 M	1–12 days 6 hours/day	0, 10, 30, 100, 300	BC, CS, BI	Hemato	10	30		Decreased peripheral lymphocytes
					Immuno	10	30		Decreased resistance to bacterial infection
Rozen et al. 1984									
34	Mouse (C57Bl/6J) 7–8 M	6 days 6 hours/day	0, 10.2, 31, 100, 301	HE, IX	Hemato		10.2[b]		Decreased peripheral lymphocyte counts; elevated peripheral RBCs
					Immuno		10.2[b]		Decreased mitogen-induced blastogenesis (function) of marrow lymphocytes

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Stoner et al. 1981									
35	Mouse Hale-Stoner 15 F	5 and 12 days 5 days/week 6 hours/day	0, 400	BC, CS, IX	Immuno		400		Decreased antibody response to tetanus toxin
Toft et al. 1982									
36	Mouse (NMRI) 5 M	2 weeks 5 days/week 8 hours/day	0, 1, 10.5, 21, 50, 95, 107	HP, BC, CS, HE	Hemato	10.5	21		Increased micronucleated polychromatic erythrocytes; decreased granulopoietic stem cells
Toft et al. 1982									
37	Mouse (NMRI) 5–6 M	1–10 days 24 hours/day	0, 21, 50, 95	HP, BC, CS, HE	Hemato			21	Decreased marrow cellularity; increased polychromatic erythrocytes; decreased marrow granulopoietic stem cells
Toft et al. 1982									
38	Mouse (NMRI) 5 M	2 weeks 5 days/week 8 hours/day	0, 14	HP, BC, CS, HE	Hemato	14			
Ungvary and Tatrai 1985									
39	Mouse (CFLP) 15 F	GDs 6–15 12 hours/day	0, 156.5, 313	DX	Develop			156.5	Fetal weight decreased by 25%
Ward et al. 1985									
40	Mouse (CD-1) 50 M, 50 F	2 weeks 5 days/week 6 hours/day	0, 1, 10, 30, 300	BC, CS, BI, BW, OW, HP	Bd wt	300			
					Hemato	30	300		Decreased peripheral WBCs and peripheral lymphocytes, histopathological lesions in spleen (extramedullary hematopoiesis) and thymus (atrophy)
					Immuno	30	300		Histopathological lesions in selected lymph nodes (lymphoid depletion)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Wells and Nerland 1991									
41	Mouse (Swiss-Webster) 4–5 M	5 days 6 hours/day	0, 3, 25, 55, 105, 199, 303, 527, 1,150, 2,290	OW, HE	Hemato	3	25		Decreased peripheral WBCs; decreased absolute spleen weight
Carpenter et al. 1944									
42	Rabbit (NS) 10 NS	3.7–36.2 minutes	45,000	CS, OF, LE	Death			45,000	100% mortality within 36.2 minutes
					Neuro			45,000	Narcosis, tremors, loss of pupillary and blink reflex, pupillary contraction
Murray et al. 1979									
43	Rabbit (New Zealand) 20 F	GDs 6–18 7 hours/day	0, 500	HE, RX, DX	Hemato	500			
					Repro	500			
					Develop	500			
Ungvary and Tatrai 1985									
44	Rabbit 11–15 F	GDs 7–20 24 hours/day	0, 156.5, 313	DX	Bd wt	156.5		313	62% decreased maternal weight gain
					Repro	156.5		313	Increased spontaneous abortions and resorptions
					Develop			156.5	Fetal weight decreased by 17% (males) and 16% (females)
INTERMEDIATE EXPOSURE									
Dow 1992									
45	Rat (Sprague-Dawley) 10 M, 10 F	3 weeks 5 days/week 6 hours/day	0, 500	HE, GN, CS	Hemato		500		Decreased peripheral WBCs and lymphocytes, increased peripheral RBCs and hemoglobin

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Harrath et al. 2022									
46	Rat (Wistar-Albino) 5 F	28 days 30 minutes/day (WB)	0, 2,000, 4,000, 8,000	BW, BC, OW, HP	Bd wt	4,000	8,000		Terminal body weight decreased by 12%
					Repro		2,000		Ovarian histopathology (degenerating follicles, increased pyknotic nuclei, abnormal oocyte structure, thickening zona pellucida) and decreased numbers of growing follicles
Kuna et al. 1992									
47	Rat (Sprague-Dawley) 26 F	10 weeks GDs 0–20 LDs 5–20 5 days/week 6 hours/day	0, 1, 10, 30, 300	BW, OW, GN, CS, DX, LE, RX	Bd wt	300			
					Repro	300			
Maltoni et al. 1982, 1983, 1985, 1989									
48	Rat (Sprague-Dawley) 70–158 M, 59–149 F	15 weeks (exposure via dam GD 12–weaning and direct post-weaning 4–5 days/week 4–7 hours/day	0, 200	LE, CS, BW, HP, HE	Cancer			200	CEL: Oral cavity carcinoma and hepatomas
Robinson et al. 1997									
49	Rat (Sprague-Dawley) 16 M	4 weeks 5 days/week 6 hour/days	0, 30, 200, 400	HE, IX, OW, BW	Immuno	200	400		Decreased total splenic cells, decreased absolute thymus weight
Songnian et al. 1982									
50	Rat (NS) 6 M, 6 F	20 weeks 6 days/week 4 hours/day	0, 4,570	BC, CS, BI	Immuno		4,570		Increased leukocyte alkaline phosphatase, decreased WBC count

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Ward et al. 1985									
51	Rat (Sprague-Dawley) 50 M, 50 F	13 weeks 5 days/week 6 hours/day	0, 1, 10, 30, 300	HP, BC, BI, BW, OW, CS, OF	Bd wt	300			
					Hemato	30	300		Decreased peripheral WBCs and lymphocytes, decreased marrow cellularity
Abplanalp et al. 2019									
52	Mouse (C57BL/6N) 20 M	6 weeks 6 hours/day (WB)	0, 50	BC, UR, OF	Endocr		50		Decreased insulin and glucose tolerance, increased glucose and insulin serum concentrations without challenge
Baarson et al. 1982									
53	Mouse (C57BL/6T) 18 M	13 weeks 5 days/week 6 hours/day	0, 300	HP, BC, IX	Hemato		300		Decreased peripheral and splenic RBCs
					Immuno		300		Decreased marrow and splenic cellularity
Baarson et al. 1984									
54	Mouse (C57BL) 5 M	24 weeks 5 days/week 6 hours/day	0, 10	BC, HP, IX	Hemato		10		Decreased peripheral lymphocytes, decreased marrow CFU-E, decreased marrow and splenic cellularity
Cronkite 1986									
55	Mouse (CBA/Ca) NS M	16 weeks 5 days/week 6 hours/day	0, 100, 300	LE, HP, BC, CS	Cancer			300	CEL: Leukemia, lymphoma
Cronkite et al. 1982									
56	Mouse (Hale-Stoner) 2–4 M	9.5 weeks 5 days/week 6 hours/day	0, 400	CS, HE, HP, BC	Hemato		400		Decreased peripheral RBCs and WBCs, decreased marrow cellularity

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Cronkite et al. 1984, 1985									
57	Mouse (C57BL/6BNL) 88–89 F	16 weeks 5 days/week 6 hours/day	0, 300	HP, BC, CS	Cancer			300	CEL: Thymic lymphoma, leukemia (all types), benign and malignant Zymbal gland epidermoid tumors and lymphoepithelioma; unspecified solid ovarian tumors
Cronkite et al. 1985									
58	Mouse (C57B1/6BNL) 5–10 B	4–16 weeks 5 days/week 6 hours/day	0, 300	HP, BC, CS, HE, LE	Hemato		300		Decreased marrow cellularity; decreased marrow CFU
Cronkite et al. 1989									
59	Mouse (CBA/Ca BNL) 57–60 M, 54–60 F	16 weeks 5 days/week 6 hours/day	0, 300	LE, CS	Cancer			300	CEL: Myelogenous neoplasms; combined non-hematopoietic tumors (Harderian and Zymbal gland, squamous cell and mammary carcinoma, papillary adenocarcinoma of the lung, benign tumors)
Cronkite et al. 1989									
60	Mouse (CBA/Ca) NS M	16 weeks 5 days/week 6 hours/day	0, 10, 25, 100, 300, 400, 3,000	HP, BC, CS, HE	Hemato	25	100		Decreased stem cells in bone marrow
					Cancer			100	CEL: Combined non-hematopoietic tumors (unspecified)
Cronkite et al. 1989									
61	Mouse (CBA/Ca BNL) NS M	20 days 5 days/week 6 hours/day	0, 316	HE, BC	Hemato		316		Decreased peripheral lymphocytes, decreased marrow CFU-S

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Das et al. 2012									
62	Mouse (Swiss-Albino) 24 M, 24 F	2 months 5 days/week 6 hours/day (WB)	0, 300	HE, HP	Hemato		300		Increased peripheral WBCs, decreased reticulocytes and neutrophils
Farris et al. 1993									
63	Mouse (CBA/Ca) 125 M	16 weeks 5 days/week 6 hours/day	0, 300	HE, HP, LE	Hemato		300		Granulocytic hyperplasia in bone marrow
					Cancer			300	CEL: Malignant lymphoma, squamous cell carcinoma of preputial gland, lung adenoma, Zymbal gland carcinoma, squamous cell carcinoma of the forestomach
Farris et al. 1997a									
64	Mouse (B6C3F1) 24 M	Up to 8 weeks 5 days/week 6 hours/day	0, 1, 10, 100, 200	HE	Hemato	10	100		Decreased peripheral RBCs, decreased marrow cellularity, decreased marrow CFU-HPP
Farris et al. 1997b									
65	Mouse (B6C3F1) 3–10 M	8 weeks 5 days/week 6 hours/day	0, 1, 10, 100, 200	HE, IX	Hemato	10	100		Decreased peripheral lymphocyte and total nucleated cell counts
Gill et al. 1980									
66	Mouse (C57B1/6) 6–15 NS	6 weeks 5 days/week 6 hours/day	0, 1,000, 2,000, 4,000	BC	Hemato		1,000		Decreased peripheral WBCs, lymphocytes, and granulocytes
Green et al. 1981a									
67	Mouse (CD-1) 11–47 M	26 weeks 6 hours/day 5 days/week	0, 302	HP	Hemato		302		Decreased marrow and spleen cellularity

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Green et al. 1981a									
68	Mouse (CD-1) 2–4 M	50 days 6 hours/day 5 days/week	0, 9.6	HP	Hemato		9.6		Increased splenic CFU-S
Green et al. 1981b									
69	Mouse (CD-1) 3–23 M	26 weeks 5 days/week 6 hours/day	0, 302	LE, HE, BW, OW	Bd wt	302			
					Hemato		302		Decreased peripheral WBCs and RBCs, altered RBC morphology, decreased absolute spleen weight
Green et al. 1981b									
70	Mouse (CD-1) 12 M	50 days 5 days/week 6 hours/day	0, 9.6	LE, HE, BW, OW	Bd wt	9.6			
					Hemato		9.6		Increased absolute spleen weight, total splenic nucleated cellularity and NRBC
Inoue and Hirabayashi 2010									
71	Mouse (C57BL/6) 23–24 NS	26 weeks 5 days/week 6 hours/day (WB)	0, 33, 100, 300	HP	Cancer			100	CEL: Thymic lymphomas
Inoue and Hirabayashi 2010									
72	Mouse (C3H/He) 23–24 NS	26 weeks 5 days/week 6 hours/day (WB)	0, 100, 300	HP	Cancer			300	CEL: AML and non-thymic lymphomas
Koshko et al. 2021									
73	Mouse (C57BL/6) 5–6 F	GDs 1-21 6 hours/day for 20 days (WB)	0, 50	DX	Neuro	50			
					Develop		50		Increased glucose, altered responses to glucose and insulin tolerance tests

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Koshko et al. 2023									
74	Mouse (C57BL/6) 3–5 F	GDs 1–19 6 hours/day for 18 days (WB)	0, 50	DX, BC	Develop	50			
Luke et al. 1988b									
75	Mouse (DBA/2, B6C3F1, C57B1/6) 6 M	13 weeks 3 or 5 days/week 6 hours/day	300	BC, CS, BI	Hemato		300		Increased frequency of MN-PCE and MN-NCE
Malovichko et al. 2021									
76	Mouse (C57BL/6) 24 M	6 weeks 5 days/week 6 hours/day (WB)	0, 50	HE, IX	Hemato		50		Decreased marrow HPCs and platelet-leukocyte aggregates, increased peripheral CD^{3+}, CD4+, and CD^{8+} T-cells
Maxwell et al. 2023									
77	Mouse (C57BL/6) 8 F	GDs 1–18 5 hours/day (WB)	0, 50	HP, RX, DX	Repro			50	Increased resorptions and pregnancy loss, altered placental labyrinth vascularity and trophoblast hyperplasia
					Develop		50		Fetal weight decreased by ~5%
Mukhopadhyay and Nath 2014									
78	Mouse (Swiss Albino) 5 M	3 or 4 weeks 5 days/week 6 hours/day (WB)	0, 300	LE, HE	Death			300	Decreased cumulative survival time by 18.6 weeks (during the post-exposure follow-up period) compared to control
					Hemato		300		Decreased peripheral lymphocytes, decreased HCT and MCV
Plappert et al. 1994a									
79	Mouse (Hybrid) 7 F	8 weeks 5 days/week 6 hours/day	0, 100, 300, 900	HE	Hemato	100	300		Decreased marrow BFU-E and CFU-E; increased CD^{4+}/CD^{8+} ratio

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Plappert et al. 1994b									
80	Mouse (Hybrid) 7 F	8 weeks 5 days/week 6 hours/day	0, 300, 900	HE	Hemato		300		Decreased marrow BFU-E and CFU-E; increased CD^{4+}/CD^{8+} ratio
Rosenthal and Snyder 1987									
81	Mouse (C57B1/6) 5 M	20 days 5 days/week 6 hours/day	0, 11.1, 29.3, 101.4	BC, IX	Immuno		11.1[c]		Delayed splenic lymphocyte response to antigens
Rosenthal and Snyder 1987									
82	Mouse (C57B1/6) 10 M	100 days 5 days/week 6 hours/day	0, 10.8, 29.3, 101.4	IX	Immuno			101.4	Depressed cell-mediated immunity against injected tumor cells (resulted in 9/10 deaths)
Seidel et al. 1989									
83	Mouse (BDF1) 4 F	8 weeks 5 days/week 6 hours/day	0, 100, 300, 900	HP, BC, CS, BI, HE	Hemato		100		Depressed marrow BFU-E and CFU-E
Snyder et al. 1988									
84	Mouse (C57BL, CD-1) 80 M	10 weeks 5 days/week 6 hours/day	0, 300, 1,200	HP, BC, CS	Cancer			1,200	CEL: Zymbal gland carcinomas in C57BL mice; leukemia/lymphoma and lung adenoma in CD-1 mice
Stoner et al. 1981									
85	Mouse (Hale-Stoner) 15 F	4–5 weeks 5 days/week 6 hours/day	0, 50, 200, 400	BC, CS, IX	Immuno	50	200		Decreased antibody response to tetanus toxoid
Vacha et al. 1990									
86	Mouse (Hybrid) 30 F	6 or 7 weeks 5 days/week 6 hours/day	0, 300	HE	Hemato		300		Decreased CFU-C, BFU-E, and CFU-E

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Ward et al. 1985									
87	Mouse (CD-1) 50 M, 50 F	13 weeks 5 days/week 6 hours/day	0, 1, 10, 30, 300	HP, BC, BI, BW, OW, CS	Bd wt	300			
					Hemato	30	300		Decreased peripheral RBCs and WBCs, decreased marrow cellularity, histopathologic lesions in spleen (extramedullary hematopoiesis)
					Immuno	30	300		Lymphoid depletion in selected lymph nodes
					Repro	30			
							300 F		Bilateral cyst in ovaries
								300 M	Atrophy/degeneration of testes; decrease in spermatozoa; increase in abnormal sperm
Zelko et al. 2021									
88	Mouse (C57BL/6) 6–11 M	6 weeks 6 hours/day 5 days/week (WB)	0, 50	OW, HP, OF	Cardio			50	Decreased fractional shortening of the left ventricle during diastole
Dow 1992									
89	Pig (Duroc-Jersey) 8 M, 8 F	3 weeks 5 days/week 6 hours/day	0, 20, 100, 500	HE, GN, CS	Hemato	20	100		Decreased peripheral WBCs and lymphocytes; increased peripheral RBCs, decreased marrow cellularity
CHRONIC EXPOSURE									
Lan et al. 2004a									
90	Human 390 B	6.1 years (average) (occupational)	<0.04, 0.57, 2.85, 28.73	HE	Hemato		0.57[d]		Decreased peripheral WBCs and platelets

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Maltoni et al. 1982, 1983, 1985, 1989									
91	Rat (Sprague-Dawley) 75–158 M, 54–149 F	104 weeks (starting on GD 12; F1 continued direct exposure post-weaning) 5 days/week 4–7 hours/day	0, 282	HP, BC, CS	Cancer			282	CEL: Zymbal gland carcinoma in F1 males and females; oral cavity carcinoma and hepatomas in F1 females
Maltoni et al. 1983									
92	Rat (Sprague-Dawley) 75 M, 65 F	104 weeks 5 days/week 4–7 hours/day	0, 200, 300	LE, CS, BW, HP, HE	Cancer			200	CEL: Hepatomas
Snyder et al. 1978, 1984									
93	Rat (Sprague-Dawley) 27–45 M	Lifetime 5 days/week 6 hours/day	0, 100, 300	LE, BW, CS, HE, GN, HP	Death			300	Median lifespan reduced 21%
					Resp	300			
					Hemato		100		Decreased peripheral RBCs and WBCs
					Hepatic	300			
					Renal	300			
Snyder et al. 1978, 1980									
94	Mouse AKR/J 50–60 M	Lifetime 5 days/week 6 hours/day	0, 100, 300	HP, BC, CS, BW	Death			300	Median lifespan reduced 72%
					Bd wt	100		300	Weight loss of 26%
					Resp	300			
					Hemato		100		Decreased peripheral RBCs, WBCs, and lymphocytes, decreased bone marrow cellularity
					Hepatic	300			
					Renal	300			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Snyder et al. 1980									
95	Mouse (C57BL) 40 M	Lifetime, 5 days/week 6 hours/day	0, 300	HP, BC, CS, BW	Death			300	Median life span decreased 45%
					Bd wt			300	Loss of body weight (20%)
					Hemato		300		Decreased peripheral lymphocytes, decreased marrow cellularity
					Cancer			300	CEL: Hematopoietic neoplasms
Snyder et al. 1982									
96	Mouse (CD-1) 40 M	Lifetime 5 days/week 6 hours/day	0, 300	NX, BW, CS, HE	Death			300	Median lifespan decreased 51%
					Bd wt		300		17% weight loss
					Hemato		300		Decreased peripheral RBCs and lymphocytes
Snyder et al. 1988									
97	Mouse (C57BL, CD-1) 60 M	Lifetime every 3rd week 7 days/week	300, 1,200	HP, BC, CS	Cancer			300	CEL: 35% increase of Zymbal gland carcinomas in C57BL mice

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Snyder et al. 1988									
98	Mouse (C57BL, CD-1) 60 M	Lifetime 6 hours/day 5 day/week	0, 300	HP, BC, CS	Hemato		300		Decreased peripheral lymphocytes

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive a provisional acute-duration inhalation MRL of 0.009 ppm. The LOAEL of 10.2 ppm was adjusted for continuous exposure and converted into a $LOAEL_{HEC}$ of 2.55 ppm and then divided by a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment, 10 for human variability); see Appendix A for more detailed information regarding the MRL.
[c]Used to derive a provisional intermediate-duration inhalation MRL of 0.007 ppm. The LOAEL of 11.1 ppm was adjusted for continuous exposure and converted into a $LOAEL_{HEC}$ of 1.98 ppm and then divided by a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment, 10 for human variability); see Appendix A for more detailed information regarding the MRL.
[d]Used to derive a provisional chronic-duration inhalation MRL of 0.002 ppm. The LOAEL of 0.57 ppm was adjusted for continuous exposure to a $LOAEL_{ADJ}$ of 0.16 ppm and then divided by a total uncertainty factor of 100 (10 for use of a LOAEL, 10 for human variability); see Appendix A for more detailed information regarding the MRL.

ADJ = adjusted; ALT = alanine aminotransferase; AML = acute myelogenous leukemia; AST = aspartate aminotransferase; B = both males and females; BC = serum (blood) chemistry; Bd wt or BW = body weight; BFU-E = erythroid burst-forming unit; BI = biochemical changes; Cardio = cardiovascular; CEL = cancer effect level; CFU = colony-forming unit; CFU-C = colony-forming unit cell; CFU-E = erythroid colony-forming unit; CFU-HPP = high-proliferative potential colony-forming unit; CFU-S = spleen colony-forming unit; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); GD = gestation day; GN = gross necropsy; HCT = hematocrit; HE = hematology; HEC = human equivalent concentration; Hemato = hematological; HP = histopathology; HPC = hematopoietic progenitor cell; Immuno = immunological; IX = immune function; LD = lactation day; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCV = mean corpuscular volume; MN-NCE = micronucleated normochromatic erythrocyte; MN-PCE = micronucleated polychromatic erythrocyte; MRL = minimal risk level; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NRBC = nucleated red blood cell; NS = not specified; NX = neurological function; OF = organ function; OW = organ weight; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive function; SLOAEL = serious lowest-observed-adverse-effect level; UR = urinalysis; (WB) = whole body; WBC = white blood cell

Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Acute (≤14 days)

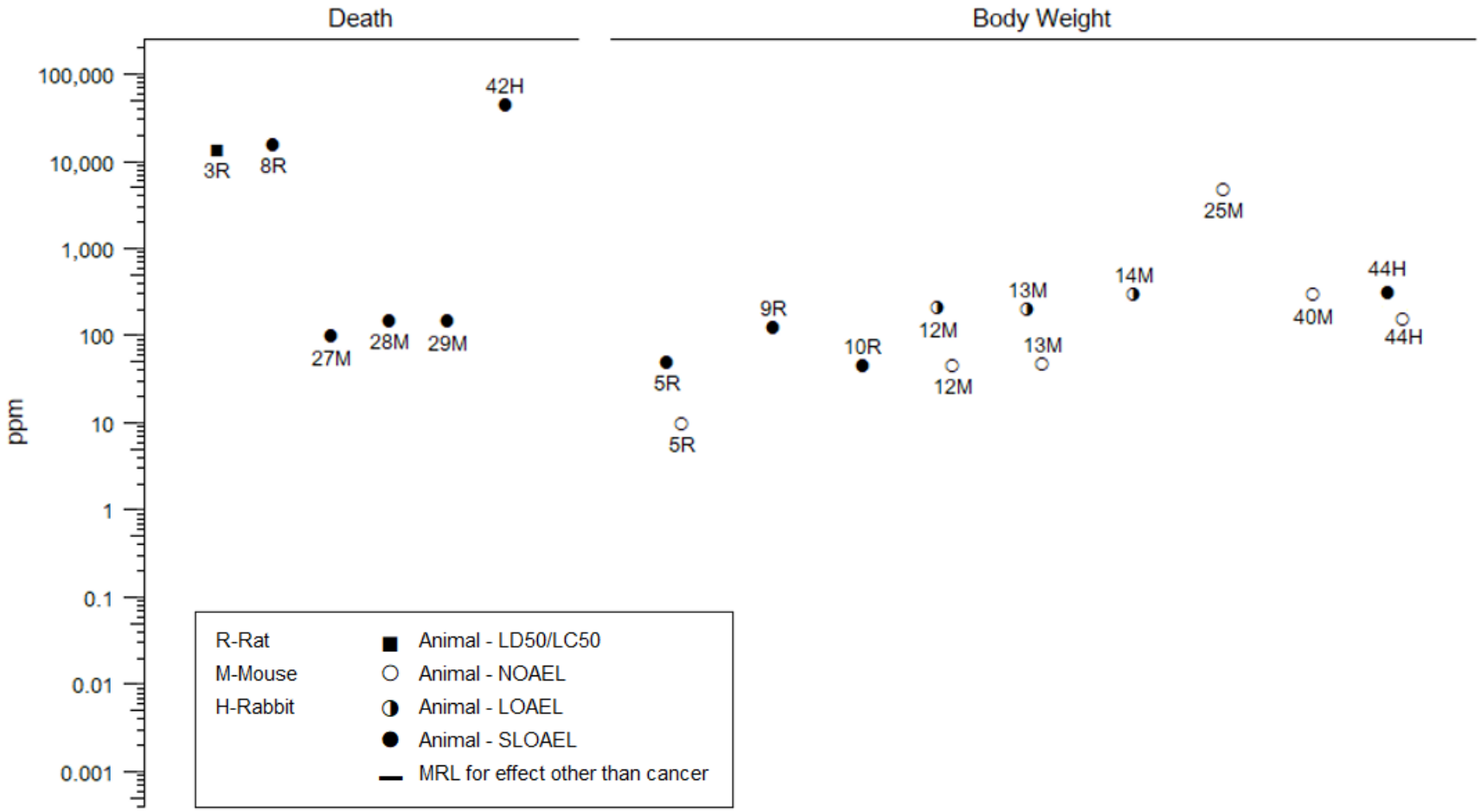


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Acute (≤14 days)

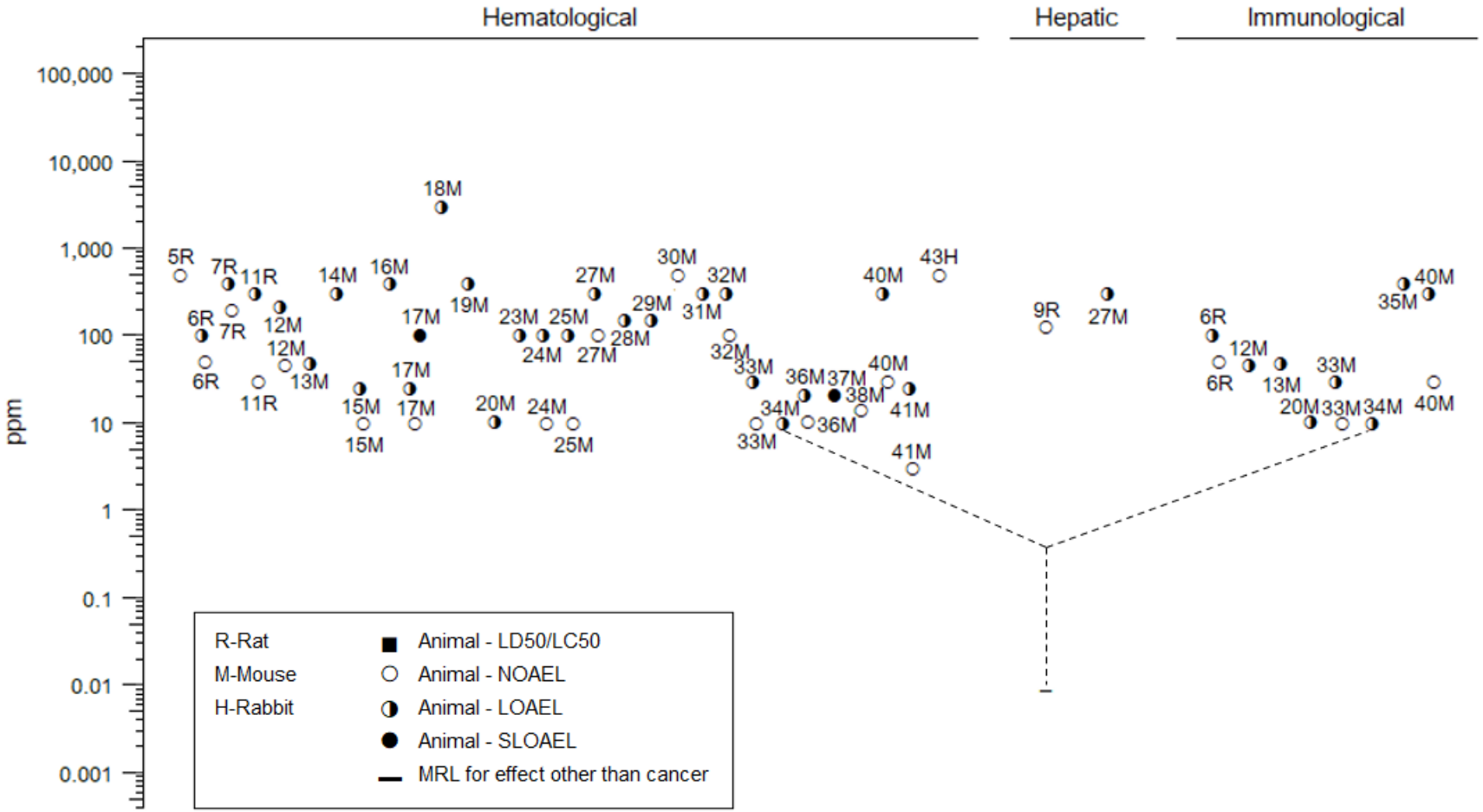


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Acute (≤14 days)

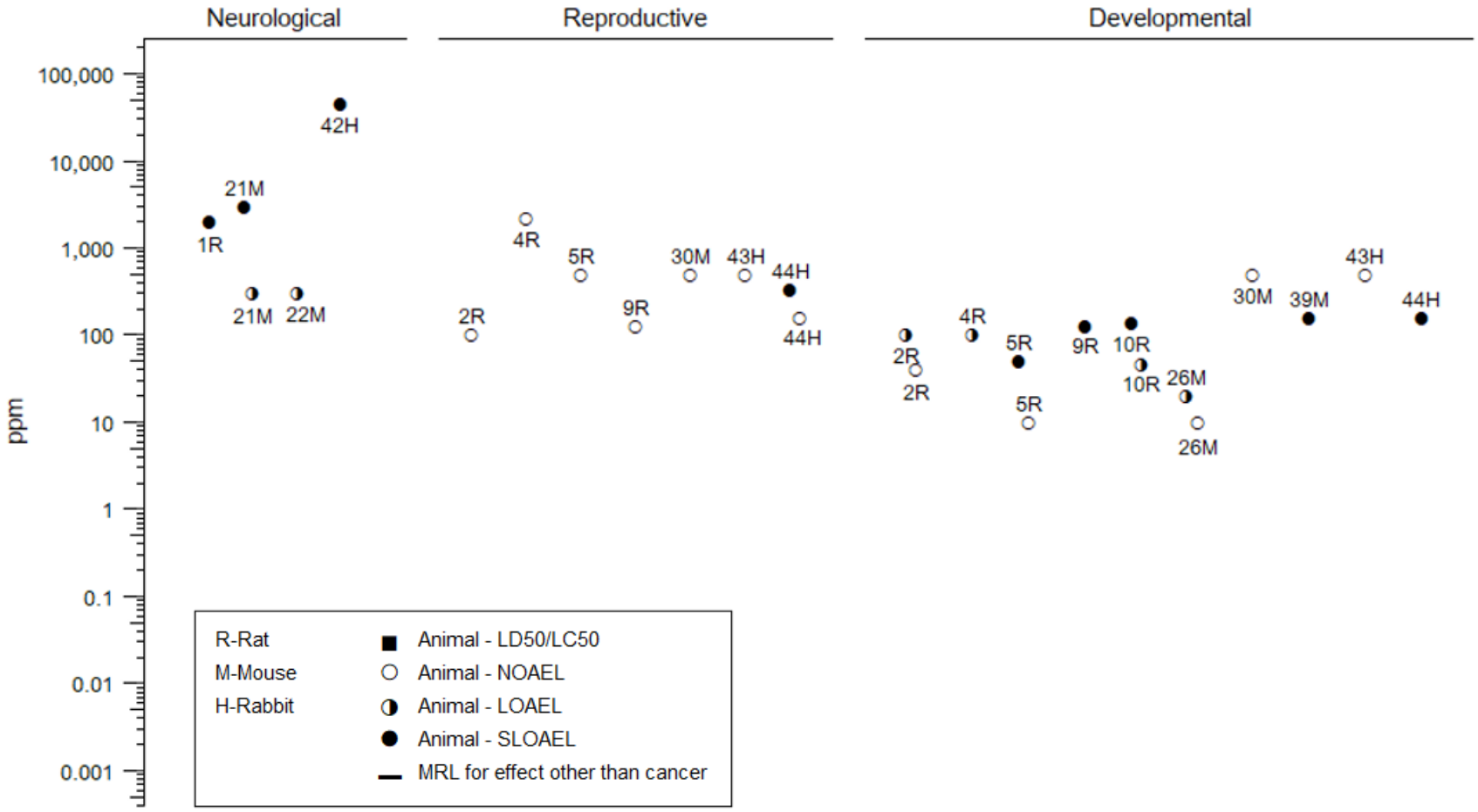


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Intermediate (15–364 days)

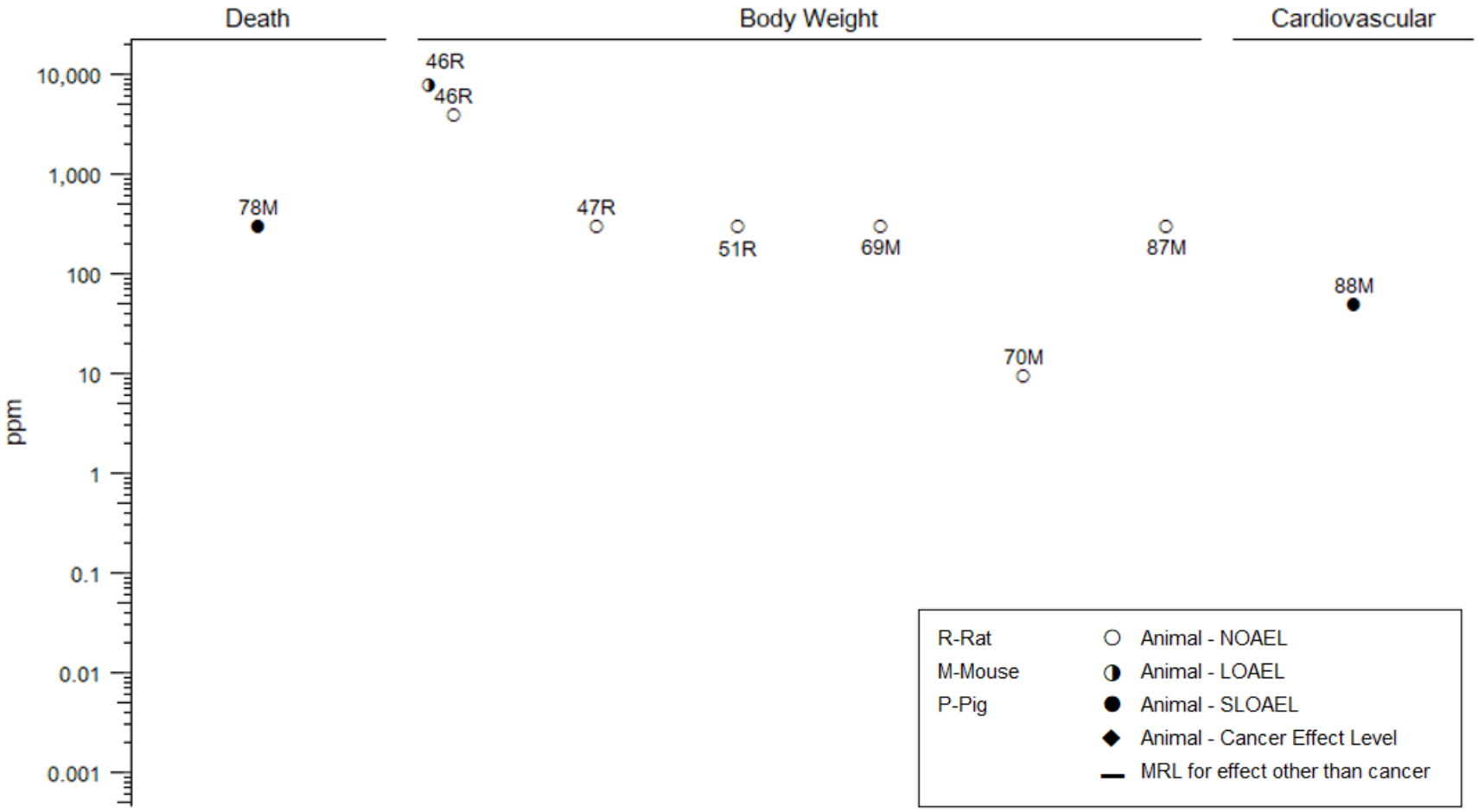


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Intermediate (15–364 days)

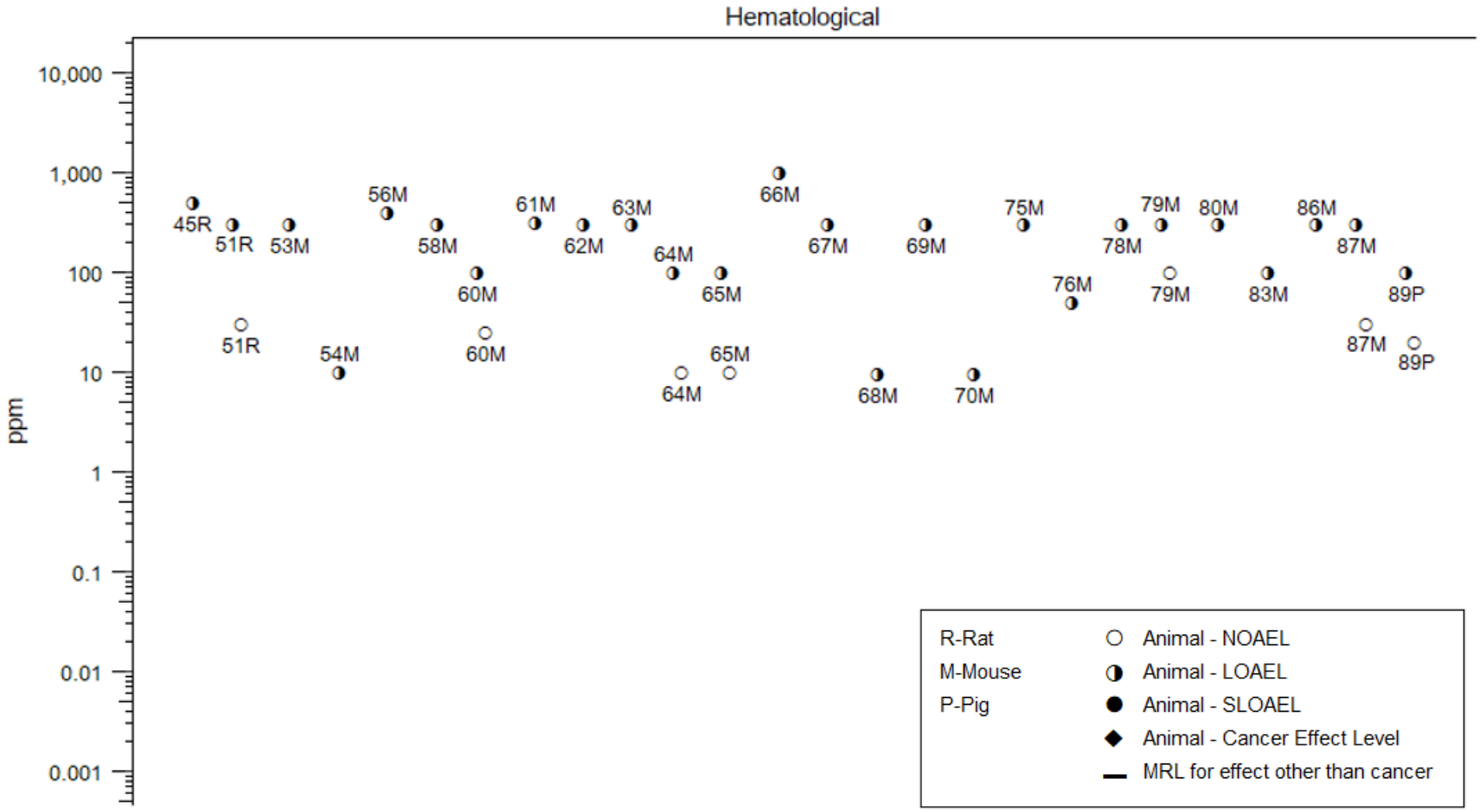


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Intermediate (15–364 days)

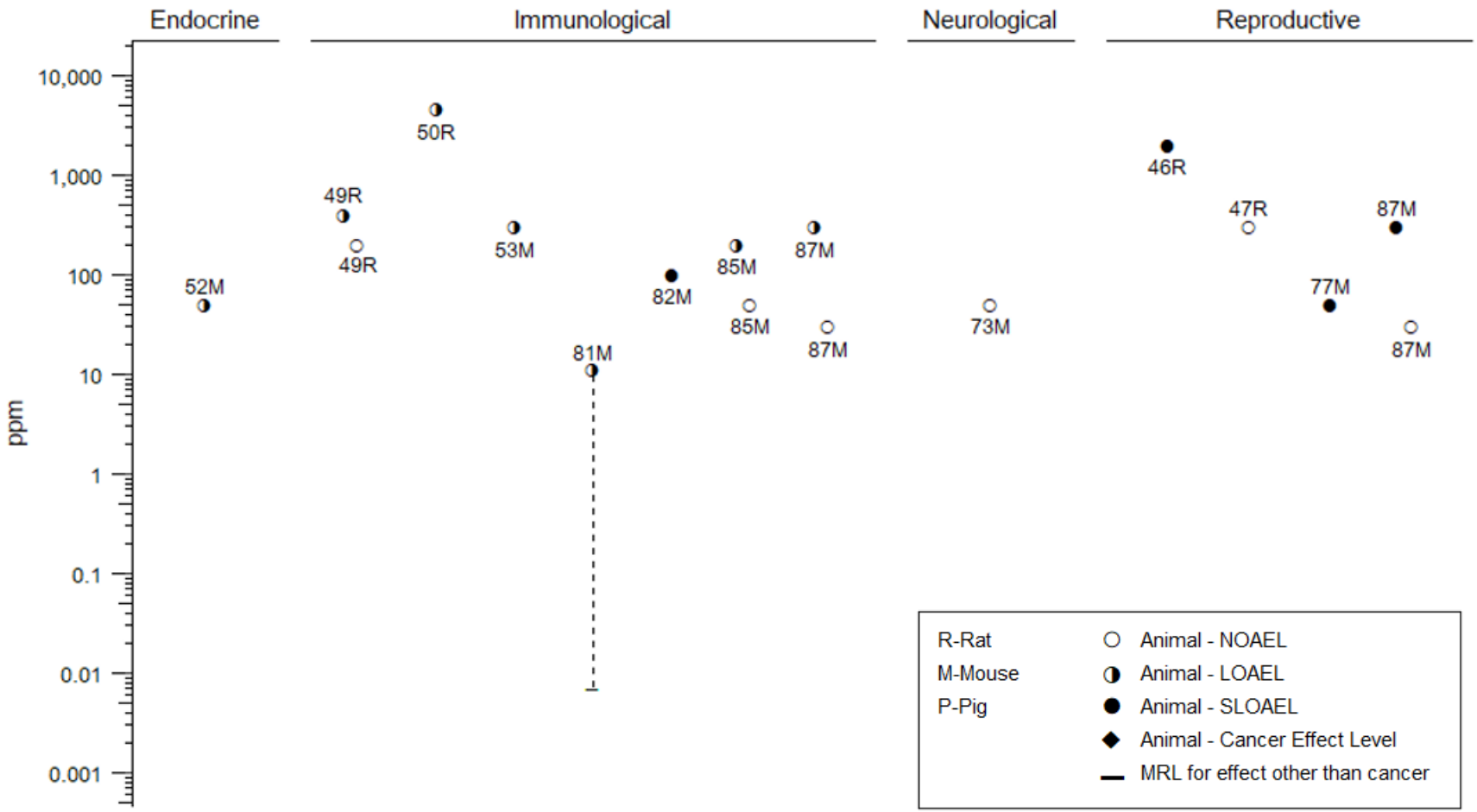


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Intermediate (15–364 days)

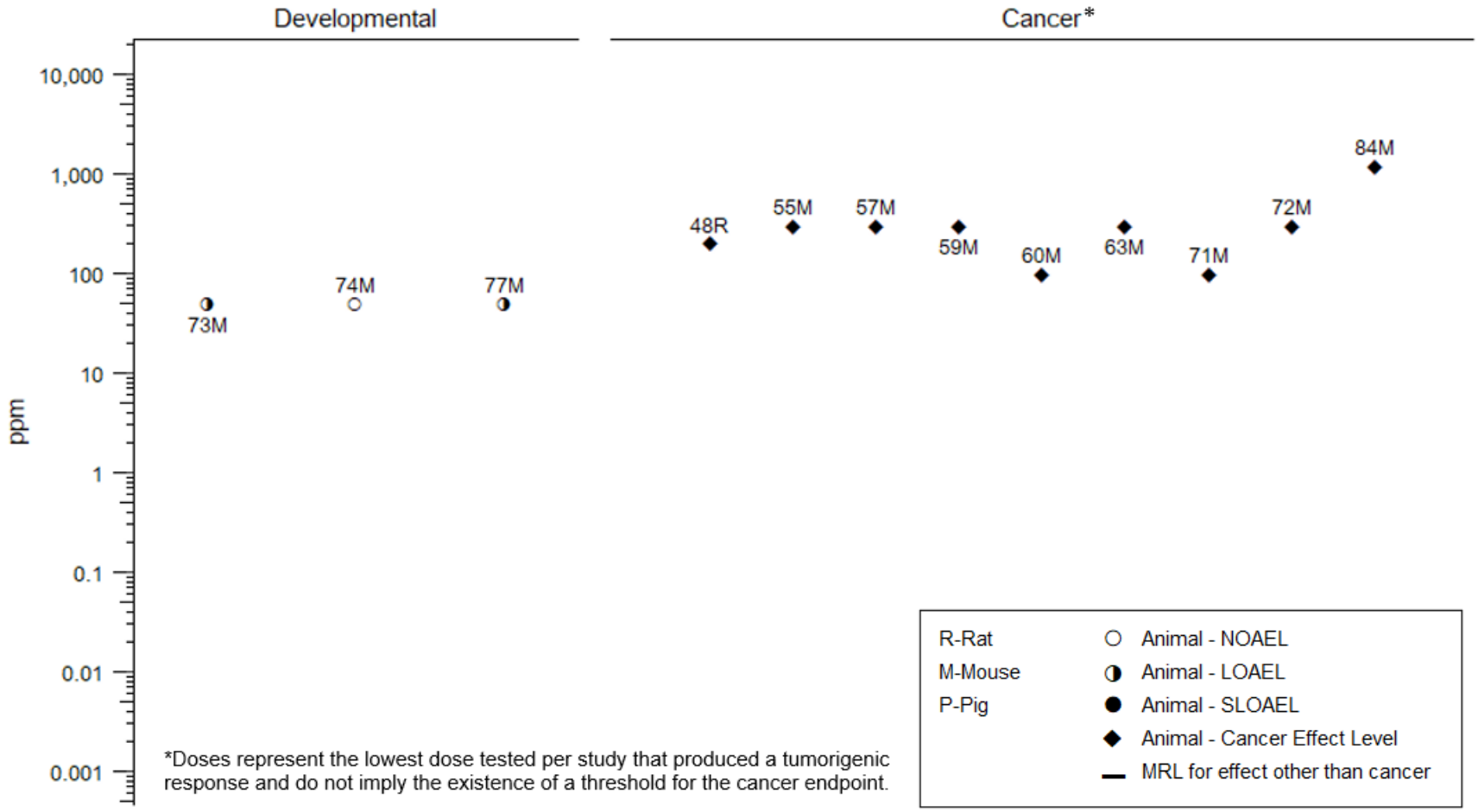


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Chronic (≥365 days)

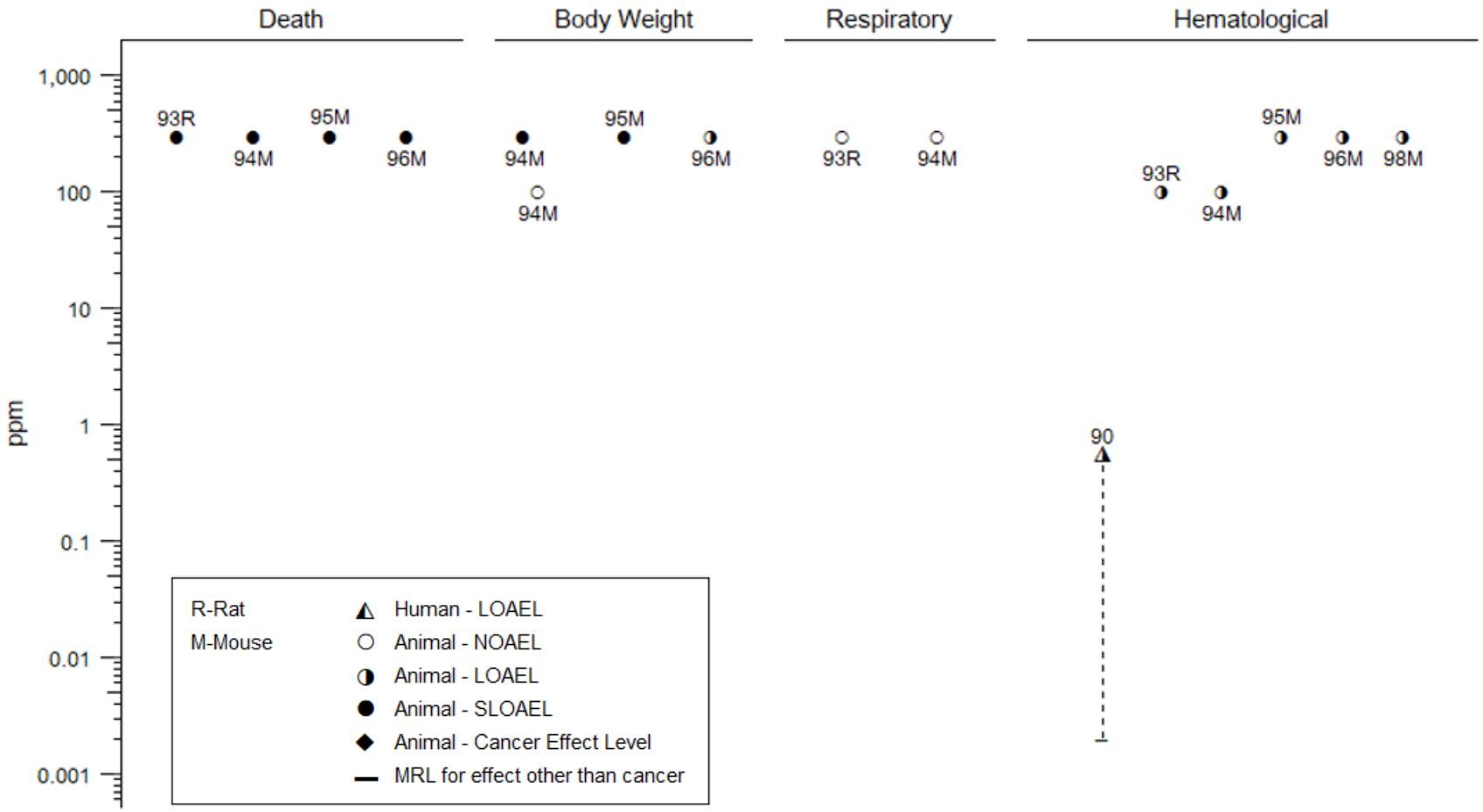
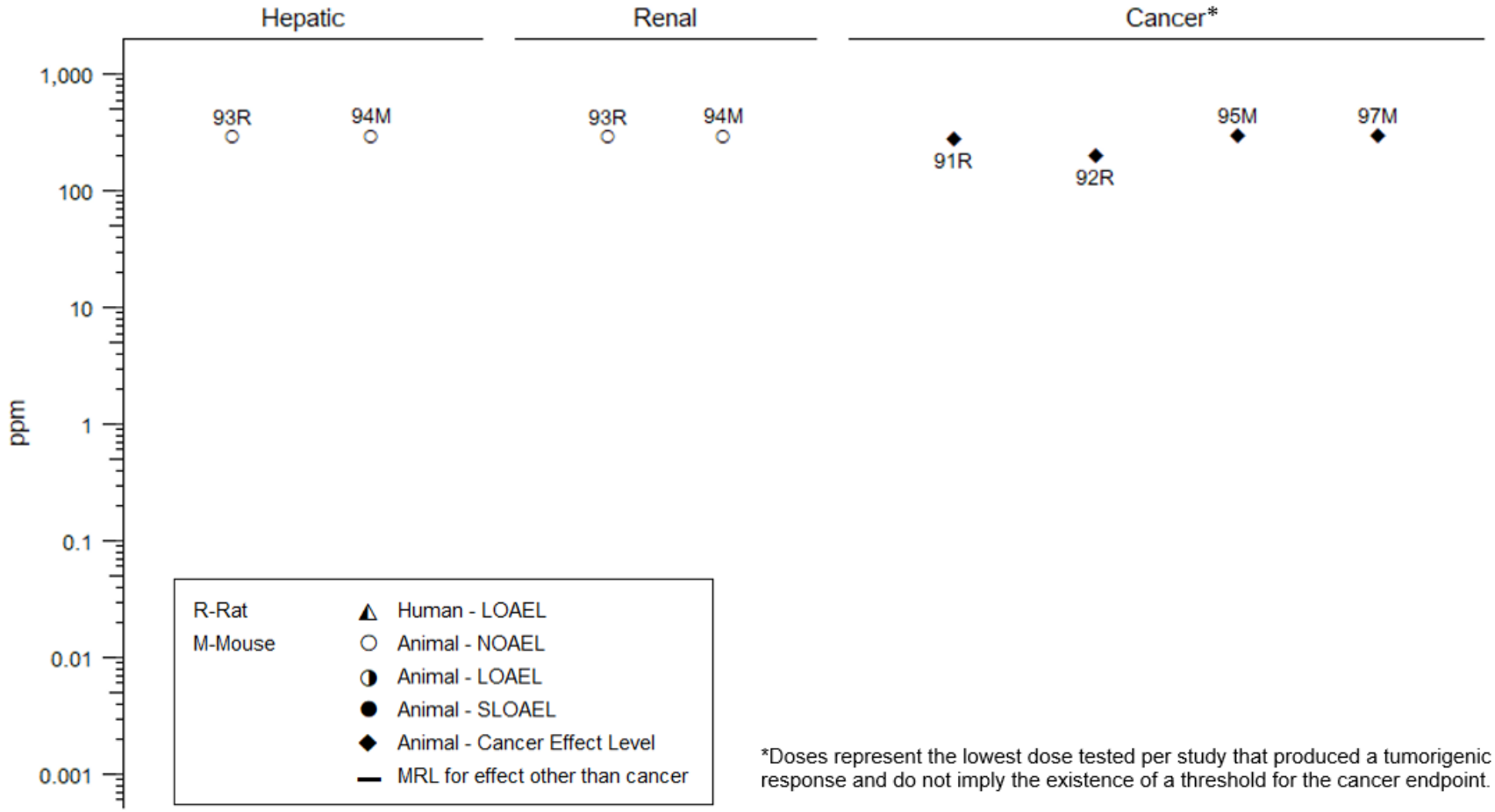


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Chronic (≥365 days)



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

Table 2-2. Levels of Significant Exposure to Benzene – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Cornish and Ryan 1965									
1	Rat (Sprague-Dawley) 10 M	Once (G)	88, 352, 760, 810, 930, 1,040, 1,870	CS, LE	Death			810	LD_{50} (fasted rats)
Exxon 1986									
2	Rat (Sprague-Dawley) 20–22 F	GDs 6–15 daily (G)	0, 50, 250, 500, 1,000	BW, FI, GN, LE, DX, OW, RX, CS	Bd wt	500	1,000		Maternal body weight decreased 11%
					Renal	1,000			
					Dermal		50		Alopecia of hindlimbs and trunk
					Repro	1,000			
					Develop	1,000			
Kanada et al. 1994									
3	Rat (Sprague-Dawley) 4–5 M	Once (G)	0, 950	BC	Neuro		950		Altered neurotransmitter concentrations in brain tissue
Kitamoto et al. 2015									
4	Rat (Crl:CD(SD)) 5 M	3 days (GO)	0, 500, 1,000, 2,000	HP	Gastro	2,000			
					Hepatic	2,000			
Wolf et al. 1956									
5	Rat (Wistar) 25 M	Once (GO)	Not reported	CS, OF, BW, BC, OW, GN, HP, LE	Death			5,600	LD_{50}
Huang et al. 2013									
6	Mouse (C57BL/6) 8 M	10 days 5 days/week 2 weeks (GO)	0, 200	HE	Hemato		200		Decreased peripheral WBCs

Table 2-2. Levels of Significant Exposure to Benzene – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Seidenberg et al. 1986									
7	Mouse (ICR/SIM) 30 F	GDs 8–12 (GO)	0, 1,300	DX, BW	Develop	1300			
INTERMEDIATE EXPOSURE									
Bahadar et al. 2015a									
8	Rat (Wistar) 6 M	4 weeks (GO)	0, 200, 400, 800	FI, WI, BW, BC, OW	Bd wt	800			
					Endocr		200		Increased plasma insulin
Bahadar et al. 2015b									
9	Rat (Wistar) 6 M	4 weeks (GO)	0, 200, 400, 800	BC, OF	Endocr		200		Hyperglycemia following glucose challenge
Heijne et al. 2005									
10	Rat (Fischer-344) 5 M	28 days (GO)	0, 10, 200, 800	BW, FI, WI, HE, BC, UR, OW, HP	Bd wt	200	800		Terminal body weights decreased by 19.5%
					Hemato	10	200		Decreased peripheral WBCs and lymphocytes, decreased relative spleen and thymus weights
					Hepatic	800			
Karaulov et al. 2017									
11	Rat (Wistar) 12–47 M	45, 90, or 135 days (W)	0, 526	BW, OW, IX	Hemato		526		Decreased splenocytes and number of CD^{4+} and CD^{4+}/CD^{8+} T-cells, decreased absolute thymus weight
					Immuno		526		Increased production of interleukins by splenic lymphocytes
NTP 1986									
12	Rat (F-344/N) 10–15 M, 10–15 F	60–120 days (3–17 weeks) 5 days/week (GO)	0, 25, 50, 100, 200, 400, 600	LE, CS, BW, FI, HE, GN, HP	Bd wt	100	200	400	At 120 days, decreased body weight gain; LOAEL: 14% in males; 16% in females; SLOAEL: 20% in males and females
					Resp	600			
					Cardio	600			

Table 2-2. Levels of Significant Exposure to Benzene – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Gastro	600			
					Hemato		25		Decreased peripheral WBCs at 120 days
					Musc/skel	600			
					Hepatic	600			
					Renal	600			
					Endocr	600			
					Immuno	100	200	600	
					Neuro	600			
Rafati et al. 2015									
13	Rat (Sprague-Dawley) 10 M	4 weeks (GW)	0, 200	HP, NX	Neuro			200	Reduced motor function, increased anxiety; decreased cerebellar volume, total cerebellar cells, Purkinje cells, glial cells, and neurons
Taningher et al. 1995									
14	Rat (Fischer-344) 6–12 M	6 weeks 5 days/week (GO)	400	HP, BW, OW	Bd wt	400			
Wolf et al. 1956									
15	Rat (Wistar) 10 F	6 months 5 days/week (GO)	0, 1, 10, 50, 100	CS, OF, BW, BC, OW, GN, HP	Hemato	1	50		Decreased WBCs and RBCs
Banik and Lahiri 2005									
16	Mouse (Swiss) 8–10 M	1 month (W)	0, 41, 82	NX	Neuro		41		Impaired short-term memory; decreased serotonin (5-hydroxytryptamine) level in serotonergic neurons
Cui et al. 2022									
17	Mouse (C57BL/6J) 11–12 M	4 weeks 6 days/week (GO)	0, 1, 10, 100	FI, BW, BC, HE, HP	Bd wt	100			
					Hemato		1		Decreased peripheral WBCs

Table 2-2. Levels of Significant Exposure to Benzene – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Fan 1992									
18	Mouse (C57BL/6) 5 M	28 days (W)	0, 27, 154	CS, FI, WI, BW	Hemato		27		Decreased splenic cellularity
					Immuno		27		Decreased splenic lymphocytes production of IL-2
Hsieh et al. 1988									
19	Mouse (CD-1) 5 M	4 weeks *ad libitum* (W)	0, 8, 40, 180	BW, FI, WI, IX	Hemato		8		Decreased peripheral RBCs and WBCs, increased MCV
					Immuno		8		Altered splenic lymphocyte proliferative response to mitogens, altered splenic lymphocyte cytotoxic response to tumor cells
Hsieh et al. 1990									
20	Mouse (CD-1) 5 M	4 weeks (W)	0, 31.5	CS, BW, FI, WI, BC, HE, GN, OW	Bd wt	31.5			
					Hemato		31.5		Decreased peripheral RBCs and WBCs, decreased relative thymus weight
					Hepatic	31.5			
					Renal	31.5			
					Immuno		31.5		Decreased splenic lymphocyte proliferative response to mitogens, decreased splenic lymphocyte cytotoxic response to tumor cells, decreased splenic lymphocyte IL-2 production in response to mitogens
Hsieh et al. 1991									
21	Mouse (CD-1) NS M	4 weeks *ad libitum* (W)	0, 8, 40, 180	BW, HP, FI, BC, WI	Immuno	8	40		Decreased splenic lymphocyte IL-2 production in response to mitogens
Li et al. 2018									
22	Mouse (Nrf2+/+) 15 M	4 weeks 6 days/week (GO)	0, 0.1, 1.0, 10.0, 100.0	HE, HP	Hemato	0.1[b]	1		Decreased numbers of WBCs, lymphocytes, neutrophils, and monocytes

Table 2-2. Levels of Significant Exposure to Benzene – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NTP 1986									
23	Mouse (B6C3F1) 10–15 M, 10–15 F	60–120 days (17 weeks) 5 days/week (GO)	0, 25, 50, 100, 200, 400, 600	LE, CS, BW, FI, HE, GN, HP	Bd wt	600			
					Resp	600			
					Cardio	600			
					Gastro	600			
					Hemato	400 F	600 F		Decreased peripheral lymphocytes
						25 M	50 M		Decreased peripheral WBCs
					Musc/skel	600			
					Hepatic	600			
					Renal	600			
					Endocr	600			
					Neuro	400	600		Intermittent tremors
					Repro	600			
Shell 1992									
24	Mouse (B6C3F1) 12 F	30 days *ad libitum* (W)	0, 12, 195, 350	BW, WI, HE, BC, OW, GN, HP	Bd wt	350			
					Hemato	12	195		Decreased peripheral WBCs
					Hepatic	350			
					Renal	350			
CHRONIC EXPOSURE									
Lan et al. 2004a									
25	Human 390 B	6.1 years (average) (occupational)	<0.04, 0.57, 2.85, 28.73	HE	Hemato		0.00091[c]		Decreased peripheral WBCs and platelets; route-to-route extrapolation from the chronic-duration oral MRL

Route-to-route extrapolation from the reported LOAEL of 0.57 ppm for occupational exposure was used by ATSDR to estimate equivalent oral dose.

Table 2-2. Levels of Significant Exposure to Benzene – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Maltoni et al. 1983									
26	Rat (Sprague-Dawley) 30–35 M, 30–35 F	52 weeks 4–5 days/week 1 time/day (GO)	0, 50, 250	LE, CS, BW, HP, GN	Death			250 F	9/35 died
					Cancer			50 F	CEL: at 144 weeks, Zymbal gland carcinoma
Maltoni et al. 1983									
27	Rat (Sprague-Dawley) 40–50 M, 40–50 F	92 weeks 4–5 days/week 1 time/day (GO)	0, 500	LE, CS, BW, HP, HE	Hemato		500		Decreased peripheral RBCs and WBCs after 84 weeks
					Cancer			500	CEL: Zymbal gland carcinoma; oral cavity carcinoma
Maltoni et al. 1985, 1989									
28	Rat (Wistar) 40 M, 40 F	104 weeks 4–5 days/week 1 time/day (GO)	0, 500	BW, OW, FI, WI, GN, HP, CS	Cancer			500	CEL: Zymbal gland carcinoma
Maltoni et al. 1985, 1989									
29	Rat (Sprague-Dawley) 30–75 M	104 weeks 5 days/week (GO)	0, 500	BW, OW, FI, WI, GN, HP, CS	Cancer			500	CEL: Zymbal gland carcinoma, oral carcinoma, and forestomach acanthomas and dysplasia in both sexes; skin carcinoma in males; forestomach in situ carcinoma in females
NTP 1986									
30	Rat (F-344/N) 50 M, 50 F	2 years 5 days/week (GO)	M: 0, 50, 100, 200; F: 0, 25, 50, 100	LE, CS, BW, FI, HE, GN, HP	Death			100 F	20/50 deaths in females
								200 M	30/50 deaths in males
					Bd wt	100 F			
						100 M		200 M	Body weights decreased 23% in 103 weeks
					Resp	100 F			
						200 M			

Table 2-2. Levels of Significant Exposure to Benzene – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Cardio	100 F			
						200 M			
					Gastro	100			
							200 M		Hyperkeratosis and acanthosis in nonglandular forestomach
					Hemato		25 F		Decreased WBCs and lymphocytes
							50 M		Decreased WBCs and lymphocytes
					Musc/skel	100 F			
						200 M			
					Hepatic	100 F			
						200 M			
					Renal	100 F			
						200 M			
					Dermal	100 F			
						200 M			
					Ocular	100 F			
						200 M			
					Endocr	100 F			
						200 M			
					Neuro	200 F			
						100 M			
					Repro	50 F	100 F		Endometrial polyps
						200 M			
					Cancer			25 F	CEL: Zymbal gland carcinomas or adenomas
								50 M	CEL: Squamous cell papillomas and carcinomas of the oral cavity

Table 2-2. Levels of Significant Exposure to Benzene – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Maltoni et al. 1985, 1989									
31	Mouse (RF/J) 45 M, 40 F	52 weeks 4–5 days/week (GO)	0, 500		Cancer			500	CEL: Lung tumors and leukemias in both sexes, mammary carcinoma in females
NTP 1986									
32	Mouse (B6C3F1) 50 M, 50 F	2 years 5 days/week (GO)	0, 25, 50, 100	LE, CS, BW, FI, HE, GN, HP	Death			100	41/50 males died; 35/50 females died
					Bd wt	50	100		Terminal body weight decreased 19% in males and 14% in females
					Resp	25 F	50 F		Alveolar hyperplasia
						50 M	100 M		Alveolar hyperplasia
					Cardio	100			
					Gastro		25		Epithelial hyperplasia and hyperkeratosis of forestomach
					Hemato		25		Decreased blood lymphocytes; increased frequency of micronucleated normochromatic peripheral erythrocytes
					Musc/skel	100			
					Hepatic	100			
					Renal	100			
					Dermal	100			
					Endocr		25		Hyperplasia of adrenal gland and harderian gland
					Neuro	100			
					Repro		25		Preputial gland hyperplasia in males; ovarian hyperplasia and senile atrophy in females

Table 2-2. Levels of Significant Exposure to Benzene – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Cancer			25	CEL: Lymphoma in both sexes; Harderian gland adenoma or carcinoma in males

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects between male and females are not indicated in Figure 2-3; only the levels of effect for the most sensitive sex are presented.
[b]Used to derive a provisional intermediate-duration oral MRL of $9x10^{-4}$ mg/kg/day for benzene; based on a NOAEL of 0.1 mg/kg/day, adjusted for continuous exposure ($NOAEL_{ADJ}$ of 0.09 mg/kg/day) and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). This MRL was also adopted for the acute-duration oral MRL. See Appendix A for more detailed information regarding the MRL.
[c]Used to derive a provisional chronic-duration oral MRL of $3x10^{-4}$ mg/kg/day for benzene; based on a route-to-route extrapolation of the chronic-duration inhalation MRL of 0.002 ppm. The chronic-duration inhalation MRL (0.002 ppm) was converted to an equivalent oral dose of $9.1x10^{-4}$ mg/kg/day using EPA (1988) human reference values for inhalation rate and body weight, and a relative bioavailability factor to adjust for differences in absorption of benzene. The equivalent oral dose was divided by a modifying factor of 3 for route-to-route extrapolation. See Appendix A for more detailed information regarding the MRL.

ADJ = adjusted; B = both males and females; BC = serum (blood) chemistry; Bd wt or BW = body weight; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); FI = food intake; (G) = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; (GO) = gavage in oil; (GW) = gavage in water; HE = hematology; Hemato = hematological; HP = histopathology; IL-2 = interleukin-2; Immuno = immunological; IX = immune function; LD_{50} = median lethal dose; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCV = mean corpuscular volume; MRL = minimal risk level; Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OF = organ function; OW = organ weight; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive function; SLOAEL = serious lowest-observed-adverse-effect level; UR = urinalysis; (W) = water; WBC = white blood cell; WI = water intake

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

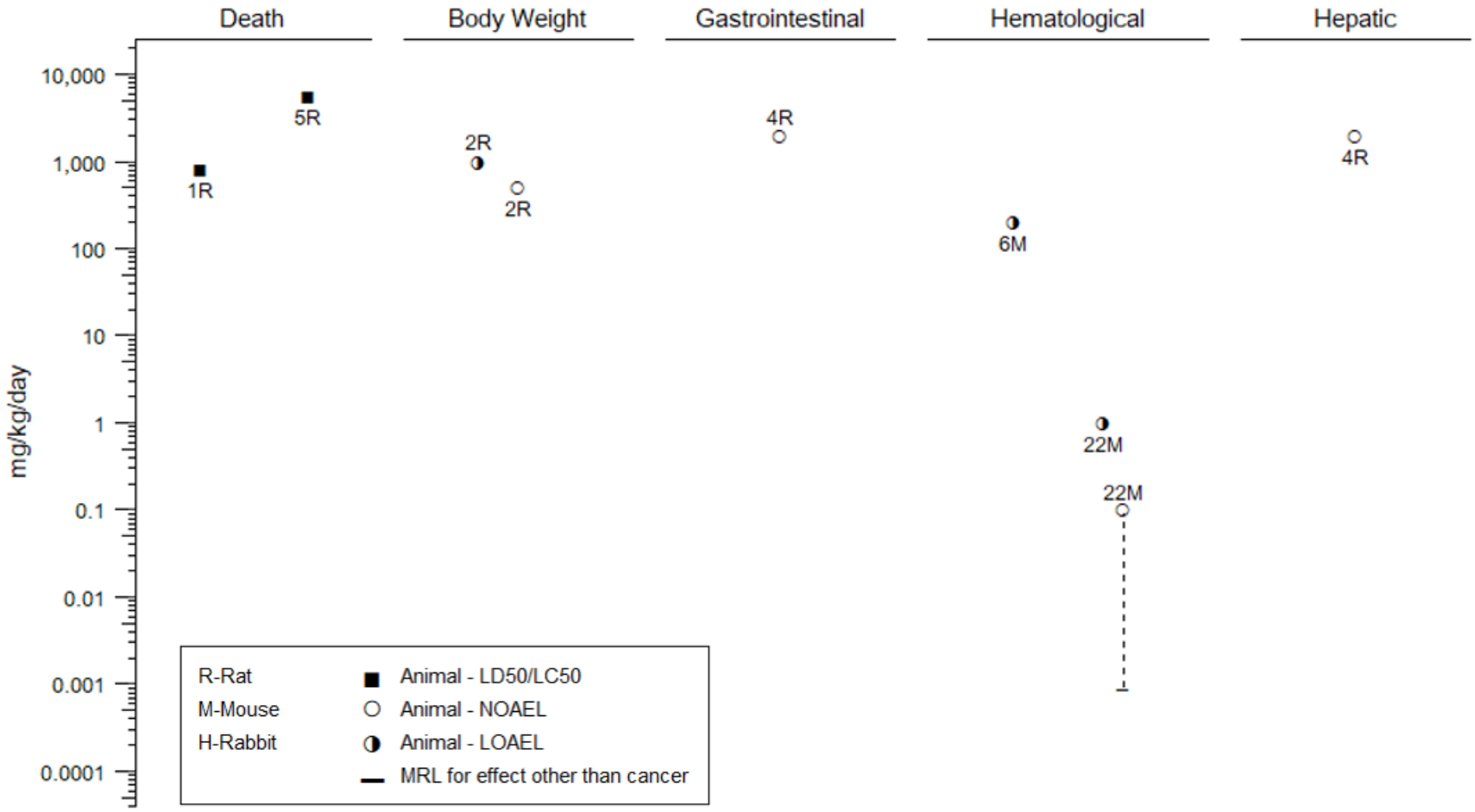


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Acute (≤14 days)

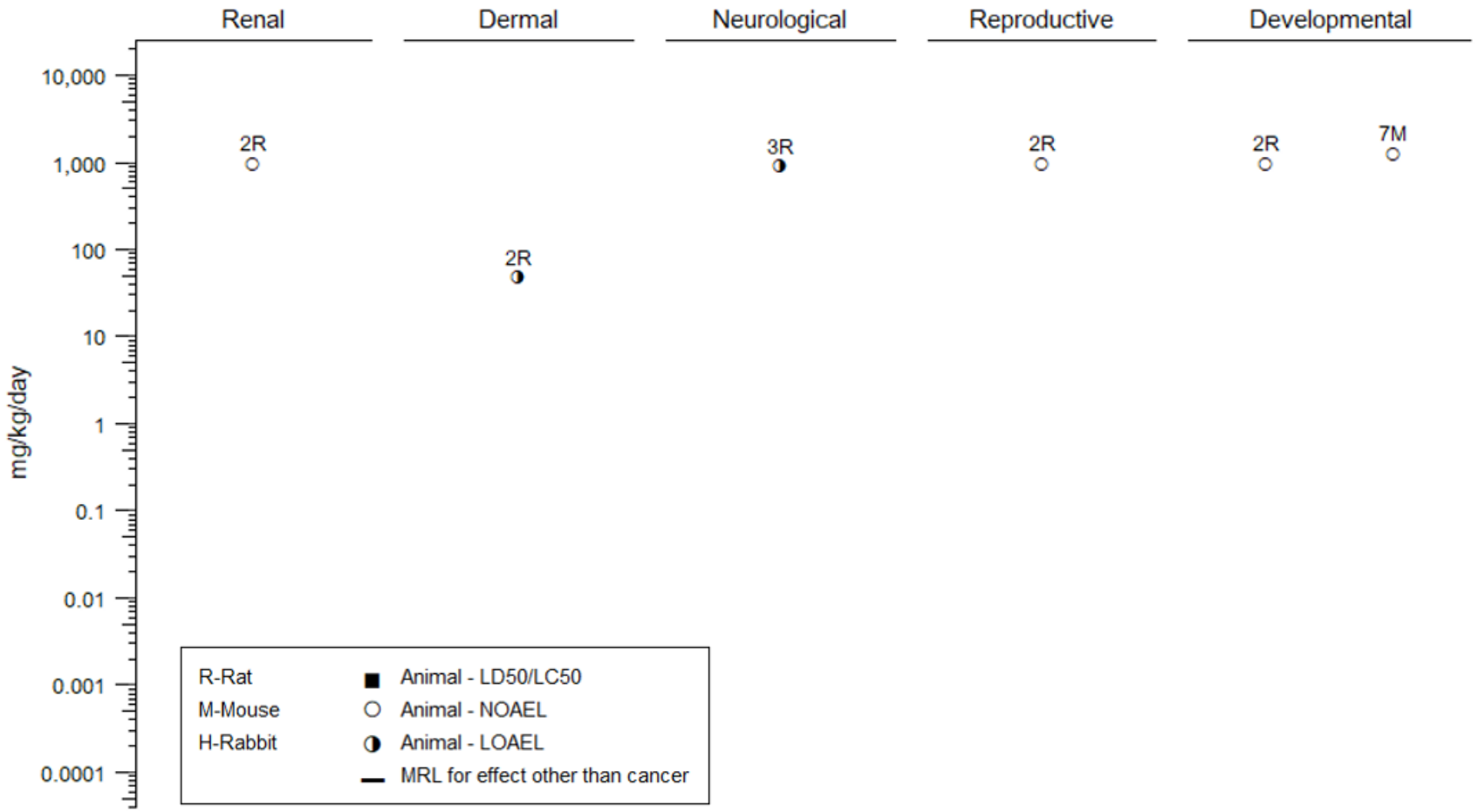


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Intermediate (15–364 days)

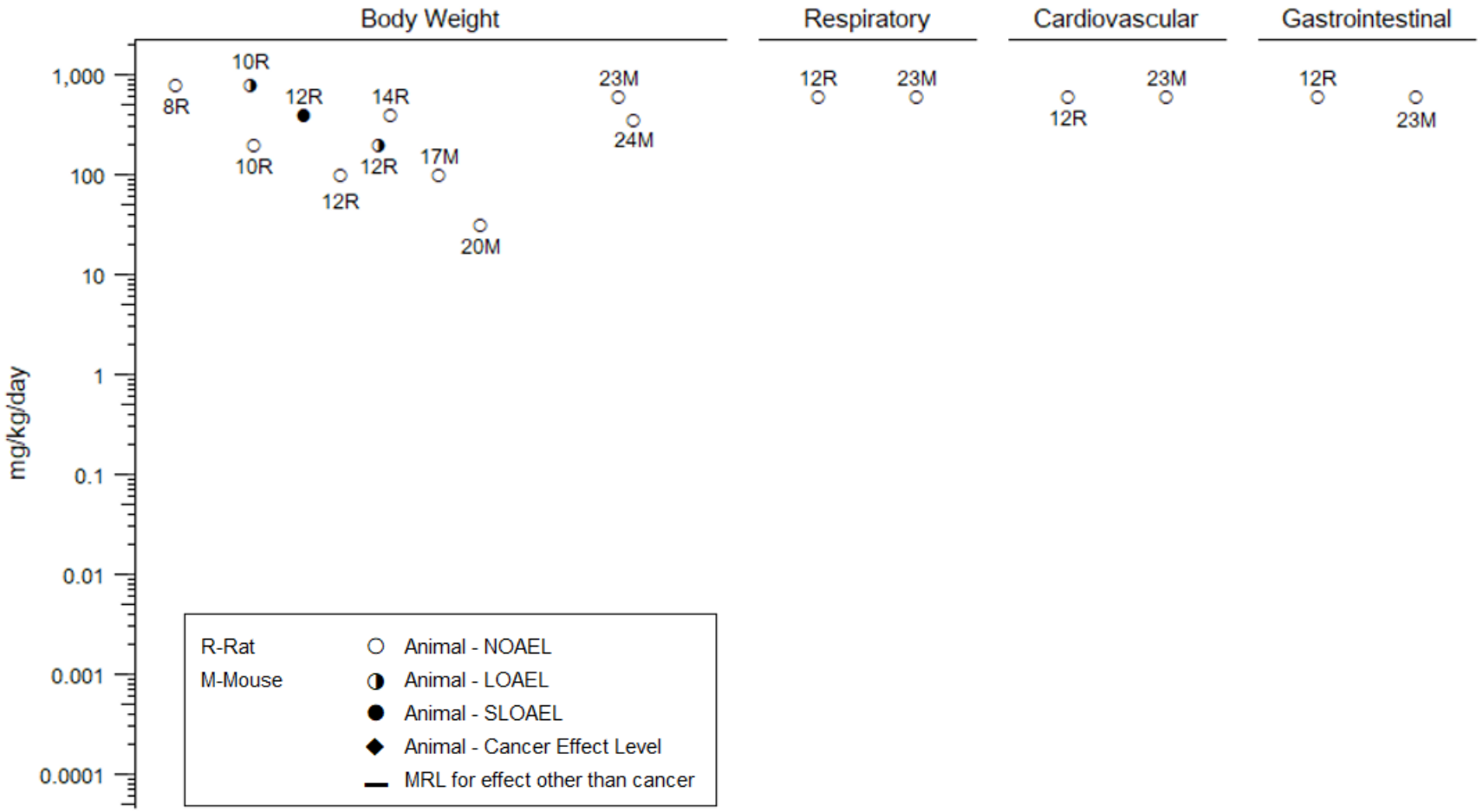


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Intermediate (15–364 days)

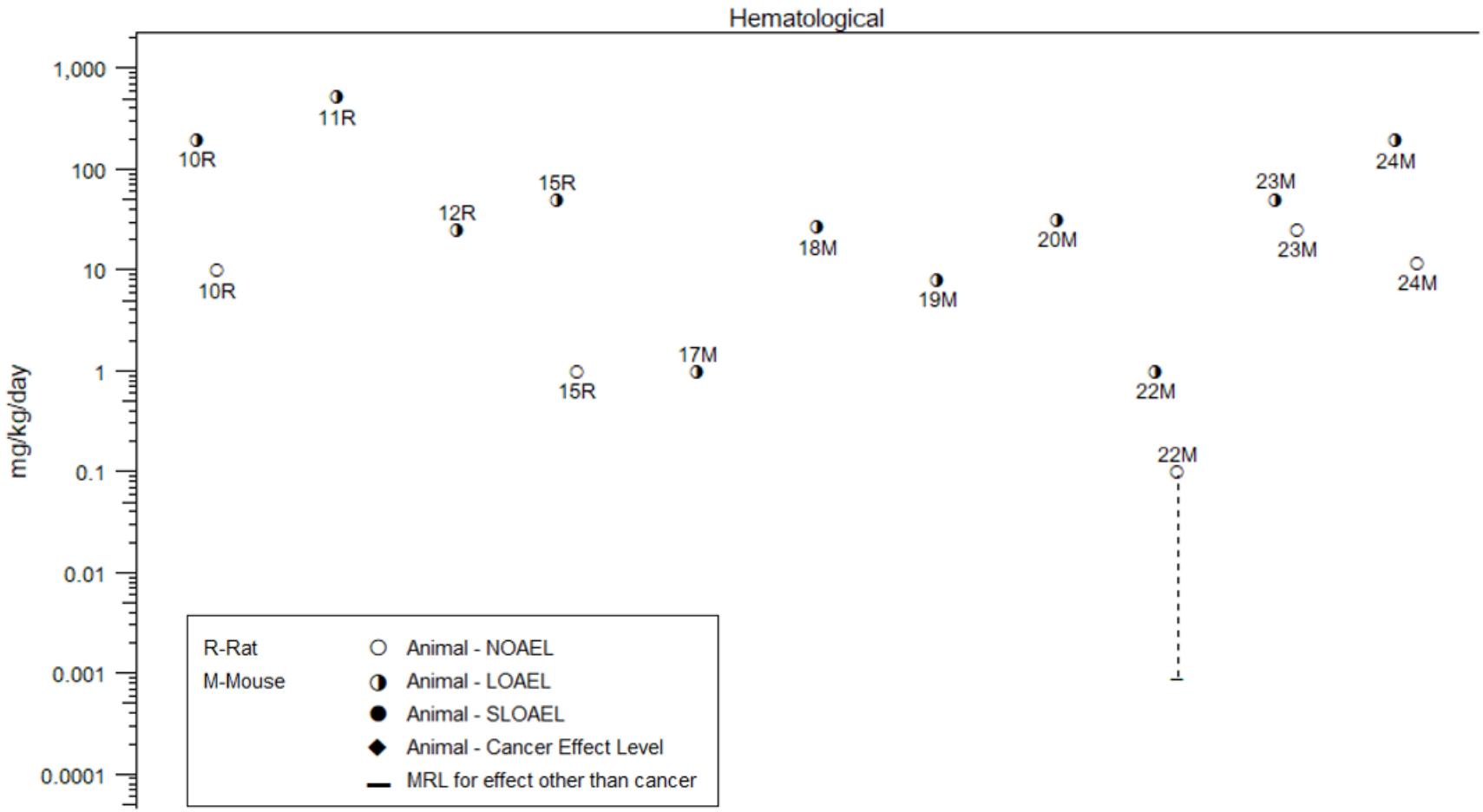


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Intermediate (15–364 days)

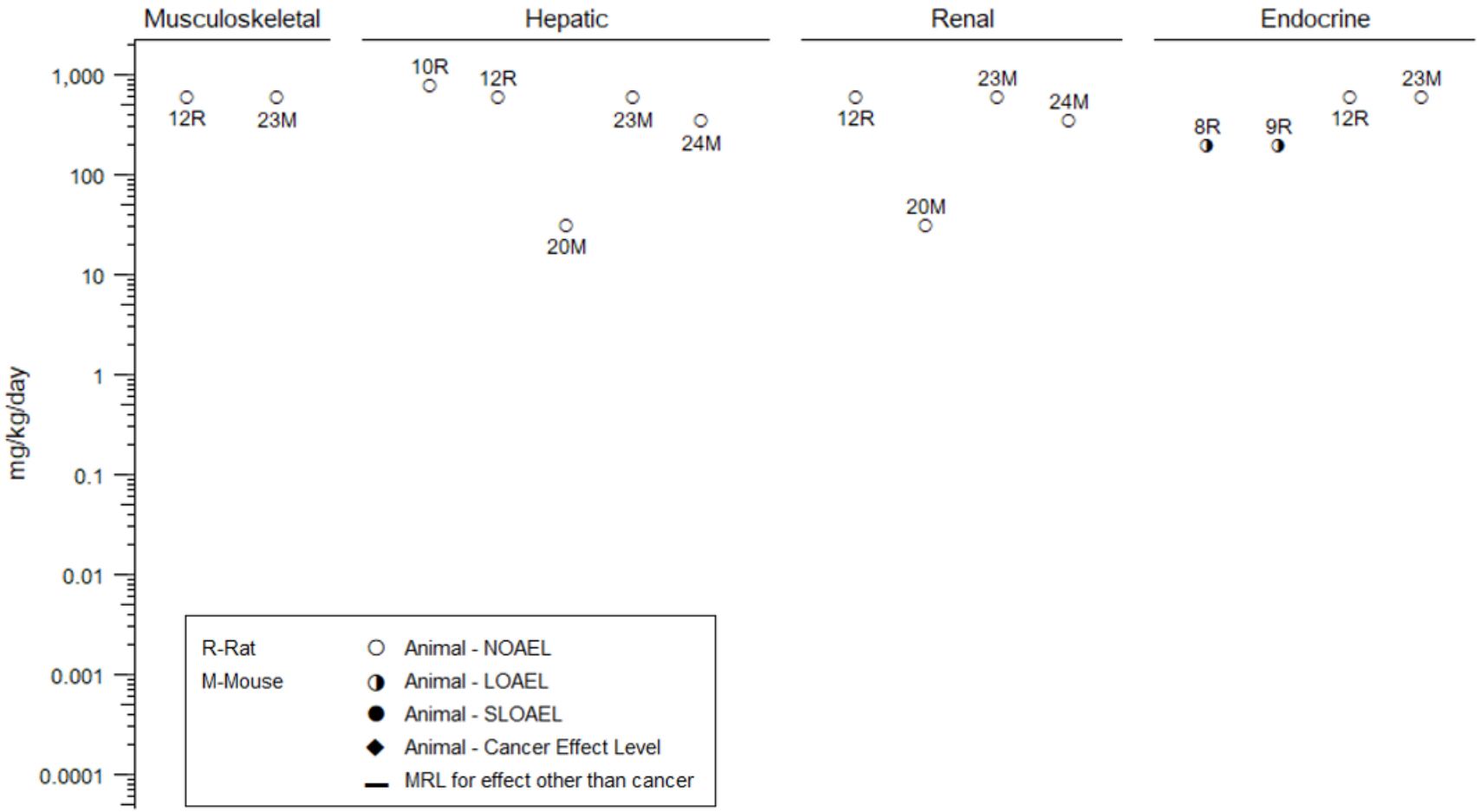


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Intermediate (15–364 days)

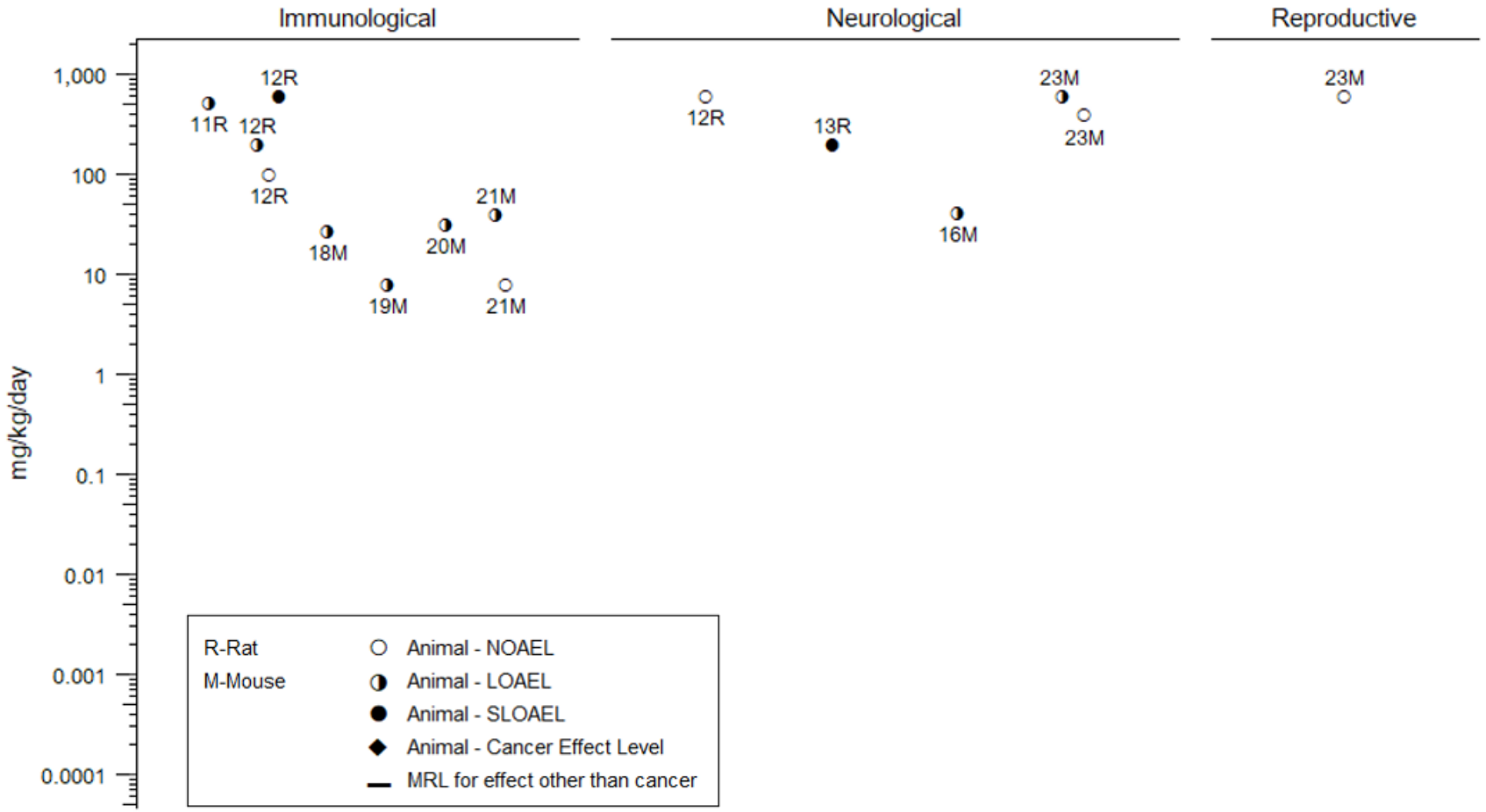


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Chronic (≥365 days)

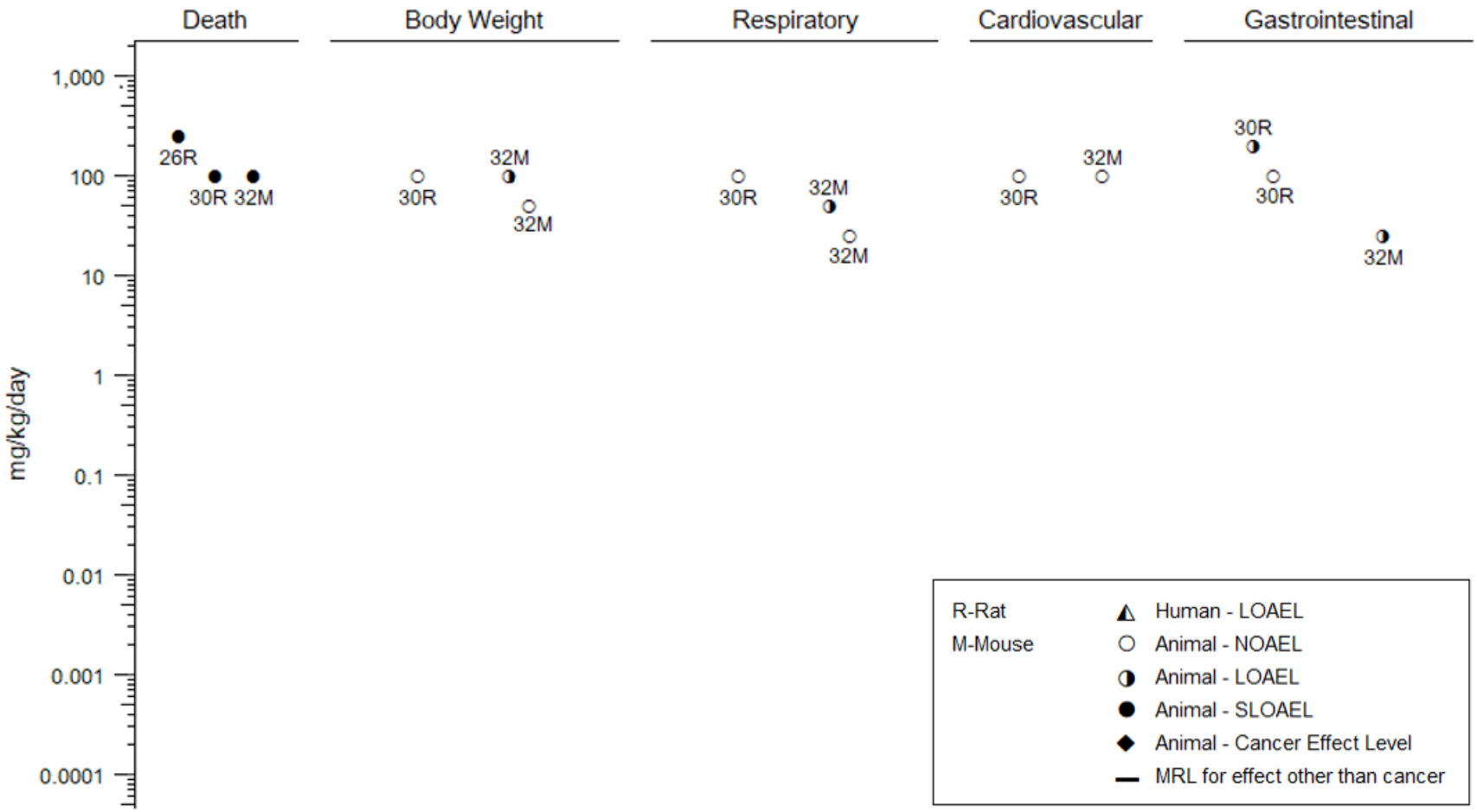


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Chronic (≥365 days)

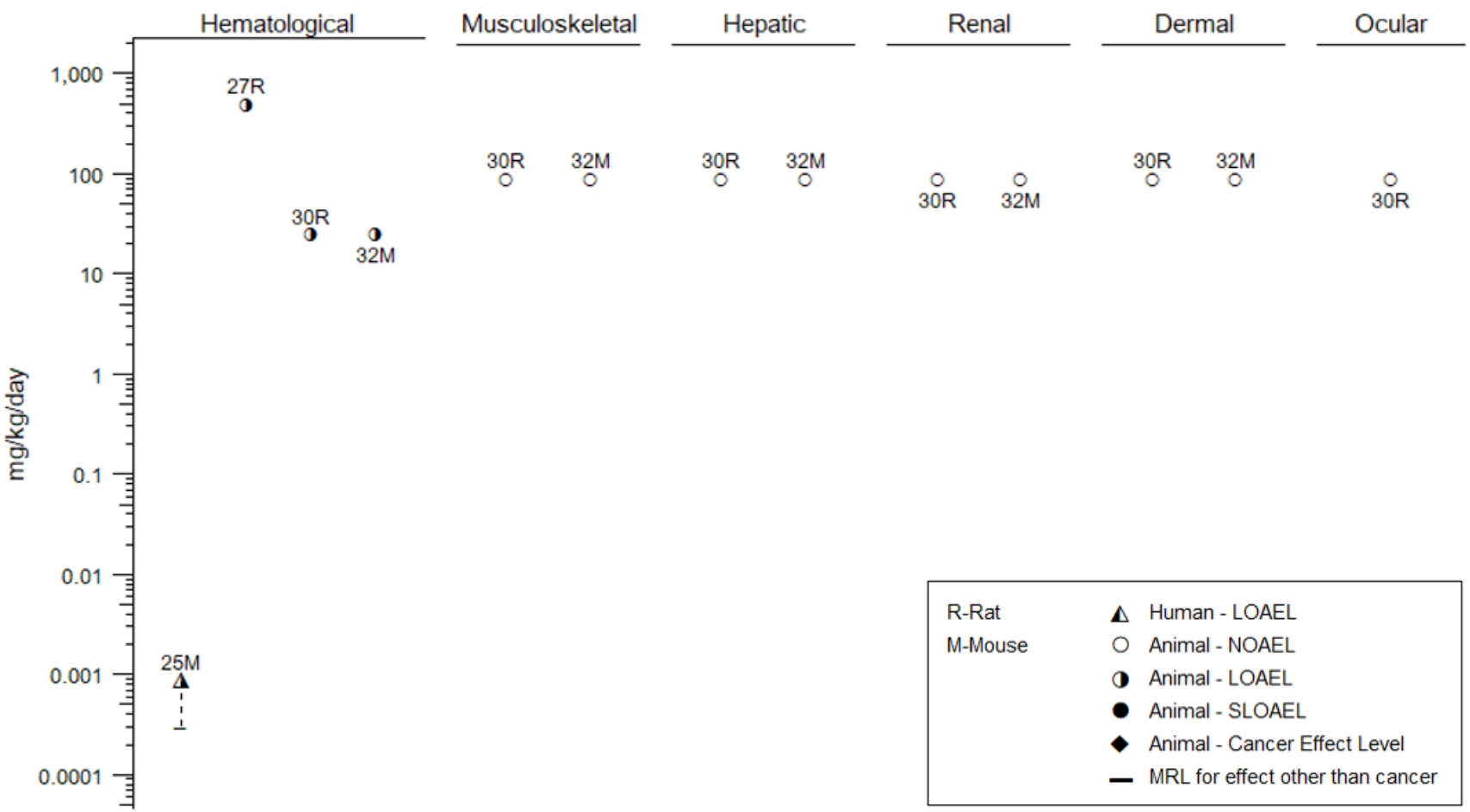


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Chronic (≥365 days)

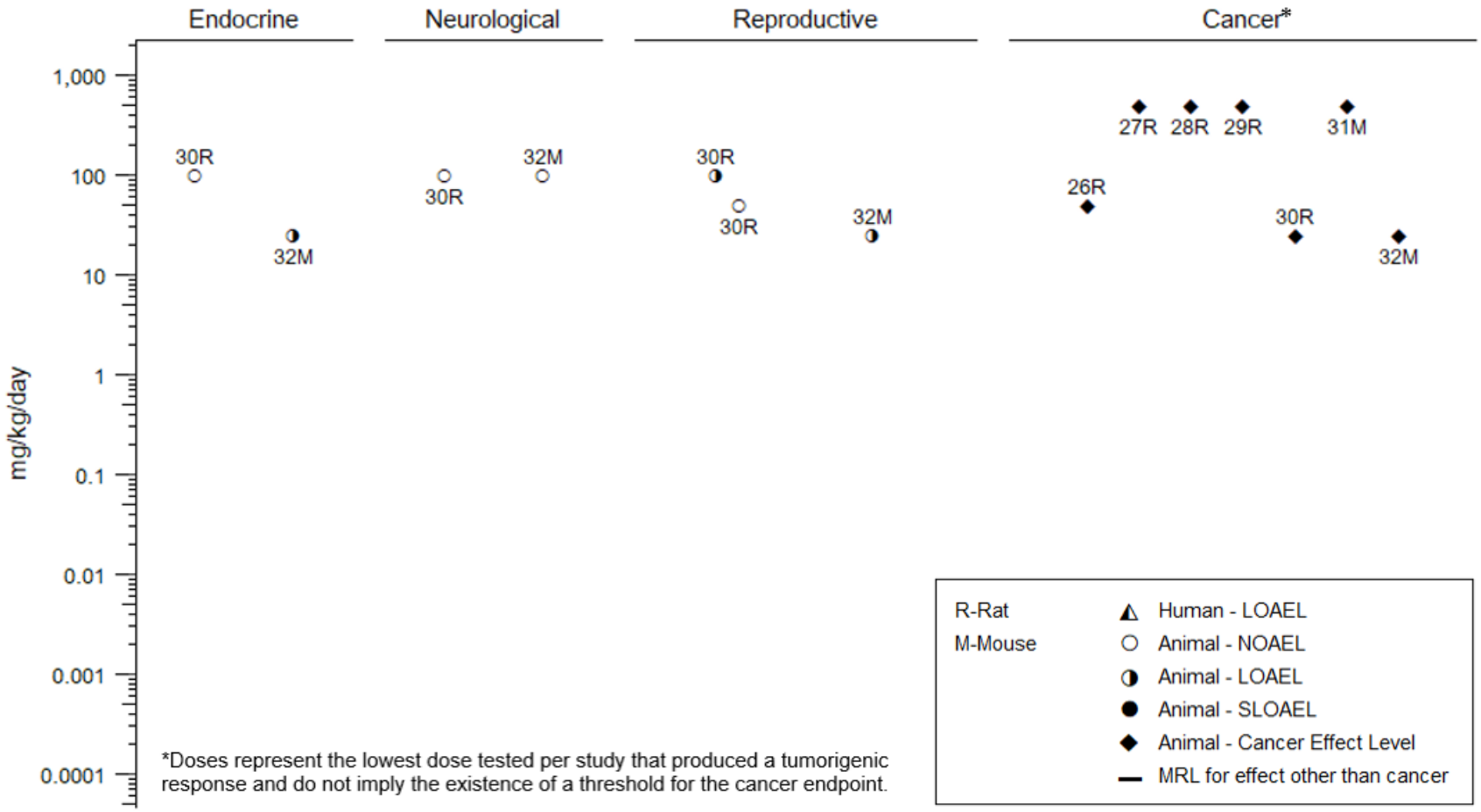


Table 2-3. Levels of Significant Exposure to Benzene – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Midzenski et al. 1992								
Human 15 M	1–21 days 2.5–8 hours/day	>60 ppm	BC, CS, HE	Dermal		>60		Mucous membrane and skin irritation after 2 days
Wolf et al. 1956								
Rabbit (NS) NS	Once	2 drops (undiluted)	CS, BW, BC, GN, OW, HP, OF	Ocular		2		Moderate conjunctival irritations; light corneal injury
INTERMEDIATE EXPOSURE								
Shell 1980								
Rat (CD) 19–20 M, 76–80 F	10 weeks 5 days/week 6 hours/day	0, 1, 10, 30, 300 ppm	LE, BW, HP, GN	Ocular	1 M	10 M		Lacrimation during the first 3 weeks of treatment
CHRONIC EXPOSURE								
Yin et al. 1987b								
Human 300 B	61 months (mean) (occupational)	M: 33 ppm; F: 59 ppm (mean time-weighted average)	CS, BC, UR, BI	Ocular		59 F 33 M		Eye irritation

B = both males and females; BW = body weight; CS = clinical signs; BC = serum (blood) chemistry; BI = biochemical changes; F = female(s); GN = gross necropsy; HE = hematology; HP = histopathology; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight; UR = urinalysis

2.2 DEATH

Studies of mortality of humans exposed to inhaled and oral benzene provide very limited quantitative data. Case reports of fatalities due to acute-duration benzene inhalation and oral exposures have appeared in the literature since the early 1900s. Following accidental inhalation exposure to high levels of benzene, 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). No studies were located regarding noncancer-related mortality in humans following long-term inhalation exposure to benzene. Cancer-related mortality data for chronic-duration human occupational exposure to benzene are presented in Section 2.18.

Acute lethal oral doses for humans have been estimated at 10 mL (8.8 g or 125 mg/kg for a 70-kg person) (Thienes and Haley 1972). Lethality in humans has been attributed to respiratory arrest, central nervous system depression, or cardiac collapse (Greenburg 1926). Accidental ingestion and/or attempted suicide with lethal oral doses of benzene have produced the following signs and symptoms: staggering gait; vomiting; shallow and rapid pulse; somnolence; and loss of consciousness, followed by delirium, pneumonitis, collapse, and then central nervous system depression, coma, and death (Thienes and Haley 1972). Ingestion of lethal doses may also result in visual disturbances and/or feelings of excitement and euphoria, which may quite suddenly change to weariness, fatigue, sleepiness, convulsion, coma, and death (NIH 1940).

Lethality of benzene in laboratory animals has been evaluated for acute-, intermediate-, and chronic-duration inhalation exposures. Note that deaths of laboratory animals due to cancer are discussed in Section 2.19. Death has been observed following acute-duration inhalation exposure to high concentrations of benzene, with little information on lethality of low concentrations. An inhalation median lethal concentration (LC_{50}) value for rats was calculated as 13,700 ppm for a 4-hour exposure (Drew and Fouts 1974). Additionally, four of six 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 benzene concentrations up to 4,862 ppm, 6 hours/day for 5 days showed no lethality. Exposure of rabbits to 45,000 ppm of benzene for up to approximately 30 minutes caused narcosis that was followed by the death of all exposed animals (Carpenter et al. 1944). There is conflicting evidence regarding lethality following repeated acute-duration exposures to lower benzene concentrations. A study in mice exposed to 300 ppm for up to 12 hours/day for 2 weeks found that survival was decreased by 5.3 weeks during a 15-week post-exposure period (Mukhopadhyay and Nath 2014). However, exposure of mice to 400 ppm for 2 weeks did not cause death in mice; this study did not have an observation period following exposure (Cronkite et al. 1985).

Intermediate-duration exposures (6 hours/day, 5 days/week for 50 days) of male CD-1 mice to benzene at doses of 9.6 ppm caused no increase in mortality, although mice exposed to 302 ppm benzene under the same regimen for a total of 26 weeks showed mortality approaching 50% (Green et al. 1981b). In male mice, exposure to 300 ppm benzene for 6 hours/day, 5 days/week for 4 weeks, the median cumulative survival time following the exposure duration was calculated as 4.8 weeks compared to 23.4 weeks in controls (Mukhopadhyay and Nath 2014).

Snyder et al. (1978, 1980, 1982) conducted a series of lifetime inhalation studies examining survival time in rats and mice exposed to benzene concentrations of 100 and/or 300 ppm benzene. In Sprague-Dawley rats that received 300 ppm benzene, the median survival time was 51 weeks compared to 65 weeks for controls (Snyder et al. 1984). Companion studies were also conducted in AKR mice exposed to 100 and 300 ppm benzene (Snyder et al. 1978, 1980) and in C57BL mice exposed to 300 ppm benzene (Snyder et al. 1980). In AKR mice, the median life span was decreased at 300 ppm (300 ppm: 11 weeks; control: 39 weeks). In C57BL mice, the median life span was 41 weeks at 300 ppm, compared to 75 weeks in controls. Median survival was also decreased in CD-1 mice exposed to 300 ppm (300 ppm: 179 days; control: 369 days) (Snyder et al. 1982).

For oral exposure of animals to benzene, data are available for all exposure duration categories. Oral median lethal dose (LD_{50}) values for rats ranged from 810 to 5,600 mg/kg; the values varied with age and strain of the animals (Cornish and Ryan 1965; Wolf et al. 1956). The LD_{50} in fasted rats was slightly lower (810 mg/kg) than in nonfasted rats (930 mg/kg) (Cornish and Ryan (1965). An intermediate-duration oral study did not find an increase in mortality in Fischer 344 rats or B6C3F1 mice treated with 600 mg/kg/day for up to 17 weeks (NTP 1986).

Chronic-duration oral exposure studies in rats were conducted by Maltoni et al. (1983) and NTP (1986). Sprague-Dawley rats were exposed to benzene in olive oil by gavage at 0, 50, or 250 mg/kg/day for 4–5 days weekly for 52 weeks and then kept under supervision until the occurrence of spontaneous death (Maltoni et al. 1983). At 250 mg/kg/day, 13 of 35 males and 9 of 35 females died. In a companion study, Sprague-Dawley rats were exposed to 500 mg/kg/day benzene in olive oil by gavage 4–5 days/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Mortality rates varied with male controls having 42% mortality compared to the 500-mg/kg/day group with 27.5% mortality. Females in the 500-mg/kg/day group had a slight increase in mortality at 6% from control animals. In a chronic-duration oral study conducted by 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 100 mg/kg/day benzene. B6C3F1 mice given 100 mg/kg/day also had increased mortality compared to control mice.

2.3 BODY WEIGHT

One study was located regarding body weight effects in humans after exposure to benzene (Zhang et al. 2020). This cross-sectional study examined 1,331 exposed petrochemical plant workers and 338 control workers in China. The primary route of exposure is assumed to be inhalation, although dermal exposure cannot be ruled out. Exposure to benzene was assessed by urinary levels of the benzene metabolite, *S*-phenylmercapturic acid (PhMA). Median levels of urinary PhMA were 0.37 and 0.18 μg/g in exposed and control groups, respectively. In exposed workers, the percentage of body fat (based on body mass index [BMI], age, and gender) was decreased by 11.2% compared to controls.

In laboratory animals, studies on effects of inhaled benzene on body weight have been conducted in rats and mice for acute and intermediate exposure durations. The study results did not show consistent effects. Decreased terminal body weight (15%) was observed in DBA/2 mice after exposure to 300 ppm benzene in air for 6 hours/day, 5 days/week for 2 weeks (Chertkov et al. 1992). Similarly, decreased terminal body weight (16 and 18% at 7 and 14 days, respectively) has also been noted in BALC/c mice exposed to 200 ppm of benzene for 6 hours/day for 7 or 14 days; no body weight effects were observed at 50 ppm (Aoyama 1986). No effects on body weight were observed in CD-1 mice exposed to concentrations up to 4,862 ppm for 6 hours/day, for 5 days (Green et al. 1981b).

Results of studies on intermediate-duration inhalation exposure show effects on body weight, but only at high exposure concentrations (>4,000 ppm). No change in body weight was observed in Sprague-Dawley

rats or CD-1 mice exposed to 300 ppm benzene for 13 weeks (Ward et al. 1985) or in CD-1 mice exposed to a lower concentration of 9.6 ppm for 50 days (Green et al. 1981b). However, at higher exposure levels, terminal body weight was decreased by approximately 12% in female Wistar-Albino rats exposed to 8,000 ppm of benzene for 30 minutes/day over 28 days (Harrath et al. 2022), although no changes were observed at exposure concentrations up to 4,000 ppm.

Effects of lifetime inhalation exposure to benzene on body weight were evaluated in a series of studies in different strains of mice (Snyder et al. 1978, 1980, 1982). In these studies, mice lost weight over the course of exposure to 300 ppm benzene. Weight losses in mouse strains AKR/J, C57BL, and CD-1 were 26, 20, and 17%, respectively. No effect on weight loss was observed in 100 ppm in AKR/J mice (Snyder et al. 1978, 1980).

Studies have also evaluated effects of inhalation exposure on maternal body weight in rats and rabbits. No effects (≥10%) on maternal body weight were reported in rats exposed by inhalation to 500 ppm benzene during gestation days (GDs) 6–15 (Kuna and Kapp 1981) or in rats exposed to doses up to 300 ppm during premating, mating, gestation, and lactation (Kuna et al. 1992). Maternal body weight gain decreased in rats exposed by inhalation to 50 ppm benzene during GDs 6–15 (Kuna and Kapp 1981). Likewise, rats exposed 0 or 125 ppm benzene for 24 hours/day on GDs 7–14, maternal weight gain was decreased by 32% compared to controls (Tatrai et al. 1980a). In a companion study, maternal weight gain was decreased by 27% compared to controls in rats exposed to 47 ppm (Tatrai et al. 1980b). Maternal weight gain was decreased by 62% compared to weight gain in controls in rabbits exposed to 313 ppm benzene on GDs 7–20 (Ungvary and Tatrai 1985).

Effects of benzene on body weight have been evaluated for acute-duration oral exposure in pregnant rats and for intermediate and chronic exposure durations in rats and mice. Pregnant Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day benzene on GDs 6–15 and killed on GD 20 (Exxon 1986). Maternal body weight was decreased by 11% at the high dose.

Most studies on intermediate-duration oral exposure to benzene did not observe effects on body weight. 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). In male C57BL/6 mice exposed to up to 85.7 mg/kg/day benzene via gavage in corn oil for 4 weeks, no effects on terminal body weights were observed (Cui et al. 2022). However, decreased white adipose tissue content and adipocytes and altered adipocyte size distribution in male C57BL/6 mice were observed at doses ≥1 mg/kg/day (Cui et al. 2022). No change in

body weight was observed in male Fischer 344 rats treated by gavage with 400 mg/kg/day benzene in corn oil for 5 days/week for 6 weeks (Taningher et al. 1995) or in male Wistar rats at 800 mg/kg/day for 4 weeks (Bahadar et al. 2015a). A study with higher doses reported decreased terminal body weight (by ~19.5%) in male F344 rats exposed to 800 mg/kg/day benzene in corn oil for 4 weeks (Heijne et al. 2005). Body weight was unaffected in male and female Fischer 344 rats given oral doses up to 100 mg/kg/day benzene in corn oil for 120 days; however, at 200 mg/kg/day, body weight gain was decreased 14 and 16% in males and females, respectively (NTP 1986). No effects on body weight were observed in female B6C3F1 mice exposed to doses up to 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). There was less than a 10% decrease in body weight of male and female B6C3F1 mice given oral doses of up to 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986).

NTP (1986) conducted 2-year oral exposure studies in rats and mice. Male rats and male and female mice exhibited body weight effects after chronic-duration exposure (NTP 1986). Terminal body weight in male rats was decreased by 23% relative to control at 200 mg/kg/day. In male and female mice given 100 mg/kg/day, terminal body weights decreased by 19 and 14%, respectively, relative to control (NTP 1986). However, female rats in the same study exposed to doses up to 100 mg/kg/day benzene did not show any change in body weight after 2 years of exposure (NTP 1986).

NTP (2007) administered benzene to groups of male and female haplo-insufficient $p16^{Ink4a}$/$p19^{Arf}$ mice (15/sex/group) by gavage (in corn oil) once/day, 5 days/week for 27 weeks at 0, 25, 50, 100, or 200 mg benzene/kg/day. Male mice exhibited dose-related lower mean body weight than controls, which was most notable for treatment weeks 14–27, at which time body weights of the 50, 100, and 200 mg/kg/day dose groups were 12, 22, and 24%, respectively, less than controls. However, no effect on body weight was observed in female mice. Note that studies on genetically altered animals are not included in the LSE table.

2.4 RESPIRATORY

Studies on respiratory effects of inhaled benzene in humans provide very limited quantitative data. Respiratory effects have been reported in humans after acute-duration (Avis and Hutton 1993; Midzenski et al. 1992; Winek and Collom 1971; Winek et al. 1967) or chronic-duration (Yin et al. 1987b) exposure to benzene vapors. The most severe respiratory effects were observed in studies with lethal exposure. After a fatal occupational exposure to benzene vapors on a chemical cargo ship for only minutes, autopsy reports on three victims revealed hemorrhagic, edematous lungs (Avis and Hutton 1993). Acute granular

tracheitis, laryngitis, bronchitis, and massive hemorrhages of the lungs were observed at autopsy of an 18-year-old male who died of benzene poisoning after intentional inhalation of benzene (Winek and Collom 1971). Similarly, acute pulmonary edema was found during the autopsy of a 16-year-old who died after sniffing glue containing benzene (Winek et al. 1967). Less severe effects were observed at nonlethal exposures. A recent case report of a car mechanic who aspirated benzene observed chemical pneumonitis; however, exposure was not estimated (Mohammed et al. 2020). Fifteen male workers employed in removing residual fuel from shipyard tanks for up to 3 weeks were evaluated for adverse effects (Midzenski et al. 1992). Mucous membrane irritation was noted in 80% and dyspnea was noted in 67% of the workers. The only information on exposure is that benzene levels were >60 ppm. In a chronic-duration study, nasal irritation and sore throat were reported by male and female workers exposed to 33 and 59 ppm benzene, respectively, for >1 year (Yin et al. 1987b).

Few studies have evaluated respiratory effects in animals after inhalation or oral exposure to benzene. Snyder et al. (1978, 1984) reported no treatment-related effects on lung tissue in male Sprague-Dawley rats exposed to 0, 100, or 300 ppm benzene 5 days/week, 6 hours/day for life. In addition, no adverse histopathological effects on lung tissue were observed in AKR/J mice exposed to 300 ppm benzene for life (Snyder et al. 1978, 1980).

Results of oral exposure studies on the respiratory system yield conflicting results. No histopathological lesions were observed in lungs, trachea, or mainstream bronchi of male and female Fischer 344 rats and B6C3F1 mice given gavage doses up to 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). NTP (1986) exposed rats and mice to oral benzene by gavage at doses up to 200 mg/kg/day (male rats) or 100 mg/kg/day (female rats, male and female mice) for 2 years. No histopathological lesions were observed in trachea, lungs, or mainstream bronchi in rats. However, in mice, the incidence of alveolar hyperplasia was increased at 50 and 100 mg/kg/day in females and at 100 mg/kg/day in males.

2.5 CARDIOVASCULAR

Few studies evaluated associations between benzene exposure and cardiovascular outcomes in humans. A cross-sectional study of adults (mean age 51 years, n=210) found that increased urinary *trans*,*trans*-muconic acid levels were associated with increased cardiovascular disease risk (Framingham Risk Score) (Abplanalp et al. 2017). However, urinary *trans*,*trans*-muconic acid is not specific for benzene, as it is also a metabolic product of preservative sorbic acid or sorbates found in food and beverages (IARC 2018). Therefore, these findings cannot be attributed to benzene alone.

Little information on cardiovascular effects of benzene in laboratory animals was located. Mice exposed to 50 ppm benzene for 6 weeks had decreased fractional shortening of the left ventricle during systole, but no histopathological lesions or other changes in cardiac function were observed (Zelko et al. 2021).

No histopathological lesions were observed in cardiac tissue from male and female Fischer 344 rats or B6C3F1 mice given oral doses up to 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). Similarly, after 2-year exposure at doses up to 200 mg/kg/day (male rats) or 100 mg/kg/day (female rats, male and female mice), no histopathological lesions were observed in the heart (NTP 1986).

2.6 GASTROINTESTINAL

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

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

Little information is available in gastrointestinal effects of benzene in animals, with only oral exposure studies identified. No histopathological lesions were observed in the stomach of rats following exposure to oral doses up to 2,000 mg/kg/day benzene in corn oil for 3 days (Kitamoto et al. 2015). No histopathological lesions were observed in esophageal and stomach tissue or in the small intestine and colon from male and female Fischer 344 rats or B6C3F1 mice given oral doses up to 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). After chronic-duration exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats, male and female mice), male rats exhibited hyperkeratosis and acanthosis in the nonglandular forestomach at 200 mg/kg/day and mice exhibited epithelial hyperplasia and hyperkeratosis in the forestomach at 25 mg/kg/day (NTP 1986).

2.7 HEMATOLOGICAL

The primary effect of benzene on the hematological system is disruption of hematopoiesis. This can lead to several types of observable changes. Cytopenia is a decline in numbers of circulating blood cells. Pancytopenia is the reduction in the number of all three major types of blood cells: erythrocytes (red

blood cells [RBCs]), thrombocytes (platelets), and leukocytes (WBCs). In adults, all three major types of blood cells are produced in the red bone marrow of the vertebrae, sternum, ribs, and pelvis. Lymphocytes are also produced in spleen and thymus, and erythrocytes are produced in the embryonic spleen. Hematopoietic tissues (marrow, spleen, thymus) contain immature cells, known as hematopoietic stem cells, that 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 bone marrow function is sufficiently impaired so that blood 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 damage progresses, bone marrow can become necrotic and filled with fatty tissue. Aplastic anemia can progress to a type of leukemia known as acute myelogenous leukemia or AML, which is discussed in Section 2.19.

Given the wide range of effects of benzene on hematopoietic tissues, delineation between hematological and immunological effects of benzene is not simple, since some effects observed in blood or hematological tissues (e.g., lymphocyte numbers) may contribute to impaired immune responses. In this discussion of hematological effects of benzene, the following types of effects have been classified as hematological, regardless of their potential impact on immunity: (1) changes in numbers of peripheral blood cells (erythrocytes, thrombocytes, leukocytes); (2) changes in cellularity of hematological tissues (marrow, spleen, thymus); (3) changes in numbers of stem cells, progenitor cells, or mature blood cells in hematological tissues; and (4) histopathological changes of hematopoietic tissues (marrow, spleen, thymus). This rather broad definition serves to allow a full discussion of effects on hematopoiesis in a single section of the profile. It also constrains the discussion of immunological effects, in Section 2.14 to the following effects: (1) changes in immune responses to antigens and (2) changes in function of cells that participate in immune responses.

No studies have provided reliable estimates of exposures that produce hematological effects following acute-duration exposure to inhaled benzene. The epidemiological evidence for hematological effects comes from studies of intermediate- or chronic-duration exposures. These studies provide evidence for hematological effects in association with exposures >0.5 ppm. Table 2-4 summarizes epidemiological studies that provide quantitative estimates of associations between intermediate- or chronic-duration exposures to benzene and hematological effects.

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to Benzene and Hematological Effects

Reference, study type, and population	Exposure concentration in air or biomarker	Outcome evaluated	Result
Workers			
Bassig et al. 2016 Cross-sectional; 250 shoe manufacture workers and 140 control workers (China)	PAir median Exposed: 1.2 ppm Controls: <0.4 (Lan et al. 2004a)	WBCs	↓
		Granulocytes	↓
		Platelets	↓
		Lymphocytes	↓
		B-cells	↓
		T-cells (CD4/CD8)	↓
		Natural killer	↓
Collins et al. 1991 Cross-sectional; 200 exposed workers, 268 control workers (United States)	Air TWA range: 0.01–1.4 ppm	RBCs	↔
		WBCs	↔
		Platelets	↔
		MCV	↔
		Hb	↔
Collins et al. 1997 Cross-sectional; 387 workers with benzene exposure and 533 controls with no occupational exposure (United States)	PAir mean Exposed: 0.55 ppm Controls: NR	MCV	↔
		Hb	↔
		WBCs	↔
		Platelets	↔
		Lymphocytes	↔
Dosemeci et al. 1996 Retrospective; 62,234 workers with benzene exposure (China)	Air multi-year mean range: 11.5–20.4 ppm	WBCs	↓
Ibrahim et al. 2014 Cross-sectional; 81 exposed workers and 83 control workers with bit exposure (Egypt)	UTMA mean Exposed: 0.22 mg/g Cr Controls: 0.043 mg/g Cr	RBCs	↓
		Platelets	↔
Irons et al. 2010 Case-case analysis; 29 MDS cases with high occupational exposure and 58 cases with no history of benzene exposure (China)	Air range Exposed: >21 ppm Controls: <0.3 ppm	MDS-unclassified	↑
		RA	↔
		RAEB	↔
		RCMD	↔

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to Benzene and Hematological Effects

Reference, study type, and population	Exposure concentration in air or biomarker	Outcome evaluated	Result
Lan et al. 2004a, 2004b Cross-sectional; 250 exposed shoe workers and 140 control workers (China)	PAir mean Low: 0.57 ppm Moderate: 2.85 ppm High: 28.7 ppm Controls: <0.04 ppm	WBCs	↓ (0.57 ppm)
		Granulocytes	↓ (0.57 ppm)
		Monocytes	↓ (0.57 ppm)
		Lymphocytes	↓ (0.57 ppm)
		CD4+ T-cells	↓ (0.57 ppm)
		CD8+ T-cells	↔
		CD4+/CD8+ ratio	↓ (0.57 ppm)
		B-cells	↓ (0.57 ppm)
		NK cells	↔
		Monocytes	↓ (0.57 ppm)
		Platelets	↓ (0.57 ppm)
		Hb	↔
Li et al. 2018 Cross-sectional, 147 exposed petrochemical workers, 122 unexposed workers (China)	UPhMA median Exposed: 100 ng/g Cr Controls: 55 ng/g Cr PAir median Exposed: 0.038 ppm Controls: <0.003 ppm	WBCs	↓ (UPhMA)[a]
		Neutrophils	↓ (UPhMA)[a]
		Lymphocytes	↔ (UPhMA)[a]
		Monocytes	↔ (UPhMA)[a]
Qu et al. 2002 Cross-sectional, workers exposed to benzene (n=131) and age and gender-matched unexposed controls (n=51) (China)	PAir median Exposed: 3.2 ppm Controls: <0.01	WBCs	↓
		Neutrophils	↓
		Lymphocytes	↓
		Monocytes	↓
		Platelets	↔
		HCT	↔
Rothman et al. 1996a, 1996b Cross-sectional: 44 exposed workers, 44 control workers	Pair median Exposed: 31 ppm Controls: NR	WBCs	↓
		Lymphocytes	↓
		Platelets	↓
		Hb	↔
		RBCs	↑
		MCV	↑
Schnatter et al. 2010 Cross-sectional; 928 exposed workers; 73 unexposed control workers (China)	PAir median Exposed: 2.3 ppm Controls: 0.003 ppm	WBCs	↓
		Lymphocytes	↓
		Neutrophils	↓
		Eosinophils	↔
		RBCs	↓
		MCV	↑
		Hb	↓
		Platelets	↓

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to Benzene and Hematological Effects

Reference, study type, and population	Exposure concentration in air or biomarker	Outcome evaluated	Result
Schnatter et al. 2012 Case-control; 29 MDS cases and 129 matched controls (Australia, Canada, United Kingdom)	Air cumulative T1: ≤0.348 ppm-year T2: 0.348–2.93 ppm T3: >2.93 ppm	MDS-unclassified	↑ (>2.93 ppm-year)
Swaen et al. 2010 Cross-sectional; 8,532 exposed workers; 12,173 unexposed control workers (Netherlands)	Air mean Exposed: 0.22 ppm Unexposed: NR	Hb	↔
		HCT	↔
		WBCs	↔
		Lymphocytes	↔
		Neutrophils	↔
		Eosinophils	↔
		Basophils	↔
		Monocytes	↔
Tsai et al. 2004 Longitudinal; 1,200 exposed workers and 3,227 unexposed workers	Pair mean Exposed: 0.60 ppm Controls: NR	WBCs	↔
		Lymphocytes	↔
		RBCs	↔
		Hb	↔
		MVC	↑
		Platelets	↔
Wang et al. 2021a Cross-sectional; 2002 exposed workers and 7942 controls (China)	Air mean Females: 0.27 ppm Males: 0.33 ppm	WBCs	↔
		Neutrophils	↔
		Platelets	↑
Wang et al. 2021b Cross-sectional; 114 exposed workers and 114 unexposed workers (China)	Air range Exposed: 0.05–0.09 ppm Control: 0.02–0.03 ppm UPhMA median Exposed: 0.44 nmol/L Control: 0.13 nmol/L	RBCs	↔ UPhMA
		WBCs	↓ UPhMA
		Lymphocytes	0 UPhMA
		Neutrophils	0 UPhMA
		Platelets	0 UPhMA
Ward et al. 1996 Case-control; 183 cases (low RBCs or WBCs), 12,209 controls, rubber workers (United States)	Air cumulative (reported in Rinsky et al. 1987) Cases: 254 ppm-years Controls: <40 ppm-years	RBCs	↓
		WBCs	↓

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to Benzene and Hematological Effects

Reference, study type, and population	Exposure concentration in air or biomarker	Outcome evaluated	Result
Zhang et al. 2020 Cross-sectional; 1,331 exposed petrochemical workers and 338 control workers (China)	UPhMA median Exposed: 0.37 µg/g Cr Control: 0.18 µg/g Cr	RBCs	↔ UPhMA
		HCT	↑ UPhMA <0.16 ↓ UPhMA >0.16
		MCHC	↑ UPhMA <0.16 ↓ UPhMA >0.16
		MCV	↔
		Hb	↔

[a]Association not evaluated against air concentration.

↑ = positive association; ↓ = inverse association; ↔ = no association; Cr = creatinine; Hb = hemoglobin; HCT = hematocrit; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MDS = myelodysplastic syndrome; NK cells = natural killer cells; NR = not reported; PAir = personal monitor air; RA = refractory anemia; RAEB = refractory anemia with excess blasts; RBC = red blood cell; RCMD = refractory cytopenia with multilineage dysplasia; T = tertile; TWA = time-weighted average; UPhMA = urinary *S*-phenylmercapturic acid; UTMA = urinary *trans,trans*-muconic acid; WBC = white blood cell

Collectively, the epidemiology studies of worker populations provide strong evidence that inhalation exposure to benzene levels >0.5 ppm for several months to several years can be associated with a reduction in the numbers of circulating blood cells (cytopenia) (Table 2-4). At higher levels of exposure (>10 ppm) clinical pancytopenia has been observed (Aksoy 1980; Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974). Continued exposure to benzene can also result in aplastic anemia or leukemia (EPA 1995a; Glass et al. 2003; IARC 2018; Rinsky et al. 2002; Yin et al. 1996b).

Depressed numbers of one or more of the circulating blood cell types (cytopenia) has been used as a biomarker of benzene toxicity to hematopoietic tissues (Cody et al. 1993; Dosemeci et al. 1996; Li et al. 2004a; Kipen et al. 1989; Uzma et al. 2008; Yin et al. 1987c). Of the occupational exposure studies meeting inclusion criteria as defined in Section 2.1, the lowest LOAEL reported for hematological effects is 0.57 ppm (Lan et al. 2004a). In this cross-sectional study, hematologic outcomes were evaluated in 250 workers exposed to benzene in shoe manufacturing industries and in 140 age- and gender-matched workers in clothing manufacturing facilities (Bassig et al. 2016; Lan et al. 2004a, 2004b). The benzene-exposed workers had been employed for an average of 6.1±2.9 (mean±standard deviation [SD]) years. Workers were stratified into four groups (<0.04 [reference], 0.57, 2.85, and 28.73 ppm) based on mean 1-month benzene (Lan et al. 2004a). Regression models of associations between benzene exposure and hematological values were adjusted to account for potential confounding factors (i.e., age, gender, cigarette smoking, alcohol consumption, recent infection, and BMI). Numbers of all types of leukocytes

studied and platelets decreased in association with increasing exposure concentrations ≥0.57 ppm (Lan et al. 2004a). The magnitude of the decrease in cell numbers at 0.57 ppm was 7–15%, with the largest effect on B-cells. At the highest exposure level (28.73 ppm), the decrease in B-cell numbers had progressed to 140 cells/μL (36% decrease), which would represent clinical B-cell deficiency (<170 cells/μL) (Mitchell et al. 2019; Morbach et al. 2010). In addition to B-cells, levels of CD4+ T-cells and the CD4+/CD8+ ratio also decreased. Decreased levels of leukocytes, granulocytes, lymphocytes, and B-cells were also noted in a subgroup (n=30) from the 0.57-ppm exposure group in which exposures to other solvents were negligible, further supporting the causal association with benzene. Lan et al. (2004a, 2004b) also evaluated proliferation and differentiation of progenitor cells in colony formation assays of blood samples collected from the cohort. A concentration-dependent decrease in colony formation was observed in the 2.85- and 28.74-ppm exposure groups. The Lan et al. (2004a) study provides strong evidence for adverse hematological effects in association with benzene exposures ≥0.57 ppm.

Several other studies of workers have reported associations between increasing benzene exposure and decreasing circulating leukocyte numbers (Irons et al. 2010; Qu et al. 2002; Rothman et al. 1996a, 1996b; Schnatter et al. 2010, 2012; Ward et al. 1996). These studies provide further support for effects of benzene at exposure concentrations >1 ppm. One of the larger studies included 928 rubber and shoe workers (median exposure: 2.3 ppm) and 73 control workers (Schnatter et al. 2010). In this study, increasing air benzene levels were associated with decreasing numbers of white blood cells (leukocytes, including lymphocytes, neutrophils) and platelets. In a cross-sectional study of 131 workers and 51 controls, increasing exposure levels (median: 3.2 ppm) were associated with decreasing counts of blood lymphocytes, neutrophils, and monocytes (Qu et al. 2002). Rothman et al. (1996a, 1996b) found similar associations at higher exposure levels (median: 31 ppm). A case-control study of 183 cases of low erythrocyte or leukocyte counts in rubber workers (254 ppm-years) found elevated odds ratios (ORs) for blood erythrocyte and leukocyte counts. A case-control study of 29 cases of myelodysplastic syndrome (MDS, failure of maturation of bone marrow progenitor cells) found an increased ORs for MDS in association with cumulative exposures exceeding 2.93 ppm-years (4.33; 95% confidence interval [CI]: 1.31–14.3) (Schnatter et al. 2012).

Further epidemiological evidence supporting associations between benzene exposure and hematological effects comes from recent studies of exposed workers that found decreasing blood cell counts in association with increasing levels of metabolites of benzene in urine. Li et al. (2018) found that peripheral leukocyte and neutrophil counts decreased in association with increasing urinary levels of SPMA (median in exposed group 100 ng/g creatinine). Wang et al. (2021b) found that peripheral

leukocyte counts decreased in association with increasing urinary levels of SPMA (median in exposed group 0.44 nmol/L; 105 ng/L). Zhang et al. (2020) found that hematocrit and mean corpuscular hemoglobin concentration decreased in association with urinary SPMA levels exceeding 0.16 µg/g Cr.

Several large studies have not found associations between benzene exposure and blood cell counts at exposures <1 ppm. One of the largest studies was a cross-sectional study of 8,532 workers (mean exposure 0.22 ppm) and 12,173 control workers (Swaen et al. 2010). In this study, no association was observed between benzene exposure and blood cell counts (including lymphocytes, neutrophils, eosinophils, basophils, and monocytes). A longitudinal study of 1,200 exposed workers (mean exposure: 0.60 ppm) and 3,227 control workers did not find exposure to be associated with blood leukocytes, including erythrocytes and lymphocyte counts (Tsai et al. 2004). A cross-sectional study of 2,002 exposed workers (mean exposure: 0.27 ppm) did not find exposure to be associated with blood leukocytes. Zhang et al. (2016) reported a benchmark dose analysis of leukocyte counts in shoe workers exposed to benzene. The estimated 95% lower confidence limits on the BMC (BMCLs) were 0.10 and 1.37 ppm-years (cumulative exposure) for benchmark responses of 5 or 10%, respectively.

Studies conducted in laboratory animals show that inhaled benzene exerts toxic effects at all phases of the hematological system, from decreasing stem cell populations in the bone marrow, to pancytopenia, to histopathological changes in the bone marrow. Hematological effects of benzene have been studied extensively in mice and, to a lesser extent, in rats (Table 2-5). The various outcomes observed in animals are consistent with disruption of hematopoiesis. These include decreases in peripheral leukocytes and erythrocytes, pancytopenia, decreases in hematopoietic stem and progenitor cells in hematopoietic tissues (e.g., marrow, spleen), impaired lymphocyte function, and hematopoietic tissue cytotoxicity.

Table 2-5. Hematological Effects of Inhalation Exposure to Benzene in Mice and Rats

Species	Time to effect[a]	Exposure concentration[b] (study duration)	Effects	Reference
Acute-duration exposure				
Mouse	6 days	10.2 (6 days)	↓ peripheral lymphocytes ↑ peripheral erythrocytes	Rozen et al. 1984
		100	↓ peripheral erythrocytes	
Mouse	5 days	10.3 (5 days)	↓ marrow erythroid CFU-E ↓ marrow CFU-E response to erythropoietin	Dempster and Snyder 1991

Table 2-5. Hematological Effects of Inhalation Exposure to Benzene in Mice and Rats

Species	Time to effect[a]	Exposure concentration[b] (study duration)	Effects	Reference
Mouse	4 days	21 (2 weeks)	↓ marrow cellularity ↓ CFUs ↑ MN-PCEs	Toft et al. 1982
Mouse	10 days	25 (16 weeks)	↓ peripheral lymphocytes	Cronkite 1986; Cronkite et al. 1985
Mouse	5 days	25 (5 days)	↓ peripheral WBCs ↓ spleen weight	Wells and Nerland 1991
Mouse	14 days	47 (14 days)	↓ peripheral WBCs ↓ spleen and thymus weights	Aoyama 1986
	7 days	208 (14 days)	↓ peripheral WBCs	
Mouse	7 days	98.5 (8 weeks)	↓ marrow progenitor cells, and differentiating hematopoietic cells	Farris et al. 1997a, 1997b
	14 days		↓ peripheral WBCs ↓ peripheral RBCs ↓ peripheral platelets	
Mouse	5 days	100 (lifetime)	↓ peripheral WBCs	Snyder et al. 1980
Mouse	10 days	100 (16 weeks)	↓ peripheral lymphocytes ↓ marrow cellularity ↓ marrow CFUs	Cronkite et al. 1984, 1989
Mouse	5 days	103 (26 weeks)	↓ peripheral lymphocytes ↓ marrow and splenic lymphocytes	Green et al. 1981a, 1981b
Mouse	4 days	302 (12 weeks)	↓ peripheral lymphocytes ↓ peripheral RBCs ↓ marrow cellularity ↓ splenic cellularity	Baarson et al. 1982
Mouse	5 days	300 (13 weeks)	↑ peripheral MN-PCEs	Luke et al. 1988b
Mouse	7 days	300 (lifetime)	↓ peripheral WBCs ↓ peripheral RBCs	Snyder et al. 1982
Rat	7 days	300 (7 days)	↓ peripheral WBCs	Li et al. 1986
Mouse	8 days	300 (lifetime)	↓ peripheral WBCs	Snyder et al. 1978, 1980
Mouse	8 days	300 (2 weeks)	↓ marrow cellularity ↓ marrow CFUs	Neun et al. 1992
Mouse	10 days	300 (2 weeks)	↓ peripheral WBCs ↓ marrow CFUs	Chertkov et al. 1992
Mouse	10 days	300 (2 weeks)	↓ peripheral lymphocytes	Mukhopadhyay and Nath 2014

Table 2-5. Hematological Effects of Inhalation Exposure to Benzene in Mice and Rats

Species	Time to effect[a]	Exposure concentration[b] (study duration)	Effects	Reference
Mouse	10 days	300 (2 weeks)	↓ peripheral WBCs ↓ peripheral RBCs	Ward et al. 1985
Rat	14 days	300 (2 weeks)	↓ peripheral WBCs	
Rat	2 weeks	500 (2 weeks)	↓ thymus weight	Robinson et al. 1997
Mouse	4 days	300 (5 days)	↓ marrow CFUs	Plappert et al. 1994a, 1994b
		900 (8 weeks)	↑ peripheral CD4+ lymphocytes	
Mouse	3 days	400 (11 days)	↓ peripheral WBCs	Cronkite et al. 1982
	5 days	400 (11 days)	↓ marrow cellularity ↓ marrow CFUs	
	11 days	400 (11 days)	↓ peripheral RBCs ↓ marrow cellularity ↓ marrow CFUs	
Mouse	4 days	900 (8 weeks)	↓ marrow CFUs ↑ peripheral CD4+ lymphocytes	Plappert et al. 1994a, 1994b
Mouse	3 days	1000 (8 days)	↓ marrow cellularity	Gill et al. 1980
	5 days	4000 (6 weeks)	↓ peripheral WBCs	
Intermediate-duration exposure				
Mouse	32 days	10.1 (24 weeks)	↓ peripheral lymphocytes ↓ marrow CFUs	Baarson et al. 1984
	66 days	10.1 (24 weeks)	↓ peripheral RBCs	
Mouse	6 weeks	50 (6 weeks)	↓ marrow HPCs	Malovichko et al. 2021
Mouse	8 weeks	100 (8 weeks)	↓ marrow CFUs	Seidel et al. 1989
Pig	3 weeks	100 (3 weeks)	↓ peripheral WBCs ↑ peripheral RBCs ↓ peripheral lymphocytes ↓ marrow cellularity	Dow 1992
Mouse	3 weeks	300 (4 weeks)	↓ peripheral lymphocytes ↓ peripheral monocytes ↓ peripheral neutrophil	Mukhopadhyay and Nath 2014
Mouse	6–7 weeks	300 (7 weeks)	↓ peripheral WBCs ↓ peripheral RBCs ↓ marrow CFUs	Vacha et al. 1990

Table 2-5. Hematological Effects of Inhalation Exposure to Benzene in Mice and Rats

Species	Time to effect[a]	Exposure concentration[b] (study duration)	Effects	Reference
Mouse	60 days	300 (2 months)	↑ peripheral lymphocytes ↓ peripheral neutrophils	Das et al. 2012
Mouse	91 days	300 (lifetime)	↓ peripheral WBCs	Snyder et al. 1984
Rat	91 days	300 (13 weeks)	↓ peripheral WBCs	Ward et al. 1985
Mouse	16 weeks	300 (16 weeks)	↑ marrow and splenic cellularity (granulocytic hyperplasia)	Farris et al. 1993
Rat	4 weeks	400 (4 weeks)	↓ splenic lymphocytes ↓ thymus weight	Robinson et al. 1997
Rat	3 weeks	500 (3 weeks)	↓ peripheral WBCs ↑ peripheral RBCs ↓ peripheral lymphocytes ↓ marrow cellularity	Dow 1992
Rat	20 weeks	4,570 (20 weeks)	↓ peripheral WBCs ↓ WBC alkaline phosphatase	Songnian et al. 1982
Chronic-duration exposure				
Mouse	2 years	100 (lifetime)	↓ marrow cellularity	Snyder et al. 1980

[a]Duration of exposure at which effect was first observed.
[b]Units are ppm.

↓ = decrease; ↑ = increase; CFU = colony-forming unit; CFU-E = erythroid colony-forming unit; HPC = hematopoietic progenitor cell; MN-PCE = micronucleated polychromatic erythrocyte; RBC = red blood cell; WBC = white blood cell

Decreases in peripheral lymphocytes, decreases in hematopoietic stem cell and progenitor cells (measured in tissue colony forming assays), and impaired lymphocyte function have been observed in mice exposed to 10.2–11 ppm for acute or intermediate durations (Baarson et al. 1984; Dempster and Snyder 1991; Rosenthal and Snyder 1987; Rozen et al. 1984). Acute- and intermediate-duration exposures to higher levels (>20 ppm) have been shown to reduce splenic and marrow cellularity (Baarson et al. 1982; Cronkite et al. 1982, 1984, 1985, 1989), which is an early indication of hematopoietic tissue failure. Intermediate-duration exposure of mice to 50 ppm decreased the number of hematopoietic progenitor cells in marrow (Malovichko et al. 2021; Seidel et al. 1989).

Marrow cytotoxicity was observed in acute-duration exposures to 22 ppm, based on increased number of micronucleated polychromatic erythrocytes (MN-PCEs) in marrow (Toft et al. 1982). At higher acute-

duration exposure levels (300 ppm), elevated numbers of MN-PCEs were observed in blood (Luke et al. 1988b).

Severity of, and recovery from, hematologic effects of benzene appear to be related to both exposure level and duration. In mice, more severe effects were observed following 2 days of exposure to 3,000 ppm compared to mice exposed for 20 days to 300 ppm. In this same study, recovery took longer after 20 days of exposure to 300 ppm compared 2–4 weeks of exposure to 3,000 ppm (Cronkite et al. 1989).

Benzene-induced cytotoxic damage in the bone marrow varied with mouse strain and exposure duration (Luke et al. 1988b). Peripheral blood smears were analyzed weekly from three strains of mice (DBA/2, B6C3F1, and C57BL/6) exposed to 300 ppm benzene for 13 weeks (6 hours/day) for either 5 days/week (Regimen 1) or 3 days/week (Regimen 2). In all three strains, an initial severe depression in rate of erythropoiesis was observed. Recovery 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-NCEs) was observed to be dependent on strain (C57BL/6=B6C3F1>DBA/2) and regimen (Regimen 1 > Regimen 2), whereas the increase in frequency of MN-PCEs was dependent on strain (DBA/2>C57BL/6=B6C3F1) but, for the most part, was not dependent on exposure regimen.

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

Studies conducted in mice have shown that oral dosing with benzene produces hematological effects similar to those observed in animals exposed by inhalation. Data are summarized in Table 2-6.

Table 2-6. Hematological Effects of Oral Exposure to Benzene in Mice and Rats (Ordered by Exposure Duration)

Species	Time of effect	Exposure[a] (study duration)	Effects	Reference
Acute-duration exposure				
Mouse	14 days	200 (14 days)	↓ peripheral WBCs ↓ peripheral lymphocytes ↓ peripheral basophils	Huang et al. 2013

Table 2-6. Hematological Effects of Oral Exposure to Benzene in Mice and Rats (Ordered by Exposure Duration)

Species	Time of effect	Exposure[a] (study duration)	Effects	Reference
Intermediate-duration exposure				
Mouse	4 weeks	1 (4 weeks)	↓ peripheral WBCs ↓ peripheral lymphocytes ↓ peripheral neutrophils ↓ peripheral monocytes	Li et al. 2018; Cui et al. 2022
Mouse	4 weeks	8 (4 weeks)	↓ peripheral lymphocytes ↓ peripheral RBCs	Hsieh et al. 1988, 1990
Rat	120 days	25 (120 days)	↓ peripheral WBCs ↓ peripheral lymphocytes	NTP 1986;
Mouse	120 days	50 (120 days)	↓ peripheral lymphocytes ↓ peripheral WBCs	
Rat	13 weeks	50 (13 weeks)	↓ peripheral lymphocytes ↓ peripheral WBCs	NTP 2007
Mouse	30 days	195 (30 days)	↓ peripheral WBCs	Shell 1992
Rat	4 weeks	200 (4 weeks)	↓ peripheral lymphocytes ↓ peripheral WBCs	Heijne et al. 2005
Rat	4 weeks	526 (135 days)	↓ splenic CD4+ lymphocytes ↓ splenic CD4+/CD8+ ratio	Karaulov et al. 2017
Chronic-duration exposure				
Mouse	18 months	25 (2 years)	↓ peripheral WBCs ↓ peripheral lymphocytes	NTP 1986
Rat	18 months	25 (2 years)	↓ peripheral WBCs ↓ peripheral lymphocytes	

[a]Units are mg/kg/day.

↓ = decrease; RBC = red blood cell; WBC = white blood cell

Gavage dosing of benzene in corn oil of 200 mg/kg/day, 5 days/week for 2 weeks decreased peripheral leukocytes, lymphocytes, and basophils. The effect on leukocytes was a 90% decrease relative to controls (Huang et al. 2013).

Intermediate-duration oral studies in animals have observed decreases in numbers of leukocytes and erythrocytes following exposure to benzene. Male and female Fischer 344 rats and B6C3F1 mice were given oral doses of 0, 25, 50, 100, 200, 400, and 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). Dose-related decreases in peripheral leukocytes and lymphocytes 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 decreases in leukocytes and lymphocytes were observed in male mice at

50 mg/kg/day and in female mice at 400 mg/kg/day for 120 days, but not for 60 days. Mice exposed to 8 mg/kg/day in the drinking water for 4 weeks had decreased numbers of erythrocytes, increased mean corpuscular volume (MCV), and decreased numbers of lymphocytes (Hsieh et al. 1988, 1990). Dose-related decreases in leukocytes were observed after 4 weeks of exposure via gavage in corn oil at ≥1 mg/kg/day in male C57BL/6J mice and at ≥200 mg/kg/day in male F344 rats (Cui et al. 2022; Heijne et al. 2005). Female B6C3F1 mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased leukocytes were also observed at 195 mg/kg/day. Decreased hemoglobin, hematocrit, leukocytes, MCV, and mean corpuscular hemoglobin (MCH) were observed at 350 mg/kg/day. Oral exposure of rats to 526 mg/kg/day benzene in drinking water decreased numbers of splenic CD4+ and CD4+/CD8+ T-cells (Karaulov et al. 2017). NTP (2007) administered benzene to groups of male and female (15/sex/group) by gavage (in corn oil) once/day, 5 days/week for 27 weeks at 0, 25, 50, 100, or 200 mg benzene/kg/day. The mice evaluated in the study were from a genetically modified strain (p16Ink4a/p19Arf) that lacks two tumor suppressor genes. All benzene-treated groups of male mice and the 100- and 200-mg/kg/day groups of female mice exhibited significantly decreased numbers of erythrocytes, leukocytes, and lymphocytes and significantly decreased MCV at weeks 13 and 27 of treatment; the 50-mg/kg/day group of female mice also exhibited significantly decreased numbers of leukocytes and lymphocytes at weeks 13 and 27. Significantly decreased hematocrit and hemoglobin were observed at weeks 13 and 27 at doses ≥50 mg/kg/day in males and in the high-dose females. At week 27 (but not week 13), significantly decreased numbers of segmented neutrophils were observed in male mice dosed at ≥50 mg/kg/day. Male mice exhibited a significantly increased incidence of hemosiderin pigmentation in bone marrow at all benzene dose levels, significantly increased incidence of bone marrow atrophy and lymphoid follicle atrophy in the spleen at the two highest dose levels, and significantly increased incidence of hematopoietic cell proliferation in the spleen at the highest dose. There were no indications of treatment-related effects on spleen or bone marrow of female mice.

One chronic-duration oral study showed that gavage doses of 25 mg/kg/day resulted in decreases in peripheral leukocytes and/or lymphocytes in both rats and mice, both at the interim sacrifices at 3–18 months and at the end of 2 years (NTP 1986). Increased frequency of micronucleated normochromic peripheral erythrocytes was observed in mice at 25 mg/kg/day after 2 years. Sprague-Dawley rats were exposed to 500 mg/kg/day benzene by ingestion (stomach tube), in olive oil, 4–5 days/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983, 1985). Decreased leukocytes and erythrocytes were observed after 84 weeks in both sexes.

2.8 MUSCULOSKELETAL

Few studies evaluating musculoskeletal effects of benzene in humans were identified. 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 Linkoping, Sweden, where a bone marrow biopsy was performed. The patient described symptoms of increasing muscle pain for 1 year, fatigue for 3 weeks and night sweats. 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 Cubatão, São 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 on 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. No exposure estimates were reported.

Little information is available regarding potential musculoskeletal effects in laboratory animals following exposure to benzene, with data available only for oral exposure. No histopathological lesions were observed in femurs from male and female rats or mice given oral doses up to 600 mg/kg/day benzene in corn oil for 120 days or in the sternebrae, femur, or vertebrae from rats and mice exposed to doses up to 200 mg/kg/day (male rats) or 100 mg/kg/day (female rats, male and female mice) for 2 years (NTP 1986).

2.9 HEPATIC

Few studies have evaluated the potential hepatic effects of benzene exposure in humans. A cross-sectional study evaluated plasma lipid profiles in 1,331 exposed petrol workers and 338 control workers in China (Zhang et al. 2020). The primary route of exposure is assumed to be inhalation, although dermal exposure cannot be ruled out. Exposure to benzene was assessed by urinary levels of the benzene metabolite, S-phenyl mercapturic acid (PhMA; also called SPMA). Median levels of urinary PhMA were 0.37 and 0.18 µg/g in exposed and control groups, respectively. No effects were observed for total plasma cholesterol or plasma triglycerides in exposed versus control workers. No differences between groups were observed for the occurrence of fatty liver, as diagnosed by ultrasound. Uzma et al. (2008) evaluated peripheral blood from 154 healthy benzene-exposed male gas station attendants (94 with <10 years of work history and 60 with >10 years of exposure); a control group of 33 healthy subjects

matched for demographics was included. Analysis included evaluation of serum total protein, albumin, total bilirubin, alkaline phosphatase, alanine transaminase (ALT), and aspartate transaminase (AST) as indicators of liver function. There were no differences between controls and filling station attendants regarding serum total protein, albumin, total bilirubin, ALT, or AST. The study is limited by small numbers of subjects, lack of measured benzene levels, and lack of accounting for exposure to other potential toxicants.

Few inhalation exposure studies have evaluated hepatic effects of benzene in laboratory animals, with data available for acute- and chronic-duration exposure. No effect on liver weight was observed in pregnant rats that were exposed to 0 or 125 ppm benzene 24 hours/day on GDs 7–14 (Tatrai et al. 1980a). Mukhopadhyay and Nath (2014) evaluated hepatic effects in male mice exposed to 300 ppm benzene for 2 weeks. Levels of AST and ALT were increased by 8.5- and 3.2-fold, respectively, compared to controls. Extended sinusoids in hepatocytic cell cords were also observed. No treatment-related non-neoplastic histopathological effects on hepatic tissue were found in male rats exposed to 300 ppm benzene 5 days/week, 6 hours/day for life (Snyder et al. 1984) or in AKR/J mice similarly exposed to 300 ppm (Snyder et al. 1978, 1980).

Oral exposure studies in animals have examined effects of acute-, intermediate-, and chronic-duration exposures. For acute-duration exposure, no abnormal histopathology was observed in the liver of male Crl:CD(SD) rats exposed to up to 2,000 mg/kg/day benzene for 3 days (Kitamoto et al. 2015).

Several intermediate-duration studies have evaluated hepatotoxicity of benzene in laboratory animals with effects generally observed at higher doses. In mice exposed to 100 mg/kg/day benzene by gavage, total plasma cholesterol was decreased by 14% and non-esterified fatty acids were increased by 21% (Cui et al. 2022). No histopathological lesions were observed in male Wistar and F344 rats administered up to 800 mg/kg/day for 28 days via gavage in corn oil (Heijne et al. 2005). Relative liver weight increased by 10%; however, the toxicological significance of this finding is uncertain as no histopathological lesions were observed (Heijne et al. 2005). The study authors suggested that increased liver weight may be due to the increased expression of drug metabolizing enzymes. No adverse liver effects, as indicated by gross necropsy, liver weights, and serum levels of hepatic enzymes, were observed in female B6C3F1 mice exposed to doses up to 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). 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 lesion effects were

observed in hepatic tissue from male and female Fischer 344 rats or B6C3F1 mice given oral doses up to 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986).

NTP (1986) evaluated hepatic effects of oral exposure of Fischer 344 rats and B6C3F1 mice to benzene for 2 years. No histopathological, non-neoplastic lesions were observed in male rats exposed to doses up to 200 mg/kg/day or in female rats and male and female mice exposed to 100 mg/kg/day (NTP 1986).

2.10 RENAL

One study described renal effects in humans after inhalation exposure to benzene. In a case report involving the death of an 18-year-old male who intentionally inhaled benzene in unknown amounts, the autopsy revealed acute kidney congestion (Winek and Collom 1971). No other details or data were given.

Chronic-duration inhalation exposure studies in laboratory animals did not find adverse renal effects following inhalation or oral exposures. No treatment-related, histopathological effects on kidney tissue were found in male Sprague-Dawley rats that were exposed to 0, 100, or 300 ppm benzene 5 days/week, 6 hours/day for life (Snyder et al. 1984) or in AKR/J mice similarly exposed to 300 ppm (Snyder et al. 1978, 1980).

Renal effects of oral exposure to benzene in laboratory animals were evaluated for acute-duration gestational, intermediate-duration, and chronic-duration exposures. No renal effects were observed in female Sprague-Dawley rats administered doses up to 1,000 mg/kg/day benzene by gavage on GDs 6–15 (and killed on GD 20) based on gross necropsy (Exxon 1986). 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). In female B6C3F1 mice exposed to 12–350 mg/kg/day benzene in drinking water for 30 days, no adverse effects were observed in the kidneys based on kidney weights, gross examination, and blood urea nitrogen and creatinine determinations (Shell 1992). 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 up to 600 mg/kg/day benzene in corn oil for 120 days or in male and female rats exposed to doses up to 200 or 100 mg/kg/day, respectively, for 2 years (NTP 1986). Similarly, no adverse effects based on histological examination were observed on renal tissues or the urinary bladder from male and female B6C3F1 mice given oral doses of 25–600 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).

2.11 DERMAL

In humans, benzene is a skin irritant. Acute fatal exposure to benzene vapors caused second-degree burns on the face, trunk, and limbs of the victims (Avis and Hutton 1993). In a study of 15 male workers who were exposed to benzene vapors (>60 ppm) over several days during the removal of residual fuel from shipyard fuel tanks (Midzenski et al. 1992), exposures to benzene ranged from 1 day to 3 weeks, 2.5–8 hours/day. Workers with >2 days (16 hours) of exposure reported skin irritation after exposure to the vapor. A case report of a nonfatal accidental poisoning reported swelling and edema of the skin (Greenburg 1926).

Few studies in animals have examined dermal effects of benzene, with studies available for oral exposure and direct dermal application. Results of oral exposure studies are conflicting. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day benzene on GDs 6–15; alopecia of the hind limbs and trunk was noted in all dose groups. (Exxon 1986). No histopathological lesions were observed in the skin of male and female Fischer 344 rats and B6C3F1 mice after a 2-years of 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).

A dermal exposure study indicates that benzene is irritating to the skin following direct application. Application-site dermal irritation was observed in male hairless rats receiving a single occlusive dermal application of benzene at 230 µL for 1 hour or repeated, unocclusive applications at 15 µL every 2 hours for 8 hours/day for 4 days (Chatterjee et al. 2005). Effects included visual signs of erythema, decreased skin moisture content, and increased transepidermal water loss, increased expression of tumor necrosis factor-α at the application site, and increased interleukin-1α in the blood. Repeated, unoccluded application produced more severe irritation than the single occluded application.

2.12 OCULAR

Eye irritation has been observed in workers exposed to benzene vapors. Three hundred solvent workers who had inhalation exposures for >1 year to benzene at 33 and 59 ppm for men and women, respectively, complained of eye irritation (Yin et al. 1987b). Solvent workers who were exposed to 33 ppm (men) or 59 ppm (women) benzene exhibited eye irritation while being exposed to the vapors.

No reliable studies in laboratory animals were identified for exposure to benzene vapor or direct ocular instillation. No histopathological lesions were noted in the eyes of male or female Fischer 344 rats and B6C3F1 mice after 2 years of 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).

2.13 ENDOCRINE

Studies on developmental endocrine effects of benzene are discussed in Section 2.17.

Few studies were located regarding endocrine effects in humans after exposure to benzene. In a cross-sectional study of elderly adults (mean age 71 years, n=505), increased urinary *trans*,*trans*-muconic acid was associated with increased ORs of insulin resistance (Choi et al. 2014; Park et al. 2022). A small cross-section study of children and adolescents (mean age 11 years, n=86) found an association between increased urinary *trans*,*trans*-muconic acid and insulin resistance (Amin et al. 2018). However, urinary *trans*,*trans*-muconic acid is not specific for benzene; therefore, these findings cannot be attributed to benzene alone.

Few studies have evaluated endocrine health effects in animals following inhalation exposure to benzene. In mice, exposure to 50 ppm benzene for 4 or 6 weeks decreased insulin tolerance, an indication of insulin resistance (Abplanalp et al. 2019; Debarba et al. 2020).

Few studies have evaluated potential endocrine effects of oral exposure to benzene, with intermediate-duration studies on insulin and blood glucose effects and intermediate- and chronic-duration studies on comprehensive endocrine tissues. Dose-related increases in plasma insulin and fasting blood glucose were observed at doses of 200–800 mg/kg/day in male Wistar rats exposed to benzene via gavage in corn oil for 4 weeks (Bahadar et al. 2015a, 2015b). Plasma insulin was increased by 1.50- and 2-fold to control at doses of 200 and 800 mg/kg/day, respectively; fasting blood glucose was increased by 1.4- and 1.5-fold compared to control at doses of 400 and 800 mg/kg/day, respectively (Bahadar et al. 2015a). In a companion study, dose-related hyperglycemia in response to glucose challenge was observed in rats exposed orally to benzene at doses of 200–800 mg/kg/day (Bahadar et al. 2015a). Blood glucose levels were increased by 1.3- and 4-fold at 200 and 800 mg/kg/day, respectively, compared to control.

For longer exposures, no histopathological lesions were observed in salivary, thyroid, parathyroid, pancreas, adrenal, or pituitary glands from male and female Fischer 344 rats or B6C3F1 mice given oral

doses up to 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). NTP (1986) also exposed male Fischer 344 male and female rats to doses up to 200 mg/kg/day (males) and 100 mg/kg/day (females) benzene, respectively, for 2 years. In mice, Zymbal gland lesions showed epithelial hyperplasia in males (0, 9, 30, and 26%) and females (2, 3, 5, and 19%) exposed to 0, 25, 50, or 100 mg/kg, respectively, for 2 years.

2.14 IMMUNOLOGICAL

Benzene disrupts hematopoiesis, leading to decreases in number of peripheral lymphocytes, which contributes to immunosuppression. However, few studies have examined effects of benzene exposure on immune function, outside of effects on peripheral lymphocyte levels and production of lymphocytes in hematologic tissues (discussed in Section 2.7, Hematological) and lymphoproliferative and bone marrow cancers (discussed in Section 2.19, Cancer). The results of these studies indicate that benzene can alter immune responses to antigens, function of peripheral lymphocytes, and levels of circulating antibodies.

A few case reports and clinical studies of workers have examined immunological endpoints other than lymphocyte numbers (Froom et al. 1994; Kirkeleit et al. 2006; Lange et al. 1973a, 1973b; Songnian et al. 1982). However, these studies have important limitations that preclude deriving reliable estimates of associations between exposures and outcomes. These include highly uncertain exposure metrics, no analysis of potential confounders of the measures of association, no estimates of confidence in the association metrics, or low numbers of subjects.

Painters who were exposed to benzene (3–7 ppm), toluene, and xylene in the workplace for 1–21 years showed increased serum levels of IgM and decreased levels of IgG and IgA (Lange et al. 1973b). The decreased levels of immunoglobulins may represent suppression of immunoglobulin-producing cells by benzene. Leukocytes agglutinins (indication of a possible antibody reaction) occurred in 10 of 35 of these workers (Lange et al. 1973a). The workers were exposed to multiple solvents, which also may have contributed to the immunological outcomes; therefore, exposure-response relationships cannot be derived from these studies.

Li et al. (2009a) measured levels of T-cell receptor excision deoxyribonucleic acid (DNA) circles in peripheral blood mononuclear cells from benzene workers. T-cell receptor excision DNA circles are formed when the T-cell receptor genes rearrange in the thymus to enable the T-cell to recognize a foreign antigen. A decrease in T-cell receptor excision DNA circles in peripheral leukocytes reflects a change in

thymic production and release of antigen-recognizing T-cells. The study included 68 benzene-exposed workers (measured benzene concentration in the workplace air averaged 37.8 mg/m^3 [11.7 ppm]). A control group consisted of 27 healthy subjects without documented benzene exposure. Levels of T-cell receptor excision DNA circles in the benzene-exposed group were significantly lower than those of controls.

Animal studies have shown that inhalation exposure to benzene affects immune responses to antigens, immunoglobulin levels, and lymphocyte function, as summarized in Table 2-7. Studies that evaluated numbers of lymphocytes in the periphery and production of lymphocytes in hematopoietic tissues are discussed in Section 2.7 (Hematological).

Table 2-7. Immunological Effects of Inhalation and Oral Exposure to Benzene in Mice and Rats

Species	Time to effect[a]	Exposure[b] (study duration)	Effects observed	Reference
Inhalation: acute-duration exposure				
Mouse	5 days	30 (12 days)	↓ resistance to bacterial infection	Rosenthal and Snyder 1985
Mouse	5 days	10.3 31 (6 days)	↓ mitogen response of marrow B-cells ↓ mitogen response of splenic T-cells	Rozen et al. 1984
Mouse	7 days	50 (14 days)	↓ splenic lymphocyte antibody production in response to antigens	Aoyama 1986
Mouse	2 weeks	300 (2 weeks)	Histopathological changes to lymph nodes, spleen, and thymus	Ward et al. 1985
Inhalation: intermediate-duration exposure				
Mouse	20 days	11.1 99.7 (100 days)	↓ splenic lymphocyte response to foreign antigens	Rosenthal and Snyder 1987
Mouse	3 weeks	99.7 101.4 (100 days)	↓ splenic lymphocyte cytotoxic response to tumor cells ↓ resistance to virus-induced tumor cells	Rosenthal and Snyder 1987
Mouse	4 weeks	200 (5 weeks)	↓ antibody response to antigens	Stoner et al. 1981
Mouse	20 weeks	4570 (20 weeks)	↑ alkaline phosphatase activity of blood leukocytes	Songnian et al. 1982

Table 2-7. Immunological Effects of Inhalation and Oral Exposure to Benzene in Mice and Rats

Species	Time to effect[a]	Exposure[b] (study duration)	Effects observed	Reference
Oral: intermediate-duration exposure				
Mouse	4 weeks	40 (4 weeks)	↓ splenic lymphocyte proliferative response to antigens ↓ splenic lymphocyte cytotoxic response to tumor cells ↓ splenic lymphocyte antibody production in response to antigens ↓ splenic lymphocyte interleukin production in response to mitogens	Hsieh et al. 1988, 1990, 1991
Mouse	3 weeks	200 (4 weeks)	↑ splenic lymphocyte cytotoxic response to tumor cells	Fan 1992
	4 weeks	200 (4 weeks)	↓ splenic lymphocyte production of interleukins in response to mitogens	
Rat	45 weeks	526 (135 days)	↑ splenic lymphocyte interleukin production in response to mitogens	Karaulov et al. 2017

[a]Exposure duration at which effect was first observed.
[b]Units for inhalation studies are in ppm and for oral studies are mg/kg/day.

↓ = decrease; ↑ = increase

Exposure of mice to benzene at ≥10.2 ppm for 6 days depressed mitogen-induced blastogenesis of B- and T-lymphocytes (Rozen et al. 1984). Pre-exposure to benzene at 30 ppm for 5–12 days increased 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. B-cells were more sensitive to benzene than T-cells on a percentage-of-control basis. These results indicate a benzene-induced delay in immune response to *L. monocytogenes*. Concentrations of 200 or 400 ppm for 4–5 weeks (5 days/week) suppressed the primary antibody response to tetanus toxin in mice, but there was no effect at 50 ppm (Stoner et al. 1981).

Rosenthal and Snyder (1987) examined the effects of benzene exposure of mice (10–100 ppm, 5 days/week for 20 days) on the response of splenic T-cells to antigens. The response to splenic T-cells foreign antigens (alloantigens) in a mixed lymphocyte reaction was delayed in mice exposed to 10.2 or 100 ppm. This delayed response was not due to the presence of benzene-induced suppressor cells and indicated that benzene impaired the functional abilities of alloreactive T-cells. Exposure of mice to100 ppm benzene 5 days/week for 3 weeks reduced tumor cytolytic activity of splenic T-cells. 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.

Splenic lymphocytes from mice exposed to 50 or 200 ppm from 7 days had a >80% decrease in IgM and IgG production in response to exposure to antigens (Aoyama 1986). Blood leukocyte alkaline phosphatase activity increased approximately 2-fold in rats exposed to 4,570 ppm for 20 weeks (Songnian et al. 1982).

Histopathological changes in spleen have been observed in mice exposed to 300 ppm benzene 6 hours/day, for 13 weeks (Ward et al. 1985). 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.

Studies of oral dosing of mice have also provided evidence of immunological effects of benzene. Hsieh et al. (1988, 1990, 1991) examined the function of splenic B- and T-cells cultured from mice exposed to benzene at oral doses of 8–180 mg/kg/day for 4 weeks. A biphasic proliferative response to B- and T-cell mitogens was observed, with an enhanced response at 8 mg/kg/day and depressed response at 40 and 180 mg/kg/day. Lymphocyte proliferation and cytotoxic response of T-lymphocytes to allogenic tumor cells also showed a similar biphasic response. Antibody production of splenic lymphocytes collected from mice exposed to 40 or 180 mg/kg/day was also decreased by 48 and 82% at the lower and higher dose, respectively (Hsieh et al. 1988). A dose-related decrease in spleen weight was observed, which was largest (21% decrease from control) in the 180-mg/kg/day group. Dose-dependent decreases in relative spleen and thymus weights were observed in rats administered oral doses ≥200 mg/kg/day for 28 days (Heijne et al. 2005). The decrease in spleen weight was 13% in rats dosed with 200 mg/kg/day and 26% in rats dosed with 800 mg/kg/day. The decrease in thymus weight was 13% in rats dosed with 800 mg/kg/day.

Exposure of mice to 27 and 154 mg/kg/day benzene in drinking water for 28 days altered the function of splenic lymphocytes (Fan 1992). After 21 days of exposure, splenic lymphocytes collected from mice exposed to either dose showed an increased cytotoxic response of cytotoxic T-lymphocytes to allogenic tumor cells, which was not evident after 28 days of exposure. After 28 days of exposure, interleukin-2 (IL-2) production of splenic lymphocytes in response to a mitogen decreased approximately 51% in mice exposed to 27 mg/kg/day and approximately 30% in mice exposed to 154 mg/kg/day. These responses returned to control levels within 21 days of cessation of exposure. Interleukin-4 and interleukin-6

production of splenic lymphocytes in response to a mitogen increased in rats exposed for 45 days to 526 mg/kg/day and production of interleukin-10 increased after 90 days of exposure (Karaulov et al. 2017).

NTP (2007) administered benzene to groups of male and female mice (15/sex/group) by gavage (in corn oil) once/day, 5 days/week for 27 weeks at 0, 25, 50, 100, or 200 mg benzene/kg/day. The mice evaluated in the study were from a genetically modified strain (p16Ink4a/p19Arf) that lacks two tumor suppressor genes. Male mice exhibited dose-related increased incidences of atrophy of thymus and lymph nodes (mandibular, mediastinal, and mesenteric) that reached the level of statistical significance in the two highest dose groups. Female mice exhibited dose-related increased incidences of mesenteric lymph node atrophy that reached the level of statistical significance in the two highest dose groups.

2.15 NEUROLOGICAL

Neurodevelopmental studies on benzene are discussed in Section 2.17.

Several case reports and a few studies in workers have evaluated neurological effects of inhaled benzene. Following acute-duration inhalation of benzene, humans exhibit signs and symptoms indicative of central nervous system effects (Cronin 1924; Flury 1928; Greenburg 1926). These signs and symptoms, reported to occur at levels of 300–3,000 ppm, include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. Acute-duration 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 1928). Lethal exposures are also associated with nonspecific neurological symptoms similar to those reported for nonlethal exposures. In reports of cases of benzene poisoning, subjects exhibited headaches, nausea, tremor, convulsions, and unconsciousness, among other neurological effects (Cronin 1924; Greenburg 1926; Midzenski et al. 1992; Tauber 1970).

Neurological effects of chronic-duration inhalation of benzene have not been well studied in humans. A study of 736 employees of a Korean petrochemical distillation factory and 172 reference office workers did not observe an association between benzene exposure and prevalence of acquired dyschromatopsia (partial color blindness) (Lee et al. 2007). Mean benzene exposures ranged from 0.27 to 2.43 ppm-years, with employment durations of >8 years for exposed workers. Another study examined eight patients (six with aplastic anemia and two with preleukemia) with previous occupational exposure to adhesives and solutions containing 9–88% benzene. Four of the six patients with aplastic anemia showed neurological

abnormalities (global atrophy of lower extremities and distal neuropathy of upper extremities) (Baslo and Aksoy 1982). Air concentrations of benzene in the workplace were reported to have reached levels of ≥210 ppm. These findings suggest that benzene may induce toxic effects on the nervous system involving peripheral nerves and/or spinal cord. The limitations of this study are that benzene exposure levels were not monitored and that there was a possibility of an additional exposure to toluene.

Studies of occupational exposure to mixtures that contain benzene (especially jet fuels) have reported neurosensory effects including hearing loss as well as vestibular and ocular effects (for example, Ödkvist et al. 1987; also reviewed by Morata et al. 2021 and Ritchie et al. 2003). In a recent cross-sectional study of 6–19-year-old participants in NHANES (2017–2020), an association was observed between hearing loss and higher concentrations of the nonspecific benzene metabolite *trans,trans*-muconic acid in urine (Benedict et al. 2024). For each doubling of the urinary concentration of *trans,trans*-muconic acid, there were increases in the odds of having slight speech frequency hearing loss (adjusted odds ratio [aOR] 1.42; 95% C: 1.05, 1.92), slight high frequency hearing loss (aOR 1.31; 95% CI 1.03, 1.66), mild speech frequency hearing loss (aOR 1.60; 95% CI 1.10, 2.32), and mild high frequency hearing loss (aOR 1.45; 95% CI 1.03, 2.04).

The neurotoxicity of benzene has not been studied extensively in animals, although some data are available for inhalation and oral exposure. Dose-related deficiencies in learning and memory, anxiety-like behavior, motor coordination, and social interaction were reported in male adolescent rats exposed to 2,000–8,000 ppm for 30 minutes (Armenta-Reséndiz et al. 2019). In rabbits, relaxation and light narcosis occurred 3.7 minutes following acute-duration exposure to benzene at 45,000 ppm (Carpenter et al. 1944). As the time after exposure progressed, additional signs were observed, including 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 mice showed a 90% decrease in hindlimb grip strength after one exposure to 1,000 or 3,000 ppm (data for 100 ppm were not reported), tremors after one exposure to 3,000 ppm that subsided 30 minutes after exposure, and increased licking of sweetened milk after 1 week of exposure to 300 ppm (Dempster et al. 1984). Hyperactivity, as indicated by increased eating and grooming and reduced sleeping and resting, were observed in mice exposed to 300 ppm for 5 days (Evans et al. 1981).

The neurological effects of intermediate- and chronic-duration oral exposure have been evaluated in rats and mice. Impaired motor function, increased anxiety, and histological changes to the brain (loss of cells

in the cortex and intracerebellar nuclei) were observed in male rats administered 200 mg/kg/day of benzene via gavage in water for 4 weeks (Rafati et al. 2015). Decreased short-term memory and decreased levels of serotonin (5-hydroxytryptamine) in serotonergic neurons were observed in adolescent male Wistar rats following exposure to 0, 41, or 82 mg/kg/day of benzene via drinking water exposure for 4 weeks (Banik and Lahiri 2005). Animals had decreased step-through latency in the passive avoidance test 15 and 30 days following the end of the exposure period to doses ≥41 mg/kg/day, indicative of impaired short-term memory.

Histological examination of the brain revealed no treatment-related lesions after gavage administration of male and female Fischer 344 rats and B6C3F1 mice with doses up to 600 mg/kg/day of benzene for 120 days (NTP 1986). In the same experiment, B6C3F1 mice exhibited tremors intermittently at doses of 400 mg/kg/day, which were more pronounced in males during the last 3 weeks of the study. No histopathological changes of the brain or spinal cord were observed in male or female Fischer 344 rats or B6C3F1 mice after oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice) for 2 years (NTP 1986).

In addition to assessments on neurological function, studies in animals have evaluated neurotransmitter concentrations in brain tissue following oral exposure. Neurochemical profiles were evaluated in rats after oral exposure to benzene (Kanada et al. 1994). Sprague-Dawley rats received a single dose of 0 or 950 mg/kg benzene by gavage and were sacrificed 2 hours after treatment. Neurotransmitters assessed in various regions of the brain were acetylcholine, 3,4-dihydroxyphenylalanine (DOPA), dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine, 3-methoxy-4-hydroxyphenylglycol (MHPG), serotonin, and 5-hydroxyindoleacetic acid (5HIAA). 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. The toxicological significance of these findings is uncertain as functional neurotoxicity assessments were not conducted.

2.16 REPRODUCTIVE

Reproductive effects of benzene have not been extensively studied. Two studies were identified that meet inclusion criteria for reliable epidemiological data (as defined in Section 2.1); study details are summarized in Table 2-8. No association between benzene exposure and spontaneous abortion was observed in 1,739 pregnancies of male partners who were exposed to benzene in a chemical plant in France (Stucker et al. 1994). Worker exposures were stratified into two groups: low (1–5 ppm) and high (≥5 ppm), based on personal air monitors. Ruckart et al. (2014) did not find an association between exposure and pre-term birth in women exposed to drinking water contaminated with benzene, trichloroethylene, and tetrachloroethylene at the Camp Lejeune Marine Corps base in North Carolina. Benzene exposure concentrations were estimated as >1 ppb based on monthly estimates.

Table 2-8. Results of Epidemiological Studies Evaluating Occupational Exposure to Benzene and Reproductive Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Ruckart et al. 2014 Retrospective; 7,829 singleton births (Marine Corps Base Camp Lejeune, North Carolina)	Drinking water mean No exposure: NR Low: <1 ppb High: >1 ppb	Pre-term birth	↔ DW (>1 ppb)
Stucker et al. 1994 Cross-sectional; 1,739 pregnancies of male chemical worker spouses (France)	Air range (PAir) Low: 1-5 ppm High: ≥5 ppm	Spontaneous abortion	↔ Air (≥ 5 ppm)

↔ = no association; DW = drinking water; NR = not reported; PAir = personal monitor air

A study by Katukam et al. (2012) evaluated semen and sperm quality in 160 benzene-exposed workers (compared to 200 controls) based on duration of employment (0–5, 5–10, and 10–15 years); however, results were assessed based on employment duration, rather than benzene levels in blood (mean for all workers: 21 ng/L). Semen volume, pH, and liquefaction were similar across all exposure durations. Duration-dependent decreases in sperm measures were observed. For the longest exposure duration, sperm count was decreased (34% of control), sperm motility was decreased (38% of control), and abnormal sperm morphology was increased (2.5-fold). Reproductive function was not assessed in this study.

In a large retrospective study (n=220,065), increasing gestational diabetes mellitus cases reported in a multi-hospital birth registry was associated with increasing modeled community benzene concentrations (Williams et al. 2019). In this study, the highest OR for gestational diabetes mellitus was in the Asian/Pacific Islander population (1.41, 99% CI 1.12, 1.77).

Animal studies have evaluated reproductive effects of inhalation and oral exposure to benzene, with conflicting results. In rats exposed to either 0 or 125 ppm benzene for 24 hours/day on GDs 7–14, no effect on implantation number was observed (Tatrai et al. 1980a). Pregnant rabbits exposed 12 hours/day to 156.5 or 313 ppm benzene on GDs 7–20 showed an increase in the number of spontaneous abortions (benzene: 6; control: 0) and percent resorptions (benzene 16.1 %; control: 5.2%) at 313 ppm (Ungvary and Tatrai 1985). However, in other reproductive studies, no effect on the number of resorptions was seen in rats at doses as high as 2,200 ppm (Green et al. 1978), or in mice or rabbits at 500 ppm (Murray et al. 1979). No adverse reproductive effects, based on number of pregnant rats and numbers of corpora lutea, implantations, and resorptions, were observed in rats exposed to up to 100 ppm on GDs 6–15 (Coate et al. 1984).

Reproductive effects have been noted in experimental animals exposed by inhalation for intermediate durations. In an intermediate-duration inhalation study, groups of male and female mice were exposed to benzene vapor concentrations of 0, 1, 10, 30, or 300 ppm, 5 days/week, 6 hours/day 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. Dose-related changes in ovarian histopathology, including degenerating follicles and decreased numbers of growing follicles were observed in female rats at exposure concentrations of 2,000–8,000 ppm for 28 days (Harrath et al. 2022). In a fertility study, female rats exposed up to 300 ppm benzene for 10 weeks during premating, mating, gestation, and lactation showed no effect on indices of fertility, reproduction, and lactation (Kuna et al. 1992). In contrast, increased resorptions, pregnancy loss, and histopathological changes (impaired vascularity and trophoblast hyperplasia) in the placenta were observed in of C57BL/6 mice exposed to 50 ppm for 5 hours/day during gestation, but not premating nor mating (Maxwell et al. 2023).

Reproductive effects of oral exposure to benzene have been examined in rats and mice. Female Sprague-Dawley rats were dosed by gavage at doses up to 1,000 mg/kg/day benzene on GDs 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 or female Fischer 344 rats or B6C3F1 mice dosed by gavage with up to 600 mg/kg/day benzene for 17 weeks (NTP 1986). In male and female Fischer 344 rats and B6C3F1 mice after oral exposure to 50–200 mg/kg/day for 2 years (male rats) or 25–100 mg/kg/day (female rats and male and female mice), a positive trend was observed for endometrial stromal polyps in female rats (NTP 1986). The incidence in the high-dose group (14/50) was greater than that in the control group (7/50). In mice, analysis of preputial gland lesions in male mice dosed at 0, 25, 50, or 100 mg/kg/day 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 neoplasia (see Section 2.19). Various non-neoplastic ovarian lesions were observed in female mice, including epithelial hyperplasia and senile atrophy (NTP 1986).

2.17 DEVELOPMENTAL

Very little information is available on developmental effects of benzene exposure in humans. A study of subjects with known benzene poisoning in Italy reported the case of one pregnant worker exposed to benzene in the air during her entire pregnancy (Forni et al. 1971). No developmental effects were reported.

Numerous inhalation studies have evaluated developmental effects in laboratory animals exposed to benzene during gestation. As discussed below, decreased fetal weight, increased skeletal variations, alterations in hematological parameters, neurodevelopmental effects, and altered glucose homeostasis have been reported. Studies have been conducted in rats, mice, and rabbits. Almost all studies evaluated effects of inhaled benzene, with few studies examining effects of oral exposure. Human data are inadequate to verify or refute findings in animals. However, given that benzene is ubiquitous in the environment and cigarette smoke is a common and important source of benzene exposure, the potential for developmental effects in humans should be considered.

In rats exposed to inhaled benzene, fetal weight was decreased by 10% and fetal crown-rump length was decreased by 5%, compared to control following exposure to 2,200 ppm benzene for 6 hours/day on GDs 6–15 (Green et al. 1978). However, no effects on body weight or crown-rump length were observed at lower concentrations of 100 or 300 ppm. An increase in the incidence of skeletal malformations was observed in all benzene groups, relative to controls. The following were observed: increased missing

sternebrae at 100 ppm; increases in delayed sternebrae ossification at 300 ppm; and increased missing sternebrae. Decreased fetal body weight has been observed at a lower benzene concentration of 50 ppm. Kuna and Kapp (1981) found decreases in fetal weight of 14 and 18%, respectively, in rats exposed to 50 and 500 ppm on GDs 6–15. No increases in skeletal variations or malformations were observed. Fetal weight was decreased by 5% of control in mice exposed to 50 ppm on GDs 1–18 (Maxwell et al. 2023). At a similar low concentration of 47 ppm (GDs 7–14) in rats, fetal weight was decreased by 5% compared to controls (Tatrai et al. 1980b), with a decrease of 28% at 141 ppm (Tatrai et al. 1980b). Fetal loss relative to implantations sites was increased in the 141-ppm group; no fetal malformations were observed in this study. Fetal weight was also decreased by 6% in males and females in another study exposing rats to 100 ppm on GDs 6–15; no fetal malformations were observed (Coate et al. 1984). In rats exposed to 125 ppm benzene on GDs 7–14, fetal weight was decreased 20% compared to control, and skeletal ossification was "retarded" (Tatrai et al. 1980a).

In mice exposed to 500 ppm benzene for 7 hours/day on GDs 6–15, decreased fetal weight (by approximately 6%, compared to controls) and increased minor skeletal variants (delayed skeletal ossifications, fused ribs, and asymmetric vertebrae) were observed in mice (Murray et al. 1979). There were no fetal malformations. A non-statistically significant increase in minor skeletal variations, including gastroschisis (an extension of intestines and sometimes other abdominal organs outside the body as a result of an abdominal wall defect), fused ribs, and minor thoracic vertebrae variations were observed in rabbits exposed to benzene 7 hours/day at 500 ppm on GDs 6–19, but no effects occurred in fetal weight (Murray et al. 1979). Exposure of mice 12 hours/day to 156.5 or 313 ppm benzene on GDs 6–15 resulted in decreased fetal weight (25 and 27% in the 156.5 and 313 ppm concentrations, respectively) and an increased percentage of rats with "skeletal retardation" (10 and 11% at the 156.5 and 313 ppm concentrations, respectively, compared to 5% in controls) (Ungvary and Tatrai 1985). No malformations were observed. A parallel study in rabbits showed that inhalation of benzene at 313 ppm caused a reduction in fetal weight (17 and 16% decreases in males and females, respectively, compared to controls) and a 2.5-fold increase in the percentage of minor fetal anomalies (Ungvary and Tatrai 1985).

Two studies have evaluated developmental effects of benzene following oral exposure. No decrease in fetal weight was observed in mice administered 1,300 mg/kg/day of benzene by gavage on GDs 8–12 (Seidenberg et al. 1986). In Sprague-Dawley rats gavaged with up to 1,000 mg/kg/day benzene on GDs 6–15, no malformations or variations were observed (Exxon 1986).

Studies assessing developmental effects on hematopoiesis, neurodevelopment, and endocrine development have been conducted in offspring of dams exposed to benzene vapor. Alterations in hematopoiesis have also been observed in the fetuses and offspring of pregnant mice exposed to inhaled benzene (Keller and Snyder 1986). Administration of 20 ppm benzene to pregnant Swiss Webster mice for 6 hours/day on GDs 6–15 caused reductions in the levels of the colony-forming units (CFUs) in fetuses, whereas 5 and 10 ppm benzene caused enhancement of these CFUs. In 2-day-old neonates, CFU 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 in the 20-ppm benzene group. In a follow-up study, Keller and Snyder (1988) conducted a series of studies in Swiss Webster mice exposed 6 hours/day on GDs 6–15 to 5, 10, or 20 ppm benzene. No effects on hematological parameters (erythrocyte and leukocyte counts, hemoglobin, and the proliferating pool of differentiating hematopoietic cells) were observed in 16-day fetuses at any of exposure level. In contrast, 2-day neonates exposed to the same concentrations of benzene exhibited a reduced number of circulating erythroid precursor cells. Furthermore, at 20 ppm, increased numbers of granulopoietic precursor cells and decreased numbers of erythropoietic precursor cells were reported. At 6 weeks of age, benzene had a similar pattern of enhanced granulopoiesis at 20 ppm, but not at 5 or 10 ppm.

Limited evidence exists for the inhalation toxicity of benzene on neurodevelopment. Hypothalamic developmental alterations in orexigenic and anorexigenic projections and impairments in leptin signaling were observed following gestational exposure to 50 ppm benzene on GDs 1–20 (Koshko et al. 2023). The toxicological significance of these findings is unclear.

Changes in glucose metabolism were reported in offspring following gestational exposure to 50 ppm benzene in C57BL/6 mice on GDs 1–21; assessments in offspring were conducted at 4 and 6 months of age (Koshko et al. 2021). Koshko et al. (2023) fed a high-fat diet to mice for 8 weeks to 5 months. In 6-month-old males, statistically increased blood glucose was observed at 30 minutes, but not at 60 or 120 minutes, after glucose challenge. At 6 months of age, females showed a significant hyperglycemic response 120 minutes after glucose challenge.

2.18 CANCER

The EPA (IRIS 2003) determined that benzene is a known human carcinogen for all routes of exposure based upon convincing human evidence as well as supporting evidence from animal studies. IARC (2018) determined that benzene is carcinogenic to humans based on sufficient evidence in humans and

animals supported by mechanistic data. HHS determined that benzene is known to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in humans (NTP 2021).

Studies conducted in workers have shown that exposure to benzene is associated with increased risk of bone marrow cancers, including myelodysplastic syndromes and AML. In studies of laboratory animals, exposure to benzene induced tumors at multiple sites in rats and mice, with a tendency towards induction of lymphomas in mice. An abundance of mechanistic evidence supports a mode of action for benzene induced bone marrow cancers that involves genotoxicity of reactive metabolites of benzene formed in hematopoietic tissue progenitor cells, as well as in liver and other tissues.

An overview of available meta-analyses and select occupational cohort studies can be found in Table 2-9. For additional details on individual worker cohorts and case-control studies, refer to the meta-analyses or IARC monographs (IARC 1982, 1987, 2012, 2018). Collectively, available epidemiological and meta-analyses studies show clear evidence of a causal relationship between occupational exposure to benzene and benzene-containing solvents and the occurrence of acute nonlymphocytic leukemia (ANLL), particularly the myeloid cell type (i.e., AML). Evidence for associations between benzene exposure and non-Hodgkin's lymphoma (NHL) from both individual studies and meta-analyses are mixed. It must be noted that available epidemiological studies are generally limited by confounding chemical exposures and methodological problems, including inadequate or lack of exposure monitoring and low statistical power (due to small numbers of cases). Many of the earlier studies are additionally limited by a lack of information on leukemia cell types other than AML, because leukemia used to be considered a single diagnostic category for epidemiological purposes, due in part to historical nomenclature, small numbers of deaths by cell type, and unavailability of cell-type-specific rates for comparison. However, a consistent excess risk of leukemia across occupational epidemiological studies indicates that benzene is the causal factor. Studies of general populations exposed to ambient levels of benzene were reviewed and excluded from discussion in this profile because of great uncertainty about causality in these studies (e.g., Janitz et al. 2017). Major uncertainty in the interpretation of these ambient exposure studies is that benzene levels (air or biomarkers) may have been a surrogate variable for exposure to "air pollution" in general (e.g., emissions from fuels and fuel combustion). These pollutants (e.g., BTEX, NO_2, PM_{10}) tend to be correlated.

Some of the strongest evidence from a single cohort for the causal link between benzene exposure and increased risk of leukemia comes from a series of studies in workers who were exposed to benzene in three rubber hydrochloride ('Pilofilm') manufacturing plants in Ohio for at least 1 day between the years

Table 2-9. Summary of Cohort Studies and Meta-Analyses Evaluating Associations Between Occupational Exposure to Benzene and Risk of Death from Lymphatic-Hematopoietic Cancers

Population (exposure)	Exposure (ppm)	Reference (n)	Cancer type										
			All hematological	All leukemia	ANLL	ANLL/ MDS	AML	CML	ALL	CLL	MM	NHL	HL
Piloform workers	Range: 0–680	Infante et al. 1977; Rinsky et al. 1981 (n=1,006 M)	↑	↑	NR	NR	NR	NR	NR	NR	NR	NR	NR
		Paxton et al. 1994a, 1994b; Wong 1995 (n=1,212 M)	↑	↑	NR	NR	↑	NR	NR	NR	↔	NR	NR
		Rinsky et al. 1987 (n=1,165 M)	↑	↑	NR	NR	NR	NR	NR	NR	↑	NR	NR
		Richardson 2008; Rinsky et al. 2002 (n=1,721 M, 124 F)	↑	↑	NR	NR	NR	NR	NR	NR	↔	↔	NR
NCI/CAPM cohort (various occupations)	Mean: 22.5	Hayes et al. 1996, 1997; Linet et al. 1996 (n=61,142 M, 49,491 F)	↑	↑	↔	↑	NR	NR	NR	NR	NR	↑	NR
	Range: 3–310	Yin et al. 1987a, 1989 (n=32,261 M, 25,153 F)	NR	↑	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Range: 3–362	Yin et al. 1987c (n=508,518 M, F)	NR	↑	NR	NR	NR	NR	NR	NR	NR	NR	NR
Italian shoemakers	Range: 0–92	Costantini et al. 2003 (n=891 M, 796 F)	NR	↑	NR	NR	NR	NR	NR	NR	NR	NR	NR
	NR	Fu et al. 1996 (n=2,008)	NR	↑	NR	NR	NR	NR	NR	NR	↔	↔	NR
	NR	Paci et al. 1989 (n=1,008 M, 1,005 F)	NR	↑ (M) ↔ (F)	NR	NR	NR	NR	NR	NR	NR	NR	NR
Chemical industry	Mean: 9.6	Bloemen et al. 2004; Collins et al. 2015 (n=2,266 M)	↔	↔	↔	NR	↔	NR	NR	↔	↔	↔	↔
	Range: 0.3–35.5	Bond et al. 1986a (n=956 M)	NR	↔	NR	NR	NR	NR	NR	NR	NR	NR	↔
		Ott et al. 1978 (n=594 M)	↔	↔	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Range: 0–50	Ireland et al. 1997 (n=4,172 M)	NR	↔	↔	NR	NR	NR	NR	↔	↔	NR	↔
Petroleum workers	<5 ppm	Glass et al. 2003, 2005 (n=15,732 M, 1,178 F; nested case-control)	NR	↑	↑	NR	NR	↔	NR	↔	↔	↔	NR

Table 2-9. Summary of Cohort Studies and Meta-Analyses Evaluating Associations Between Occupational Exposure to Benzene and Risk of Death from Lymphatic-Hematopoietic Cancers

Population (exposure)	Exposure (ppm)	Reference (n)	Cancer type										
			All hematological	All leukemia	ANLL	ANLL/ MDS	AML	CML	ALL	CLL	MM	NHL	HL
	<1 ppm	Raabe and Wong 1996 (n=208,741; meta-analysis of 19 cohorts)	NR	NR	NR	NR	↔	↔	↔	↔	NR	NR	NR
	NR	Rushton et al. 2014 (n=140 cases, 586 controls; meta-analysis of three case-control studies)	NR	NR	NR	NR	↔	NR	NR	↔	NR	NR	NR
	0.2–0.3	Schnatter et al. 2012 (n=370 cases,1,587 controls; meta-analysis of three case-control studies)	NR	NR	NR	NR	↔	↔	NR	↔	NR	NR	NR
	NR	Sonoda et al. 2001 (n=3,878 cases, 8,797 controls; meta-analysis of six case-control studies)	NR	NR	NR	NR	NR	NR	NR	NR	↔	NR	NR
	NR	Wong and Raabe 1997 (n=250,816; meta-analysis of 22 cohorts)	NR	NR	NR	NR	NR	NR	NR	NR	↔	NR	NR
	NR	Wong and Raabe 2000 (n=308,199; meta-analysis of 26 cohorts)	NR	NR	NR	NR	NR	NR	NR	NR	NR	↔	NR
Vehicle maintenance workers	NR[a]	Hunting et al. 1995 (n=335 M)	↔	↑	NR	NR	NR	NR	NR	NR	NR	NR	NR
Any industry	NR	Alexander and Wagner 2010 (meta-analysis; 8 cohort and 14 case-control studies)	NR	NR	NR	NR	NR	NR	NR	NR	NR	↔	NR
	NR	Infante 2006 (meta-analysis; eight cohorts)	NR	NR	NR	NR	NR	NR	NR	NR	↑	NR	NR

Table 2-9. Summary of Cohort Studies and Meta-Analyses Evaluating Associations Between Occupational Exposure to Benzene and Risk of Death from Lymphatic-Hematopoietic Cancers

Population (exposure)	Exposure (ppm)	Reference (n)	Cancer type										
			All hematological	All leukemia	ANLL	ANLL/ MDS	AML	CML	ALL	CLL	MM	NHL	HL
	NR	Kane and Newton 2010 (meta-analysis; 6 cohort, 16 case-control, 2 other study types)	NR	NR	NR	NR	NR	NR	NR	NR	NR	↔	NR
	NR	Khalade et al. 2010 (meta-analysis; 13 cohort and 3 case-control)	NR	↑	NR	NR	↑	↔	NR	↑	NR	NR	NR
	NR	Lamm et al. 2005 (meta-analysis; 7 cohort and 14 case-control)	NR	NR	NR	NR	NR	NR	NR	NR	NR	↔	NR
	NR	Lamm et al. 2009 (meta-analysis; 6 case-control)	NR	NR	NR	NR	NR	↔	NR	NR	NR	NR	NR
	NR	Rana et al. 2021 (meta-analysis; 8 cohort and 20 case-control)	NR	NR	NR	NR	NR	NR	NR	NR	NR	↑	NR
	NR	Sonoda et al. 2001 (meta-analysis; 9 case-control)	NR	NR	NR	NR	NR	NR	NR	NR	↔	NR	NR
	NR	Vlaanderen et al. 2011 (meta-analysis; 44 cohorts)	NR	NR	NR	NR	↑	NR	↑	↔	↔[b]	↔	↔
	NR	Vlaanderen et al. 2012 (meta-analysis; 17 cohorts)	NR	NR	NR	NR	NR	↔	NR	NR	NR	NR	NR

Table 2-9. Summary of Cohort Studies and Meta-Analyses Evaluating Associations Between Occupational Exposure to Benzene and Risk of Death from Lymphatic-Hematopoietic Cancers

Population (exposure)	Exposure (ppm)	Reference (n)	Cancer type										
			All hematological	All leukemia	ANLL	ANLL/ MDS	AML	CML	ALL	CLL	MM	NHL	HL
Any industry or household exposure[c]	NR	Carlos-Wallace et al. 2016 (meta-analysis; 20 cohort and case-control studies)	NR	↑	NR	NR	↑	NR	↑	NR	NR	NR	NR

[a]Multi-route exposure. Workers regularly used gasoline to clean parts and wash their hands, were exposed via inhalation, and occasionally siphoned gasoline by mouth.
[b]An association was observed when there was a reported association with AML.
[c]Evaluating childhood leukemia.

↑ = association; ↔ = no association; ALL = acute lymphoid leukemia; AML = acute myeloid leukemia; ANLL = acute nonlymphocytic leukemia; CAPM = Chinese Academy of Preventive Medicine; CLL = chronic lymphatic leukemia; CML = chronic myeloid leukemia; F = females; HL = Hodgkin's lymphoma; M = males; MDS = myelodysplastic syndrome (precursor lesion); MM = multiple myeloma; NCI = National Cancer Institute; NHL = non-Hodgkin's lymphoma; NR = not reported

of 1940 and 1975 (Infante et al. 1977; Paxton et al. 1994a, 1994b; Richardson 2008; Rinsky et al. 1981, 1987, 2002; Wong 1995). In comparison to other published studies, the Pilofilm workers had the fewest reported co-exposures to other potentially carcinogenic substances and experienced a greater range of estimated exposures to benzene (EPA 1998). Findings from the Piloform cohort are reported in a series of studies using various expansions and follow-ups of the cohort (ranging from 748 to 1,291 subjects). Some studies estimated worker exposures based on available occupational hygiene data and job titles, although these estimates often had to fill in large data gaps. Reported exposure levels at various timepoints, locations, and job titles ranged from 0 to 640 ppm (Rinsky et al. 1981). Schnatter et al. (1996) reviewed the available exposure assessments available for these cohorts, including estimated cumulative exposure assessments. Collectively, these studies show a positive association between cumulative exposure to benzene and excess mortality from all leukemias (combined) and AML. The greatest susceptibility was observed in the 10 years immediately following exposure and in individuals exposed at ages ≥45 years old (Richardson 2008). No consistent associations were observed for multiple myeloma or NHL. Schnatter et al. (1996) determined an increased risk of AML after occupational exposure to benzene at concentrations between 50 and 60 ppm using median exposure estimates or between 20 and 25 ppm using the lowest (most conservative) exposure estimates for this cohort.

A large collaborative study between the National Cancer Institute (NCI) and the Chinese Academy of Preventive Medicine (CAPM) also provides strong evidence of a causal link between occupational exposure to benzene and incidence of leukemia, including both occurrence of disease and cause of death (Hayes et al. 1996, 1997; Linet et al. 1996). The joint NCI/CAPM study is an expansion of earlier studies performed by CAPM alone (Yin et al. 1987a, 1987c, 1989). The joint NCI/CAPM study evaluated lymphohematopoietic malignancies and other hematologic disorders in a cohort of 74,828 benzene-exposed and 35,805 nonexposed workers employed in 672 factories in 12 cities in China between the years of 1972 and 1987 (Hayes et al. 1996, 1997, 2001; Linet et al. 1996). Workers were exposed to mean benzene levels of 22.5 ppm for a mean employment duration of 9.3 years in various job categories using benzene as a solvent for paints, varnishes, glues, coatings, and other products. Outcomes of the exposed and unexposed workers were followed for an average of 10.5 and 11.7 years, respectively. Analysis of this cohort found an association between benzene exposure and elevated risk for all hematological neoplasms, all leukemias, and ANLL and precursor MDS combined, with a borderline association with ANLL alone. Further analysis showed that risk was associated with increasing average and cumulative levels of exposure; no associations were observed with duration of exposure. The risk of NHL was increased only in individuals in the highest exposure group (≥25 ppm) or those exposed the longest (≥10 years). While findings from this study are confounded by likely concurrent exposure to

many other chemicals, analysis by occupational group (coatings, rubber, chemical, shoe, other/mixed) showed that the observed increases in risks were consistent across the spectrum of industries studied, suggesting that the associations were due to the common exposure to benzene rather than other industry-specific exposures. Additionally, this cohort it is one of the largest of its type undertaken and evaluated many thousands of benzene-exposed workers, enabling detection of elevated risks at relatively low levels of exposure.

Additional occupational studies contribute to the weight of evidence for increased risk of death from leukemia following high occupational exposure to benzene. Findings include associations between risk of death from leukemia and exposure to benzene in Italian shoemakers (Costantini et al. 2003; Fu et al. 1996; Paci et al. 1989), increased risk of death from leukemia in a small cohort of male vehicle maintenance workers (Hunting et al. 1995). Case series and case reports also reported incidences of leukemia in shoe factory and rotogravure plant workers exposed to high benzene levels during its use as a solvent (Aksoy 1987; Aksoy et al. 1974; IARC 1982; Vigliani and Forni 1976).

Epidemiological studies of chemical companies with lower benzene exposures have not observed associations between occupational exposure to benzene and increased risk of death from leukemia. For example, no increase in the risk of death due to leukemia was observed in a prospective study of 2,266 male chemical workers who were exposed to benzene in various Dow Chemical Company manufacturing processes between 1938 and 1970 (Bloemen et al. 2004; Collins et al. 2015). Bloemen et al. (2004) followed the workers from 1940 to 1996 and reported average duration of exposure, intensity of exposure, and cumulative exposure of 4.8 years, 9.6 ppm, and 39.7 ppm-years, respectively. Collins et al. (2015) extended the follow-up through 2009. There were no significant increases in risk for any lymphohematopoietic malignancies, including all leukemias, ANLL, total lymphoid leukemia, chronic lymphatic leukemia (CLL), total myeloid leukemia, AML, NHL, Hodgkin's disease, or multiple myeloma. Earlier investigations of this cohort did not observe clear associations between increased risk of death from lymphohematopoietic malignancies, leukemia, or Hodgkin's lymphoma in exposed workers (Bond et al. 1986a; Ott et al. 1978). Similarly, no increases in risk of mortality from all leukemias, ANLL, CLL, multiple myeloma, or Hodgkin's lymphoma were observed in a cohort of 4,172 male chemical workers who were exposed to benzene in various Monsanto Company manufacturing processes between 1940 and 1977 (Ireland et al. 1997). Reported benzene levels were 0–50 ppm, with a median cumulative exposure of 36 ppm-months.

Similar to the chemical industry workers, no significant increases in lymphohematopoietic cancers were found in petroleum industry workers exposed to lower levels of benzene (means generally <1 ppm) based on findings from several meta-analyses. A meta-analysis was conducted on 19 cohorts of petroleum workers in the United States and the United Kingdom that were pooled into a single database for cell-type-specific leukemia analysis (Raabe and Wong 1996). The combined cohort consisted of 208,741 workers, mainly refinery employees who contributed >4.6 million person-years of observation. Benzene exposures were mainly from handling gasoline and the estimated mean and cumulative exposures for the most exposed jobs were <1 ppm and <45 ppm-years, respectively. No increased risks were found for mortality from AML, chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), or CLL. Analyses limited to studies of refinery workers or studies with at least 15 years of follow-up yielded similar results. However, a nested-case control study of the Health Watch petroleum workers cohort (n=16,910) from Australia observed an increased risk of leukemia, specifically ANLL, with exposure to benzene at concentrations >0.8 ppm or cumulative exposures >16 ppm-years (Glass et al. 2003, 2005, 2006). No associations were observed for CML, CLL, NHL, or multiple myeloma.

No increases in mortality from multiple myeloma or NHL were observed in meta-analyses of 22 cohorts of petroleum workers (n=250,816) or 26 cohorts of petroleum workers (n=308,199), respectively, from the United States, Canada, the United Kingdom, and Australia (Wong and Raabe 1997, 2000). Meta-analyses of case-control studies did not observe associations between case status (AML, CML, CLL, multiple myeloma) and occupational exposure to petroleum products (Rushton et al. 2014; Schnatter et al. 2012; Sonoda et al. 2001). However, an association between cumulative benzene exposure and MDS was observed (Li and Schnatter 2018; Rushton et al. 2014; Schnatter et al. 2012).

Several meta-analyses have evaluated the potential association between occupational benzene exposure (from any industry) and risk of one or more lymphatic-hematopoietic cancers. A meta-analysis of 12 cohort and 3 case-control studies showed a clear increased risk of death from leukemia in benzene-exposed workers; further analysis of the 9 cohort studies that provided estimates of cumulative exposure showed a clear dose-related increase (Khalade et al. 2010). For individual leukemia types, meta-analysis showed increased overall risk for AML and CLL in benzene-exposed workers, but not CML. Vlaanderen et al. (2011) evaluated possible associations between occupational exposure to benzene from any industry and risk of lymphoma subtypes (AML, Hodgkin's lymphoma, NHL, multiple myeloma, ALL, and CLL) in a meta-analysis of 44 cohort studies that reported results for one or more of the lymphoma subtypes. Occupational benzene exposure was associated with increased risk of AML and ALL only when all studies were considered. When only studies with strong AML associations were included, an association

was also observed for multiple myeloma and borderline associations were observed for CLL. No associations were observed for Hodgkin's lymphoma or NHL. Vlaanderen et al. (2012) similarly designed a meta-analysis of possible associations between occupational exposure to benzene and risk of CML based on 17 cohort studies. Occupational exposure to benzene was not associated with CML. A meta-analysis of eight cohorts of benzene-exposed workers observed an association between benzene exposure and increased risk of death from multiple myeloma (Infante 2006), while meta-analyses of case-control studies indicate that occupational benzene exposure (from any industry) is not likely to be causally related to the risk of multiple myeloma (Sonoda et al. 2001) or CML (Lamm et al. 2009). A single case-control study also did not find associations between occupational benzene exposure and case status for hairy cell leukemia, a rare B-lymphoid chronic leukemia (Clavel et al. 1996).

Four meta-analyses evaluated possible associations between occupational exposure to benzene (from any industry) and risk of NHL (Alexander and Wagner 2010; Kane and Newton 2010; Lan et al. 2005; Rana et al. 2021). The meta-analysis of Alexander and Wagner (2010) included 8 cohort studies and 14 case-control studies and did not observe any associations between benzene exposure and risk of NHL for measures of any measure of benzene exposure (i.e., any benzene exposure, highest level of benzene exposure, and meta-analysis of 5 studies that reported results for ≥60 ppm-years). The meta-analysis of Kane and Newton (2010) included 6 cohort studies, 16 case-control studies, and 2 studies of other designs. Random-effects meta-analysis did not show any association between benzene exposure and risk of NHL. Among six studies for which benzene exposure was estimated from historical measurements, there were no statistically significant associations between benzene exposure and risk of NHL relative to increasing cumulative, average, peak, or duration of benzene exposure. The meta-analysis by Lamm et al. (2005) included 7 cohort studies, 3 nested occupation-based case-control studies, and 11 population-based case-control studies. When cases were combined, no excess risk of NHL was associated with exposure to benzene using the full data set or when excluding studies with known multiple chemical exposures. In contrast to earlier meta-analyses, a systematic review and meta-analysis by Rana et al. (2021) reported increased relative risk of NHL, especially diffuse large B-cell lymphoma, in highly exposed workers. This meta-analysis included 8 cohort studies and 20 case-control studies. Potential associations were observed for follicular lymphoma or hairy cell leukemia, but findings were inconclusive.

Carlos-Wallace et al. (2016) conducted a meta-analysis to evaluate the potential association between exposure to benzene and risk of childhood leukemia. The meta-analysis included cohort and case-control studies of benzene exposure from occupational or household use of benzenes and solvents (n=20); traffic density and traffic-related air pollution (n=12); and residential proximity to gas stations (n=3). Meta-

analysis showed that the risk of childhood leukemia was associated with paternal and/or maternal benzene exposure from occupational or household use of benzenes and solvents, and with traffic density and traffic-related air pollution. For both metrics, benzene exposure was associated with a higher risk for AML compared with ALL. There was no association between childhood leukemia and residential proximity to gas stations.

Numerous studies show that benzene is a multi-site carcinogen in rodents following intermediate- or chronic-duration inhalation or oral exposure.

Results of cancer bioassays in rats and mice following inhalation exposure are summarized in Table 2-10. Data are limited for rats. No exposure-related tumors were observed in male rats following lifetime exposure to concentrations up to 300 ppm (Snyder et al. 1978, 1984). In a study that exposed pregnant dams starting on GD 12 through lactation and then continued exposure of dams and offspring for a total exposure of 104 weeks, no exposure-related tumors were observed in dams (Maltoni et al. 1982, 1983, 1985, 1989). However, Zymbal gland carcinomas were observed in both F1 males and females at natural death, and F1 females also had increased incidence of oral cavity carcinomas and hepatomas. Exposure in this study was 200 ppm for the first 19 weeks followed by 300 ppm for the remaining 85 weeks (TWA of 282 ppm). If exposure was only for 15 weeks (starting on GD 12), no exposure-related tumors were observed in F1 males at natural death; however, oral cavity carcinomas and hepatomas were still induced in F1 females.

Numerous cancer bioassays have been conducted in mice following inhalation exposure. Consistent with human data, increased incidence of hematopoietic neoplasms, lymphoma, and leukemia are common findings following intermediate- or chronic-duration exposure to benzene at 300 ppm in various mouse strains (Cronkite 1986; Cronkite et al. 1984, 1985, 1989; Farris et al. 1993; Inoue and Hirabayashi 2010; Kawasaki et al. 2009; Snyder et al. 1980, 1988). No exposure-related changes in leukemia incidence were observed in AKR/J mice, a strain with very high (85–95%) spontaneous rates of leukemia (Snyder et al. 1978, 1980). In mice with tumor suppressor genes turned down (*Trp-53* deficient), the incidence of AML and thymic and non-thymic lymphomas were increased in male mice following exposure to 300 ppm for 26 weeks, compared to exposed wild-type mice (Inoue and Hirabayashi 2010; Kawasaki et al. 2009). Additional tumor sites in mice following intermediate- or chronic-duration inhalation exposure to ≥300 ppm include the Zymbal gland, Harderian gland, lung, forestomach, preputial gland, mammary gland, and ovary (Cronkite et al. 1984, 1985, 1989; Farris et al. 1993; Snyder et al. 1988).

Table 2-10. Summary of Inhalation Studies Evaluating Tumor Response in Rodents

Species (sex, n)	Concentration (ppm)	Duration	Exposure-related tumor outcomes		Reference
			Males	Females	
Sprague-Dawley rat (27–45 M)	0, 100, 300	Lifetime (mean lifetime of 51–80 weeks) 5 days/week 6 hours/day	No exposure-related tumors	NA	Snyder et al. 1978, 1984
Sprague-Dawley rat (54–60 F0F; 75–158 F1M, 65–149 F1F)	0, 282[a]	104 weeks (starting on GD 12; F1 continued direct exposure post-weaning) 5 days/week 4–7 hours/day	Tumors in F1 animals: Zymbal gland carcinomas 0 ppm: 2/152 282 ppm: 6/75[b]	Tumors in F1 animals: Zymbal gland carcinomas 0 ppm: 0/148 282 ppm: 8/65[b] Oral cavity carcinoma 0 ppm: 0/47 282 ppm: 10/22[b] Hepatomas 0 ppm: 0/146 282 ppm: 7/59[b] No exposure-related tumors in F0 dams	Maltoni et al. 1982, 1983, 1985, 1989
Sprague-Dawley rat (70–158 M, 59–149 F)	0, 200	15 weeks (exposure via dam GD 12–weaning and direct post-weaning) 4–5 days/week 4–7 hours/day	No exposure-related tumors.	Oral cavity carcinoma 0 ppm: 0/47 200 ppm: 6/21[b] Hepatomas 0 ppm: 0/146 200 ppm: 5/59[b]	

Table 2-10. Summary of Inhalation Studies Evaluating Tumor Response in Rodents

Species (sex, n)	Concentration (ppm)	Duration	Exposure-related tumor outcomes		Reference
			Males	Females	
C57BL mouse (40 M)	0, 300	Lifetime 5 days/week 6 hours/day	Hematopoietic neoplasms 0 ppm: 2/40 300 ppm: 8/40[d] Thymic lymphoma 0 ppm: 0/40 300 ppm: 6/40[d]	NA	Snyder et al. 1980
AKR/J mouse[c] (50–60 M)	0, 100, 300	Lifetime 5 days/week 6 hours/day	No exposure-related tumors	NA	Snyder et al. 1978, 1980
C57BL mouse (60 M)	0, 300	Lifetime every 3rd week 7 days/week	Zymbal gland carcinoma 0 ppm: 0/46 300 ppm: 19/54[d] All malignant tumors 0 ppm: 2/46 300 ppm: 24/54[d]	NA	Snyder et al. 1988
CD-1 mouse (60 M)	0, 300	Lifetime every 3rd week 7 days/week	Lung adenoma 0 ppm: 3/46 300 ppm: 14/54[d] Leukemia/lymphoma 0 ppm: 1/46 300 ppm: 7/54[b] All malignant tumors 0 ppm: 1/46 300 ppm: 12/54[d]	NA	

Table 2-10. Summary of Inhalation Studies Evaluating Tumor Response in Rodents

Species (sex, n)	Concentration (ppm)	Duration	Exposure-related tumor outcomes		Reference
			Males	Females	
CD-1 mouse (40 M)	0, 300	Lifetime 5 days/week 6 hours/day	No exposure-related tumors		Snyder et al. 1982
C57BL/6 mouse (wild-type) (18–20 NS)	0, 33, 100, 300	26 weeks 5 days/week 6 hours/day	Thymic lymphomas 0 ppm: 0/20 33 ppm: 0/19 100 ppm: 2/19 300 ppm: 5/18[d] Neoplasms of hematopoietic and lymphoid tissues 0 ppm: 2/20 33 ppm: 4/19 100 ppm: 3/19 300 ppm: 10/18[d]	NA	Inoue and Hirabayashi 2010; Kawasaki et al. 2009
C57BL/6 mouse (heterozygous *Trp-53* deficient) (24–27 NS)	0, 33, 100, 300	26 weeks 5 days/week 6 hours/day	Thymic lymphomas 0 ppm: 0/24 33 ppm: 1/27 100 ppm: 4/25 300 ppm: 19/26[d]	NA	
C3H/He mouse (wild-type) (23–24 NS)	0, 100, 300	26 weeks 5 days/week 6 hours/day	AML 0 ppm: 0/23 100 ppm: 0/24 300 ppm: 2/23 Non-thymic lymphoma 0 ppm: 2/23 100 ppm: 2/24 300 ppm: 5/23	NA	
C3H/He mouse (heterozygous	0, 100, 300	26 weeks 5 days/week	AML 0 ppm: 2/24	NA	

Table 2-10. Summary of Inhalation Studies Evaluating Tumor Response in Rodents

Species (sex, n)	Concentration (ppm)	Duration	Exposure-related tumor outcomes		Reference
			Males	Females	
Trp-53 deficient) (24 NS)		6 hours/day	100 ppm: 2/24 300 ppm: 9/24[d] Non-thymic lymphoma 0 ppm: 3/24 100 ppm: 6/24 300 ppm: 10/24[d]		
CBA/Ca mouse (125 M)	0, 300	16 weeks 5 days/week 6 hours/day	Malignant lymphoma 0 ppm: 2/119 300 ppm: 14/118[d] Squamous cell carcinoma of preputial gland 0 ppm: 0/118 300 ppm: 71/118[d] Lung adenoma 0 ppm: 17/119 300 ppm: 42/118[d] Zymbal gland carcinoma 0 ppm: 1/125 300 ppm: 14/125[b] Forestomach squamous cell carcinoma 0 ppm: 0/125 300 ppm: 9/125[b]		Farris et al. 1993

Table 2-10. Summary of Inhalation Studies Evaluating Tumor Response in Rodents

Species (sex, n)	Concentration (ppm)	Duration	Exposure-related tumor outcomes		Reference
			Males	Females	
CBA/Ca mouse (NS M)	0, 300	16 weeks 5 days/week 6 hours/day	Leukemia-lymphoma[e] 0 ppm: 0% 300 ppm: 25% Overall neoplasms[e] 0 ppm: 10% 300 ppm: 70%	NA	Cronkite 1986
CBA/Ca mouse (NS B)	0, 100	16 weeks 5 days/week 6 hours/day	Overall neoplasms 0 ppm: 1% 100 ppm: 10% One control and three exposed showed leukemia	Overall neoplasms 0 ppm: 20% 100 ppm: 60%	
CBA/Ca BNL mouse (75–85 M)	0, 100	16 weeks 5 days/week 6 hours/day	Combined non-hematopoietic tumors (unspecified) 0 ppm: 14/70 100 ppm: 38/85[d]	NA	Cronkite et al. 1989
CBA/Ca BNL mouse (57–60 M, 54–60 F)	0, 300	16 weeks 5 days/week 6 hours/day	Myelogenous neoplasms 0 ppm: 0/60 300 ppm: 11/57[d] Combined non-hematopoietic tumors (Harderian and Zymbal gland, squamous cell and mammary carcinoma, papillary adenocarcinoma of the lung, benign tumors) 0 ppm: 13/60 300 ppm: 30/57[d]	Myelogenous neoplasms 0 ppm: 1/60 300 ppm: 6/54[d] Combined non-hematopoietic tumors (Harderian and Zymbal gland, squamous cell and mammary carcinoma, papillary adenocarcinoma of the lung, benign tumors) 0 ppm: 21/60 300 ppm: 43/54[d]	

Table 2-10. Summary of Inhalation Studies Evaluating Tumor Response in Rodents

Species (sex, n)	Concentration (ppm)	Duration	Exposure-related tumor outcomes		Reference
			Males	Females	
C57Bl/6 mouse (88–89 F)	0, 300	16 weeks 5 days/week 6 hours/day	NA	Thymic lymphoma 0 ppm: 1/88 300 ppm: 10/89[b] Leukemia (all types) 0 ppm: 8/88 300 ppm: 20/89[b] Zymbal gland epidermoid tumors and lymphoepithelioma 0 ppm: 1/88 300 ppm: 16/89[b] Ovarian solid tumors (unspecified) 0 ppm: 0/88 300 ppm: 8/89[b]	Cronkite et al. 1984, 1985

Table 2-10. Summary of Inhalation Studies Evaluating Tumor Response in Rodents

Species (sex, n)	Concentration (ppm)	Duration	Exposure-related tumor outcomes		Reference
			Males	Females	
C57BL mouse (80 M)	0, 1,200	10 weeks 5 days/week 6 hours/day	No exposure-related tumors		Snyder et al. 1988
CD-1 mouse (80 M)	0, 1,200	10 weeks 5 days/week 6 hours/day	Zymbal gland carcinoma 0 ppm: 0/71 300 ppm: 4/71[d] Lung adenoma 0 ppm: 17/71 300 ppm: 33/71[d]		

[a]Time-weighted average concentration; exposure was 200 ppm for the first 19 weeks and 300 ppm for the remaining 85 weeks.
[b]Statistically significant compared to control ($p<0.05$) based on 2-tailed Fisher's Exact Probably Test, conducted for this review (GraphPad).
[c]AKR/J mice spontaneously develop leukemia (spontaneous incidence of 85–95%)
[d]$p<0.05$ for dose compared to control group (as reported by the study authors).
[e]Estimated from graphically-presented data.

AML = acute myeloid leukemia; B = both (sexes); F = female(s); GD = gestation da; M = male(s); NA = not applicable; NS = not specified

Results of cancer bioassays in rats and mice following oral exposure are summarized in Table 2-11. In rats exposed to benzene for chronic durations, exposure-related tumors were observed in the Zymbal gland (adenoma, carcinoma), oral cavity (carcinoma, papilloma), and forestomach (acanthomas and dysplasia, *in situ* carcinomas) in both sexes (Maltoni et al. 1983, 1985, 1989; NTP 1986) and in the skin (squamous cell carcinoma or papilloma) in males (Maltoni et al. 1985, 1989; NTP 1986). The lowest dose associated with tumor induction was 25 mg/kg/day (NTP 1986). Zymbal gland carcinomas were observed at natural death following intermediate-duration exposure to 50 mg/kg/day in female rats (Maltoni et al. 1983, 1985, 1989). Tumors observed at natural death following intermediate-duration exposure to 500 mg/kg/day included leukemia and pulmonary tumors in both sexes and mammary carcinomas in females (Maltoni et al. 1989).

In mice, exposure-related tumors following chronic-duration exposure included malignant lymphoma, leukemia, Zymbal gland carcinoma, Harderian gland adenoma or carcinoma, and pulmonary tumors in both sexes (Maltoni et al. 1989; NTP 1986). Additional tumor sites in mice were adrenal gland, forestomach, and preputial gland in males and ovary and mammary gland in females (Maltoni et al. 1989; NTP 1986). In mice with tumor suppressor genes turned off (haploinsufficient $p16^{Ink4a}/p19^{Arf}$ mice), malignant lymphomas were observed in males following exposure to 200 mg/kg/day after only 27 weeks (NTP 2007). Multiple organs including the spleen, thymus, lymph node, kidney, lung, and/or brain were infiltrated with neoplastic cells.

Application of benzene to the skin of animals has not produced evidence of carcinogenicity, although most studies were inadequate for evaluation. As summarized by IARC (1982, 1987, 2012, 2018), many dermal carcinogenicity studies of chemicals other than benzene used benzene as a vehicle and treated large numbers of control animals (mice) with benzene alone. None of these studies indicated that benzene induced skin tumors; however, all possible tumor sites usually were not examined.

Table 2-11. Summary of Oral Studies Evaluating Tumor Response in Rodents

			Exposure-related tumor outcomes		
Species (sex, n)	Dose (mg/kg/day)	Duration	Males	Females	Reference
F-344/N rats (50 M, 50 F)	M: 0, 50, 100, 200 F: 0, 25, 50, 100	2 years 5 days/week	Zymbal gland carcinoma 0 mg/kg/day: 2/32 50 mg/kg/day: 6/46 100 mg/kg/day: 10/42 200 mg/kg/day: 17/42[a,b] Zymbal gland adenoma or carcinoma 0 mg/kg/day: 2/32 50 mg/kg/day: 7/46 100 mg/kg/day: 10/42 200 mg/kg/day: 18/42[a,b] Oral cavity squamous cell carcinoma or papilloma 0 mg/kg/day: 1/50 50 mg/kg/day: 9/50[a] 100 mg/kg/day: 16/50[a] 200 mg/kg/day: 19/50[a,b] Skin squamous cell carcinoma or papilloma 0 mg/kg/day: 1/50 50 mg/kg/day: 7/50[a] 100 mg/kg/day: 5/50 200 mg/kg/day: 12/50[a,b]	Zymbal gland carcinoma 0 mg/kg/day: 0/45 25 mg/kg/day: 5/40[a] 50 mg/kg/day: 5/44 100 mg/kg/day: 14/46[a,b] Zymbal gland adenoma or carcinoma 0 mg/kg/day: 0/45 25 mg/kg/day: 5/40[a] 50 mg/kg/day: 6/44[a] 100 mg/kg/day: 15/46[a,b] Oral cavity squamous cell carcinoma or papilloma 0 mg/kg/day: 1/50 25 mg/kg/day: 5/50 50 mg/kg/day: 12/50[a] 100 mg/kg/day: 9/50[a,b]	NTP 1986
Wistar rat (40 M, 40 F)	0, 500	104 weeks 4–5 days/week	Zymbal gland carcinoma[c] 0 mg/kg/day: 0/40 500 mg/kg/day: 7/40[d]	Zymbal gland carcinoma[c] 0 mg/kg/day: 0/40 500 mg/kg/day: 6/40[d]	Maltoni et al. 1989

Table 2-11. Summary of Oral Studies Evaluating Tumor Response in Rodents

Species (sex, n)	Dose (mg/kg/day)	Duration	Exposure-related tumor outcomes		Reference
			Males	Females	
Sprague-Dawley rat (40–50 M, 40–50 F)	0, 500	104 weeks 4–5 days/week	Zymbal gland carcinoma[c] 0 mg/kg/day: 1/50 500 mg/kg/day: 18/40[d] Oral cavity carcinoma[c] 0 mg/kg/day: 0/50 500 mg/kg/day: 21/40[d] Forestomach acanthomas and dysplasia 0 mg/kg/day: 0/50 500 mg/kg/day: 10/40[d] Skin carcinoma 0 mg/kg/day: 0/50 500 mg/kg/day: 9/40[d]	Zymbal gland carcinoma[c] 0 mg/kg/day: 0/50 500 mg/kg/day: 16/40[d] Oral cavity carcinoma[c] 0 mg/kg/day: 0/50 500 mg/kg/day: 20/40[d] Forestomach acanthomas and dysplasia 0 mg/kg/day: 0/50 500 mg/kg/day: 7/40[d] Forestomach *in situ* carcinomas 0 mg/kg/day: 0/50 500 mg/kg/day: 6/40[d]	Maltoni et al. 1985, 1989
Sprague-Dawley rat (40–50 M, 40–50 F)	0, 500	92 weeks 4–5 days/week	Zymbal gland carcinoma[c] 0 mg/kg/day: 1/48 500 mg/kg/day: 6/40[d] Oral cavity carcinoma[c] 0 mg/kg/day: 0/48 500 mg/kg/day: 7/40[d]	Zymbal gland carcinoma[c] 0 mg/kg/day: 0/49 500 mg/kg/day: 6/40[d] Oral cavity carcinoma[c] 0 mg/kg/day: 0/49 500 mg/kg/day: 4/40[d]	Maltoni et al. 1983
Sprague-Dawley rat (30–35 M, 30–35 F)	0, 50, 250	52 weeks 4–5 days/week	No exposure-related tumors	Zymbal gland carcinoma[c] 0 mg/kg/day: 0/30 50 mg/kg/day: 2/30 250 mg/kg/day: 8/32[d]	Maltoni et al. 1983, 1985, 1989

Table 2-11. Summary of Oral Studies Evaluating Tumor Response in Rodents

			Exposure-related tumor outcomes		
Species (sex, n)	Dose (mg/kg/day)	Duration	Males	Females	Reference
B6C3F1 mice (50 M, 50 F)	0, 25, 50, 100	2 years 5 days/week	Harderian gland adenoma or carcinoma 0 mg/kg/day: 1/49 25 mg/kg/day: 10/46[a] 50 mg/kg/day: 13/49[a] 100 mg/kg/day: 14/48[a,b] Alveolar/bronchiolar carcinoma 0 mg/kg/day: 5/49 25 mg/kg/day: 11/48 50 mg/kg/day: 12/50 100 mg/kg/day: 14/49[b] Zymbal gland carcinoma 0 mg/kg/day: 0/43 25 mg/kg/day: 3/34 50 mg/kg/day: 12/40[a] 100 mg/kg/day: 10/39[a,b] Lymphoma, all malignant 0 mg/kg/day: 4/49 25 mg/kg/day: 9/48 50 mg/kg/day: 9/50 100 mg/kg/day: 15/49[a,b]	Harderian gland adenoma or carcinoma 0 mg/kg/day: 5/48 25 mg/kg/day: 6/44 50 mg/kg/day: 10/50 100 mg/kg/day: 10/47[a,b] Alveolar/bronchiolar carcinoma 0 mg/kg/day: 0/49 25 mg/kg/day: 3/42 50 mg/kg/day: 6/50[a] 100 mg/kg/day: 6/49[a,b] Zymbal gland carcinoma 0 mg/kg/day: 0/43 25 mg/kg/day: 0/32 50 mg/kg/day: 1/37 100 mg/kg/day: 3/31[a,b] Lymphoma, all malignant 0 mg/kg/day: 15/49 25 mg/kg/day: 24/45[a] 50 mg/kg/day: 24/50 100 mg/kg/day: 20/49	NTP 1986

Table 2-11. Summary of Oral Studies Evaluating Tumor Response in Rodents

Species (sex, n)	Dose (mg/kg/day)	Duration	Exposure-related tumor outcomes: Males	Exposure-related tumor outcomes: Females	Reference
			Lymphoma or leukemia 0 mg/kg/day: 4/49 25 mg/kg/day: 10/48 50 mg/kg/day: 10/50 100 mg/kg/day: 15/49[a,b]	Lymphoma or leukemia 0 mg/kg/day: 15/49 25 mg/kg/day: 25/45[a] 50 mg/kg/day: 26/50 100 mg/kg/day: 22/49	
			Adrenal pheochromocytoma 0 mg/kg/day: 1/47 25 mg/kg/day: 1/48 50 mg/kg/day: 7/49[a] 100 mg/kg/day: 4/46	Ovarian granulosa cell tumor or carcinoma 0 mg/kg/day: 1/47 25 mg/kg/day: 1/44 50 mg/kg/day: 6/49[a] 100 mg/kg/day: 8/48[a,b]	
			Forestomach squamous cell papilloma 0 mg/kg/day: 2/45 25 mg/kg/day: 1/42 50 mg/kg/day: 2/44 100 mg/kg/day: 5/38[b]	Ovarian mixed tumor, benign 0 mg/kg/day: 0/47 25 mg/kg/day: 1/44 50 mg/kg/day: 12/49[a] 100 mg/kg/day: 7/48[a,b]	
			Preputial gland carcinoma 0 mg/kg/day: 0/21 25 mg/kg/day: 5/28 50 mg/kg/day: 19/29[a] 100 mg/kg/day: 31/35[a,b]	Mammary gland carcinoma 0 mg/kg/day: 0/49 25 mg/kg/day: 2/45 50 mg/kg/day: 5/50[a] 100 mg/kg/day: 10/49[a,b]	
				Mammary gland carcinosarcoma 0 mg/kg/day: 0/49 25 mg/kg/day: 0/45 50 mg/kg/day: 1/50 100 mg/kg/day: 4/49[a,b]	

Table 2-11. Summary of Oral Studies Evaluating Tumor Response in Rodents

Species (sex, n)	Dose (mg/kg/day)	Duration	Exposure-related tumor outcomes		Reference
			Males	Females	
Swiss mouse (40 M, 40 F)	0, 500	78 weeks 4–5 days/week	Pulmonary tumors[c] 0 mg/kg/day: 3/40 500 mg/kg/day: 17/40[d] Zymbal gland carcinoma or dysplasia[c] 0 mg/kg/day: 0/40 500 mg/kg/day: 7/40[d]	Pulmonary tumors[c] 0 mg/kg/day: 4/40 500 mg/kg/day: 15/40[d] Mammary carcinomas[c] 0 mg/kg/day: 2/40 500 mg/kg/day: 19/40[d]	Maltoni et al. 1989
RF/J mouse (45 M, 40 F)	0, 500	52 weeks 4–5 days/week	Pulmonary tumors[c] 0 mg/kg/day: 5/45 500 mg/kg/day: 23/45[d] Leukemias[c] 0 mg/kg/day: 17/45 500 mg/kg/day: 26/45	Pulmonary tumors[c] 0 mg/kg/day: 3/40 500 mg/kg/day: 18/40[d] Leukemias[c] 0 mg/kg/day: 14/40 500 mg/kg/day: 24/40[d] Mammary carcinomas[c] 0 mg/kg/day: 1/40 500 mg/kg/day: 9/40[d]	
B6129 mice (Haploinsufficient $p16^{Ink4a}/p19^{Arf}$) (15 M, 15 F)	0, 25, 50, 100, 200	27 weeks 5 days/week	Malignant lymphoma 0 mg/kg/day: 0/15 25 mg/kg/day: 0/15 50 mg/kg/day: 0/15 100 mg/kg/day: 0/15 200 mg/kg/day: 5/15[b]	No exposure-related tumors	NTP 2007

[a]p<0.05 for dose compared to control group (Incidental Tumor Tests).
[b]p<0.01 for dose-response related trend (as reported by the study authors).
[c]Incidence calculated for this review based on reported percent incidence (%) and animal number (n).
[d]Statistically significant compared to control (p<0.05) based on 2-tailed Fisher's Exact Probably Test, conducted for this review (GraphPad).

F = female(s); M = male(s)

2.19 GENOTOXICITY

The genotoxic effects of benzene have been studied extensively. The *in vivo* and *in vitro* data are summarized in Tables 2-12 and 2-13, respectively. In chronically-exposed humans, benzene primarily causes chromosomal aberrations. Chromosomal aberrations in humans are frequently demonstrated in peripheral blood lymphocytes and bone marrow. Although inhalation, oral, and dermal routes are all potential pathways of exposure relevant to humans, available *in vivo* human data are usually drawn from occupational settings in which inhalation and dermal exposure routes are most prevalent. In most of these studies, chromosome abnormalities were detected in workers exposed to high concentrations of benzene. However, Qu et al. (2003a, 2003b) noted a concentration-related increase in chromosomal aberrations across a wide range of exposure concentrations, including workers with relatively low-level benzene exposure. Limitations of many of the occupational studies include lack of exposure data, possible exposure to other chemicals, and lack of appropriate control groups.

Table 2-12. Genotoxicity of Benzene *In Vivo*

Species (test system)	Endpoint	Result	Reference
Prokaryotic cells			
Escherichia coli (host mediated DNA repair)	DNA synthesis	–	Hellmér and Bolcsfoldi 1992a
Insects			
Drosophila melanogaster	Sex-linked recessive lethal	–	Kale and Baum 1983
Spermatocytes	Recombination	–	
Spermatogonia	Recombination	+	
Spermatocytes	Heritable translocation	–	
Mammalian cells (nonhuman)			
Mouse (bone marrow)	Chromosomal aberrations	+	Giver et al. 2001; Shelby and Witt 1995; Siou et al. 1981
	Chromosomal aberrations	+	Meyne and Legator 1980
	Chromosomal aberrations	(+)	Tice et al. 1980, 1982
	Chromosomal aberrations	+	Mukhopadhyay and Nath 2014
Mouse (spleen lymphocytes)	Chromosomal aberrations	+	Au et al. 1991; Rithidech et al. 1987
Mouse (lymphoid cells, myeloid cells)	Chromosomal aberrations	+	Giver et al. 2001
Rat (bone marrow)	Chromosomal aberrations	+	Fujie et al. 1992; Hoechst 1977; Mukhopadhyay and Nath 2014; Philip and Jensen 1970; Styles and Richardson 1984
	Chromosomal aberrations	+	Anderson and Richardson 1981[b]
	Chromosomal aberrations	–	Hoechst 1977

Table 2-12. Genotoxicity of Benzene *In Vivo*

Species (test system)	Endpoint	Result	Reference
Chinese hamster (bone marrow)	Chromosomal aberrations	+	Siou et al. 1981
Rabbit (bone marrow)	Chromosomal aberrations	+	Kissling and Speck 1972, 1973
Mouse (bone marrow)	Micronuclei	+	Shelby and Witt 1995; Shelby et al. 1993
Mouse (bone marrow PCEs)	Micronuclei	+	Ciranni et al. 1988
	Micronuclei	+	Suzuki et al. 1989
	Micronuclei	+	Au et al. 1990; Barale et al. 1985; Chen et al. 1994; Diaz et al. 1980; Erexson et al. 1986; Farris et al. 1996; Harper et al. 1984; Hite et al. 1980; Siou et al. 1981; Toft et al. 1982
	Micronuclei	+	Meyne and Legator 1980
	Micronuclei	+[a]	Eastmond et al. 2001
	Micronuclei	+	Farris et al. 1996
	Micronuclei	+[a]	Eastmond et al. 2001
Mouse (pregnant/bone marrow PCEs)	Micronuclei	(+)	Ciranni et al. 1988
Mouse (peripheral blood)	Micronuclei	+	Hayashi et al. 1992; Healy et al. 2001
Mouse (peripheral blood PCEs)	Micronuclei	+	Farris et al. 1996
	Micronuclei	+[a]	Luke et al. 1988a
	Micronuclei	+	Barale et al. 1985; Choy et al. 1985; Farris et al. 1996; Rithidech et al. 1988
	Micronuclei	+[a]	Luke et al. 1988a
Mouse (peripheral PCEs and erythrocytes)	Micronuclei	+	French et al. 2015
Mouse (lung fibroblasts)	Micronuclei	+	Ranaldi et al. 1998
Mouse (fetus/liver cells)	Micronuclei	+	Ciranni et al. 1988
Rat (lymphocytes)	Micronuclei	+	Erexson et al. 1986
Rat (bone marrow PCEs)	Micronuclei	+	Kitamoto et al. 2015
Chinese hamster (bone marrow)	Micronuclei	+	Siou et al. 1981
Mouse (bone marrow)	Sister chromatid exchange	+	Tice et al. 1980, 1982
Mouse (pregnant/bone marrow)	Sister chromatid exchange	+	Sharma et al. 1985
Mouse (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
Mouse (fetus/liver cells)	Sister chromatid exchange	+	Sharma et al. 1985
Rat (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
Mouse (spleen lymphocytes)	Mutations	+	Ward et al. 1992
Mouse (lung tissue)	Mutations	+	Mullin et al. 1998
Mouse embryo (premelanocytes)	Mutations (deletions)	+	Schiestl et al. 1997
Mouse (white blood cells)	DNA adducts	+	Lévay et al. 1996

Table 2-12. Genotoxicity of Benzene *In Vivo*

Species (test system)	Endpoint	Result	Reference
Mouse (liver)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Mani et al. 1999; Turteltaub and Mani 2003
Rat (bone marrow)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Pathak et al. 1995; Turteltaub and Mani 2003
Rat (liver)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lutz and Schlatter 1977; Mani et al. 1999; Mazzullo et al. 1989; Turteltaub and Mani 2003
Mouse (peripheral blood lymphocytes)	DNA strand breaks	+	Tuo et al. 1996
Rat (lymphocytes, bone marrow, spleen, liver)	DNA strand breaks	+	Lee et al. 2005
Mouse (peripheral blood lymphocytes, bone marrow)	DNA damage	+	Chang et al. 2005
Mouse (lymphocytes)	DNA damage	+	Mukhopadhyay and Nath 2014
Rat (fetal liver)	DNA damage	+	Holmes and Winn 2022
Rat (liver, stomach, bone marrow)	DNA damage	-	Kitamoto et al. 2015
Rat (bone marrow)	DNA oxidative damage	+	Kolachana et al. 1993
Mouse (bone marrow)	DNA synthesis inhibition	+	Lee et al. 1988
Rabbit (bone marrow)	DNA synthesis inhibition	+	Kissling and Speck 1972
Mouse (bone marrow)	RNA synthesis inhibition	+	Kissling and Speck 1972
Rat (liver mitochondria)	RNA synthesis inhibition	+	Kalf et al. 1982
Human cells			
Mouse (spermatogonia)	Sperm head abnormality	+	Topham 1980
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Bogadi-Šare et al. 1997; Ding et al. 1983; Forni et al. 1971; Kašuba et al. 2000; Picciano 1979; Sasiadek 1992; Sasiadek and Jagielski 1990; Sasiadek et al. 1989; Smith et al. 1998; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Yang et al. 2010; Zhang et al. 1998a, 1999
	Chromosomal aberrations	(+)	Yardley-Jones et al. 1990
	Chromosomal aberrations	–	Bogadi-Šare et al. 1997; Jablonická et al. 1987; Lovreglio et al. 2014;
Human (lymphocytes)	Micronuclei	+	Robertson et al. 1991

Table 2-12. Genotoxicity of Benzene *In Vivo*

Species (test system)	Endpoint	Result	Reference
Human (occupational exposure/lymphocytes)	Micronuclei	+	Cao et al. 2023; Kim et al. 2010; Liu et al. 1996; Lovreglio et al. 2014; Zhang et al. 2014
	Micronuclei	–	Pitarque et al. 1996; Surrallés et al. 1997
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	+	Popp et al. 1992; Tunsaringkarn et al. 2011
	Sister chromatid exchange	–	Kašuba et al. 2000; Pitarque et al. 1997; Seiji et al. 1990; Yardley-Jones et al. 1988
Human (sperm)	Mutation	+	Katukam et al. 2012
Human (bone marrow)	Mutations (gene-duplicating)	+	Rothman et al. 1995
	Mutations (gene-inactivating)	–	Rothman et al. 1995
	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Pathak et al. 1995; Turteltaub and Mani 2003
Human (occupational exposure/lymphocytes)	DNA strand breaks	+	Andreoli et al. 1997; Nilsson et al. 1996; Sul et al. 2002
Human (occupational exposure/lymphocytes)	DNA repair efficiency	_	Hallberg et al. 1996
Human (occupational exposure/lymphocytes)	DNA oxidative damage	+	Liu et al. 1996
		–	Lovreglio et al. 2014

[a]Increase in micronuclei was exposure duration-dependent.

+ = positive result; – = negative result; (+) = weakly positive result; DNA = deoxyribonucleic acid; PCE = polychromatic erythrocyte; RNA = ribonucleic acid

Table 2-13. Genotoxicity of Benzene *In Vitro*

		Result		
Species (test system)	Endpoint	With activation	Without activation	Reference
Prokaryotic organisms				
Salmonella typhimurium (Ames test)	Gene mutation	–	–	De Flora et al. 1984
S. typhimurium (histidine reversion)	Gene mutation	+	–	Glatt et al. 1989
S. typhimurium (azaquanine reversion)	Gene mutation	+	No data	Kaden et al. 1979; Seixas et al. 1982
Bacillus subtilis (histidine reversion)	Gene mutation	–	–	Tanooka 1977

Table 2-13. Genotoxicity of Benzene *In Vitro*

Species (test system)	Endpoint	Result		Reference
		With activation	Without activation	
Escherichia coli (DNA polymerase 1/cell-free DNA synthetic system)	DNA synthesis inhibition	No data	–	Lee et al. 1988
E. coli (host meditated DNA repair)	DNA synthesis	No data	No data	Hellmér and Bolcsfoldi 1992b
Plasmid DNA ΦX-174 RF I	DNA degradation	No data	+	Li et al. 1995
Saccharomyces cerevisiae	Intrachromosomal recombination	No data	+	Sommers and Schiestl 2006
Eukaryotic organisms (nonhuman)				
Fungi:				
Aspergillus nidulans (methionine suppressors)	Gene mutation	No data	–	Crebelli et al. 1986
Mammalian (non-human) cells:				
Mouse (L5178Y cells/TK test)	Gene mutation	–	–	Oberly et al. 1984
Chinese hamster (ovary cell culture)	Chromosomal aberrations	–	–	Gulati et al. 1989; Pandey et al. 2009a, 2009b
Chinese hamster (ovary cell culture)	Micronuclei	–	–	Douglas et al. 1985
Chinese hamster (ovary cell culture)	Sister chromatid exchange	–	–	Douglas et al. 1985; Gulati et al. 1989
Rabbit (bone marrow mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984
Rat (liver mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984
Calf thymus DNA	DNA adducts	No data	+	Chenna et al. 1995
Rat (hepatocytes)	DNA breaks	No data	–	Bradley 1985
Chinese hamster (ovary cell culture)	DNA breaks	+	+	Douglas et al. 1985; Pandey et al. 2009a, 2009b
	DNA breaks	+	+[a]	Lakhanisky and Hendricks 1985
Chinese hamster (V79 cell culture)	DNA breaks	–	–	Swenberg et al. 1976
Mouse (L5178Y cell culture)	DNA breaks	No data	–	Pellack-Walker and Blumer 1986
Rat liver epithelial cells	DNA hyperphos-phorylation	No data	+	Dees and Travis 1994
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	(+)	Glauert et al. 1985
	Unscheduled DNA synthesis	No data	–	Probst and Hill 1985; Williams et al. 1985

Table 2-13. Genotoxicity of Benzene *In Vitro*

Species (test system)	Endpoint	Result		Reference
		With activation	Without activation	
Mouse (bone marrow cell culture)	DNA synthesis inhibition	No data	+	Lee et al. 1988
	DNA synthesis inhibition	+	(+)	Lee et al. 1989
Calf (thymus DNA polymerase α/cell-free DNA synthetic system)	DNA synthesis inhibition	No data	+	Lee et al. 1988
Mouse (spleen lymphocytes)	RNA synthesis inhibition	No data	+	Post et al. 1985
Rat (liver mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982
Cat, rabbit (bone marrow mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982
Human cells:				
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	+	Eastmond et al. 1994; Morimoto 1976
	Chromosomal aberrations	No data	–	Gerner-Smidt and Friedrich 1978; Holeckova et al. 2008
Human (lymphoblastoid culture)	Intrachromosomal recombination	No data	+	Aubrecht et al. 1995
Human (whole blood cells)	Micronuclei	–	–	Zarani et al. 1999
Human (lymphocyte cell culture)	Micronuclei	No data	_	Holeckova et al. 2008
Human (whole blood cells)	Micronuclei	–	–	Zarani et al. 1999
Human (lymphocyte cell culture)	Micronuclei	No data	_	Holeckova et al. 2008
Human (lymphocyte cell culture)	Sister chromatid exchange	+	No data	Morimoto 1983
	Sister chromatid exchange	No data	–	Gerner-Smidt and Friedrich 1978
	Sister chromatid exchange	+	+	Siviková et al. 2005
Human (bone marrow)	DNA adducts	No data	+	Bodell et al. 1993; Lévay and Bodell 1992
Human (leukemia cells)	DNA adducts	No data	+	Bodell et al. 1993; Lévay and Bodell 1992
Human (lymphocyte cell culture)	DNA breaks	+	No data	Peng et al. 2012
Human (leukemia cells)	DNA oxidative damage	No data	+	Kolachana et al. 1993
Human (A549 cell culture)	DNA damage	No data	–	Zhang et al. 2017

Table 2-13. Genotoxicity of Benzene *In Vitro*

Species (test system)	Endpoint	Result		Reference
		With activation	Without activation	
Human (lymphocyte cell culture)	DNA repair	No data	_	Hallberg et al. 1996
Human (HeLa S3 cells)	Unscheduled DNA synthesis	–	–	Barrett 1985
Human (HeLa cells)	DNA synthesis inhibition	–	–	Painter and Howard 1982

[a]Effect of benzene on DNA breaks was reduced when metabolic activators were used.

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

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

DNA repair efficiency was evaluated in blood lymphocytes collected from exposed or unexposed workers in a petrochemical plant (Hallberg et al. 1996). Lymphocytes from exposed or unexposed workers did not show significant differences in their ability to repair light-damaged DNA; however, the study authors suggested that the sample population was too small to detect any differences given the large individual variations in repair capacity (Hallberg et al. 1996).

Results of *in vivo* studies in animals and *in vitro* studies in eukaryotic and prokaryotic cells provide convincing evidence of benzene's genotoxicity. Consistent, positive findings for chromosomal aberrations in bone marrow and lymphocytes in animals support the human case reports and epidemiological studies in which chromosomal damage was linked to benzene exposure. Positive results were observed in all studies testing for increased micronuclei frequencies. Although no human studies were located that reported increased sister chromatid exchange in exposed individuals, increases in sister chromatid exchange have been reported in mice and rats (Erexson et al. 1986; Sharma et al. 1985; Tice et al. 1980, 1982).

2.20 MECHANISMS OF ACTION

It has been established that the toxicity of benzene is primarily due to its toxic metabolites. Although numerous mechanisms are involved in the toxicity of benzene, it is likely that nearly all effects are due to cellular damage of reactive benzene metabolites. Thus, there are numerous studies investigating the role of benzene metabolites in benzene-induced toxicity. The role of reactive metabolites in the toxicity of benzene has been extensively reviewed by IARC (2018); the following information is summarized from this review.

Metabolism of benzene results in the formation of multiple reactive electrophilic intermediates and pro-oxidant metabolites. Reactive metabolites include epoxides, muconaldehyde and other open-ring compounds, and quinones and semiquinones. These reactive oxygen species interact with, and damage, cellular molecules and structures, including proteins, DNA, and ribonucleic acid (RNA), resulting in altered cell function. Thus, oxidative stress is an important mechanism for benzene-induced toxicity, including hematological effects, immunotoxicity, genotoxicity, and cancer. Reactive metabolites produce genomic instability, including damage to DNA (binding to DNA, strand breaks, gene mutations), chromosomal damage, altered chromosome translations, and decreased DNA repair.

All tissues have the capacity to metabolize benzene. However, the liver is the primary tissue for benzene metabolism. Reactive metabolites are transported to extrahepatic sites, including bone marrow. Reactive metabolites are also be generated within the bone marrow.

Reactive metabolites of benzene are made less toxic through glutathione conjugations. Certain polymorphisms of glutathione transferases (GSTs) may result in a decrease in conjugation reactions, leading to increased toxicity. Polymorphisms of other enzymes involved in the metabolism of benzene,

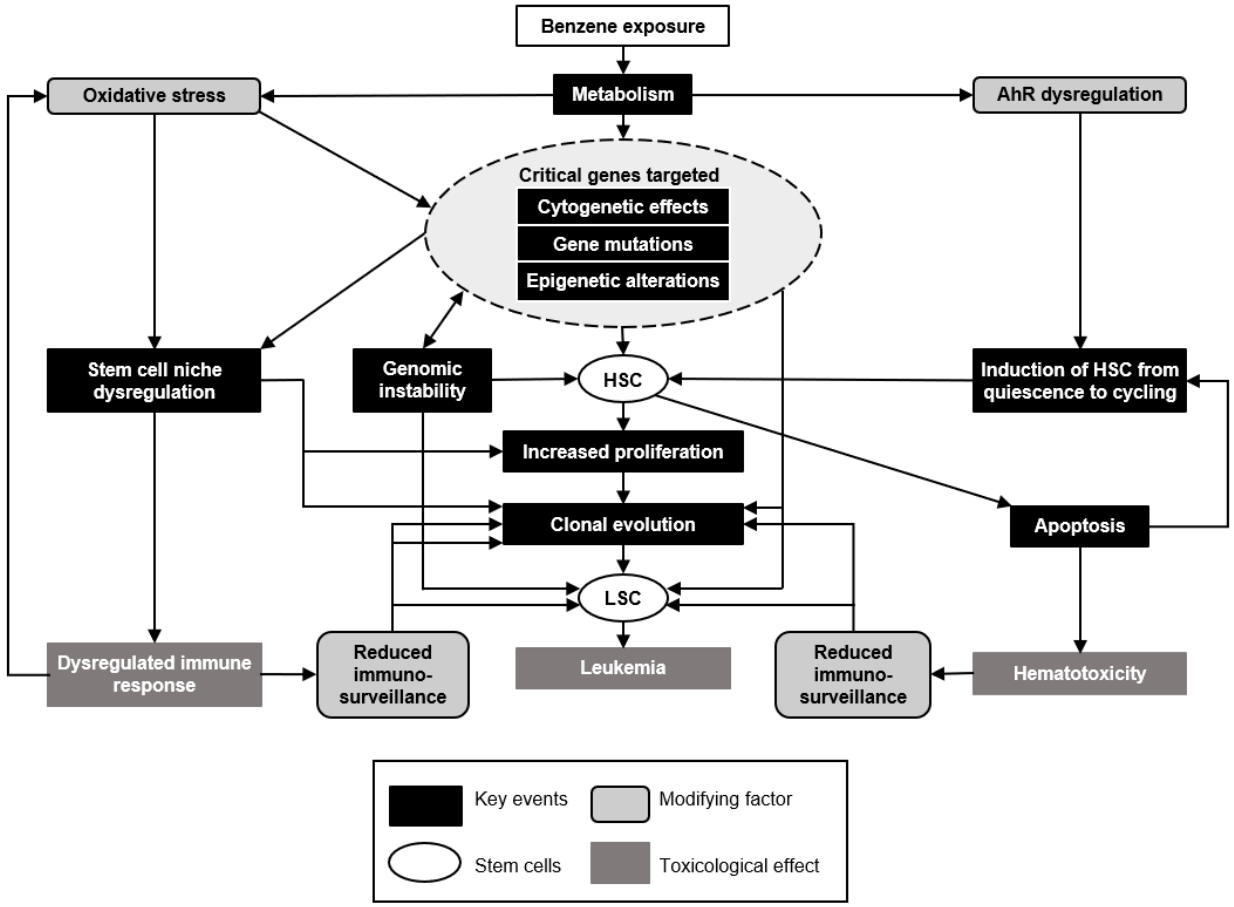
including NQO1, epoxide hydrolase, and MPO, may alter enzyme activity and thereby alter the levels of reactive metabolites. This is discussed in more detail in Section 3.2.

Other mechanisms involved in the toxicity of benzene include altered cell proliferations, apoptosis, chronic inflammation, epigenetic alterations, and polymorphisms of cytokines and vascular adhesion molecules. In addition to IARC (2018), other reviews include additional proposed mechanisms, such as receptor dysregulation, altered intracellular gap-junction communication, altered protein phosphorylation, stem cell dysregulation, and epigenetic modifications (DNA methylation, post-translational modifications, and altered microRNA expression) (Cordiano et al. 2022; Fenga et al. 2016; McHale et al. 2012; Mozzoni et al. 2023). Decreased expression of protein phosphatase 2 (deletion of the Ppp2r1a gene) in mice down-regulated CYP2E1 and decreased sensitivity of mice to hematological effects of benzene (Chen et al. 2019). However, it is likely that the initiating event for all mechanisms is due to reactive metabolic interactions with cellular macromolecules.

As discussed in previous sections of the profile, hematotoxicity, immunotoxicity, and leukemia are well-established and sensitive effects of benzene. Although the mechanisms of benzene toxicity have not been fully elucidated, McHale et al. (2012) has proposed the mechanistic scheme shown in Figure 2-4 to demonstrate the critical role of benzene metabolites in the development of these effects.

Disruption of gene expression and its consequences have been associated with hematological toxicity of benzene. This includes aberrant regulation of long non-coding and micro RNAs, damage to DNA, and abnormalities in DNA repair response (Kaina et al. 2018; Tian et al. 2020; Wang et al. 2012, 2021c). Increased mitochondrial DNA copy numbers and chromosomal telomere length, indicative of a “cell survival or longevity” response, have been observed in workers exposed to benzene (Li et al. 2020; Ren et al. 2020) and may contribute to nonlinear dose-response relationships for hematological toxicity observed in some studies of worker populations (Cox et al. 2021; Vermeulen et al. 2023). Induction of deacetylation and autophagy and metabolomic abnormalities have also been associated with benzene hematological toxicity (Guo et al. 2022; Qian, 2019).

Figure 2-4. Mechanisms of Action for Benzene-Induced Hematological and Immunological Effects and Leukemogenesis



HSC = hematopoietic stem cell; LSC = leukemic stem cell

Source: McHale et al. 2012 by permission of Oxford University Press

Wang et al. (2024) proposed a mechanistic scheme that connects various early events observed in workers to hematological and immunological effects. In this scheme, events observed in workers include epigenetic alterations, cytotoxicity, gene mutations, oxidative stress increased chromosome telomere lengths, and increased mitochondrial DNA copy numbers. These changes contribute to apoptosis/ autophagy, genomic instability, disruption of hematopoiesis, impaired DNA repair responses, and decreased immune surveillance. The downstream consequences are altered gene expression of cell signaling pathways increased hematopoietic cell proliferation and clonal evolution, and leukemia.