Exhibit 187

Toxicological Profile for Benzene

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Agency for Toxic Substances and Disease Registry

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DISCLAIMER

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FOREWORD

This toxicological profile is prepared in accordance with guidelines developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance and the associated acute, intermediate, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine the levels of exposure that present a significant risk to human health due to acute-, intermediate-, and chronic-duration exposures; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public. ATSDR plans to revise these documents in response to public comments and as additional data become available. Therefore, we encourage comments that will make the toxicological profile series of the greatest use.

Electronic comments may be submitted via: www.regulations.gov. Follow the on-line instructions for submitting comments.

Written comments may also be sent to: Agency for Toxic Substances and Disease Registry
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The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA Section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health-related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under Section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staffs of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and is being made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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VERSION HISTORY

Date	Description
October 2024	Draft for public comment toxicological profile released
June 2015	Addendum to the toxicological profile released
August 2007	Final toxicological profile released
September 1997	Final toxicological profile released
April 1993	Final toxicological profile released
May 1989	Final toxicological profile released

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ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

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CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

1.1 OVERVIEW AND U.S. EXPOSURES

Benzene is ubiquitous in the environment. It is a flammable organic compound and is formed from human activities and by natural processes. Benzene is slightly soluble in water and evaporates rapidly into air, with outdoor air concentrations ranging from 0.082 to 4.66 ppbv. The odor recognition threshold in air is 97 ppm. Therefore, populations can be exposed excessively to benzene without knowledge of the exposure or exposure-associated health hazards.

Benzene is widely distributed in the environment. The exposure scenario of most concern to the general public is low-level inhalation over long periods. This is because the general population is exposed to benzene mainly through inhalation of contaminated air, particularly in areas of heavy traffic and around gas stations, through inhalation of tobacco smoke from both active and passive smoking, and in some cases, from poorly ventilated indoor air. Smoking has been identified as the single most important source of benzene exposure for the estimated 40 million U.S. smokers. Smoking accounts for approximately half of the total benzene exposure of the general population. Individuals employed in industries that make or use benzene, or products containing benzene, are probably exposed to the highest concentrations of benzene. In addition, benzene is a common combustion product of wood and organic material, providing high inhalation exposure potential for firefighters. Of the general population, those residing around certain chemical manufacturing sites or living near waste sites containing benzene or near leaking fuel tanks may be exposed to concentrations of benzene that are higher than background air concentrations. In private residences, benzene levels in the air have been shown to be higher in houses with attached garages, where the inhabitants smoke inside the house, or where gas stoves or ovens are used.

Benzene may be present in food, beverages, and water; however, benzene is at low levels in these items and, therefore, not considered a major exposure. Benzene contamination of well water may occur from leakage of underground gasoline storage tanks and seepage from landfills and hazardous waste sites. People with contaminated tap water can be exposed from drinking the water or eating foods prepared with it. In addition, exposure can also occur via inhalation during showering, bathing, or cooking with contaminated tap water. Showering and bathing with benzene-contaminated water can also contribute to dermal exposure.

1.2 SUMMARY OF HEALTH EFFECTS

Exposure to benzene is associated with numerous adverse effects in several organ systems. This is due to highly reactive metabolites of benzene that are widely distributed throughout the body. However, the primary and most sensitive targets of benzene are the hematopoietic and immune systems. Hematotoxicity, immunotoxicity, and hematopoietic cancer (acute myelogenous leukemia or AML) are well-established health effects of benzene. The hematological effects of benzene were reported in workers in the early 1900s, with leukemia first reported in 1928 (Smith 2010). Since those initial reports, numerous studies have confirmed associations between occupational exposures to benzene and hematotoxicity, immunotoxicity, and leukemia, with support from several studies in laboratory animals. As illustrated in Figures 1-1 and 1-2, the most sensitive effects of benzene are on the hematological and immunological systems. A systematic review of these endpoints (Appendix C) resulted in the hazard identification conclusion that hematological effects are a known health effect for humans.

- ***Hematological:*** The primary effect of benzene on the hematological system is disruption of hematopoiesis (production of blood cells). 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).

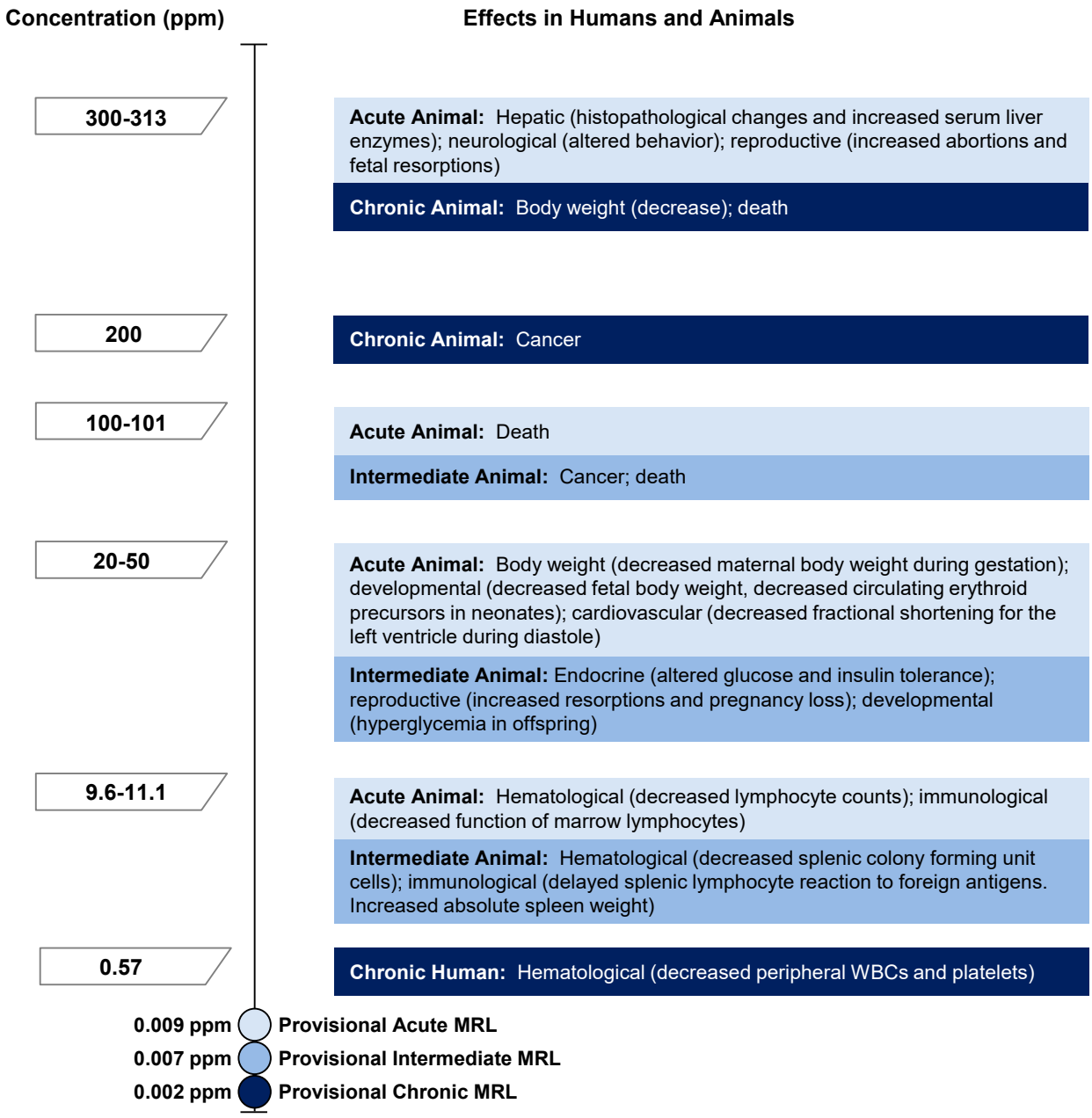
The systematic review identified immunological effects as a presumed health effect for humans.

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

Studies evaluating developmental effects and cancer from benzene did not undergo formal systematic review; however, the following conclusions are drawn.

- ***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 to 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.

Figure 1-1. Health Effects Found in Animals Following Inhalation Exposure to Benzene*



*Health effect displayed only at the most sensitive dose.

Figure 1-2. Health Effects Found in Animals Following Oral Exposure to Benzene*

Dose (mg/kg/day)	Effects in Animals
810-1,000	**Acute:** Death; neurological (altered brain neurotransmitters); body weight (decreased)
200-250	**Acute:** Hematological (decreased peripheral white blood cells) **Intermediate:** Endocrine (hyperglycemia and altered insulin response); body weight (decreased); cancer; death
100	**Intermediate:** Hepatic (increased fatty acids) **Chronic:** Body weight (decreased); death
41-50	**Acute:** Dermal (alopecia) **Intermediate:** Hematological (decreased erythrocytes); neurological (impaired short-term memory) **Chronic:** Respiratory (alveolar hyperplasia)
25	**Chronic:** Hematological (decreased white blood cells and lymphocytes, increased frequency of micronucleated erythrocytes); gastrointestinal (forestomach epithelial hyperplasia); endocrine (hyperplasia of adrenal and harderian glands); cancer
1-8	**Intermediate:** Hematological (decreased WBCs, lymphocytes, neutrophils, and monocytes); immunological (decreased splenic lymphocyte function)
9×10^{-4} mg/kg/day	**Provisional Acute and Intermediate MRL**
3×10^{-4} mg/kg/day	**Provisional Chronic MRL**

*Health effect displayed only at the most sensitive dose.

- ***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 show that exposure to benzene induced tumors at multiple sites in rats and mice, with a tendency towards induction of lymphomas in mice.

The Department of Health and Human Services (HHS) has determined that benzene is a known human carcinogen (NTP 2021). The International Agency Research on Cancer (IARC 2018) has classified benzene as a Group 1 (carcinogenic to humans) agent, and the U.S. Environmental Protection Agency (EPA) has classified benzene in Group A (known human carcinogen) (IRIS 2003).

1.3 MINIMAL RISK LEVELS (MRLs)

The inhalation database was considered adequate for derivation of an acute-, intermediate- and chronic-duration inhalation MRLs for benzene, with hematological and immunological effects as the most sensitive and well-studied effects. The toxicity of benzene following oral exposure has been much less studied compared to inhalation exposure. Available oral data identify hematological and immunological effects as the most sensitive. Adequate data are available to derive an intermediate-duration oral MRL. No adequate oral exposure studies were identified to derive acute- or chronic-duration oral MRLs. However, the intermediate-duration oral MRL was adopted for the acute-duration oral MRL. For the chronic-duration oral MRL, the intermediate-duration oral MRL was adopted with application of a modifying factor. For both the inhalation and oral databases, hematological effects are the most sensitive, as shown in Figures 1-3 and 1-4, respectively. The provisional MRLs are summarized in Table 1-1.

Figure 1-3. Summary of Sensitive Targets of Benzene – Inhalation

Available data indicate that the hematological and immunological systems are the most sensitive targets of benzene inhalation exposure.

Numbers in triangles and circles are the lowest LOAELs (ppm) among health effects in humans and animals, respectively.

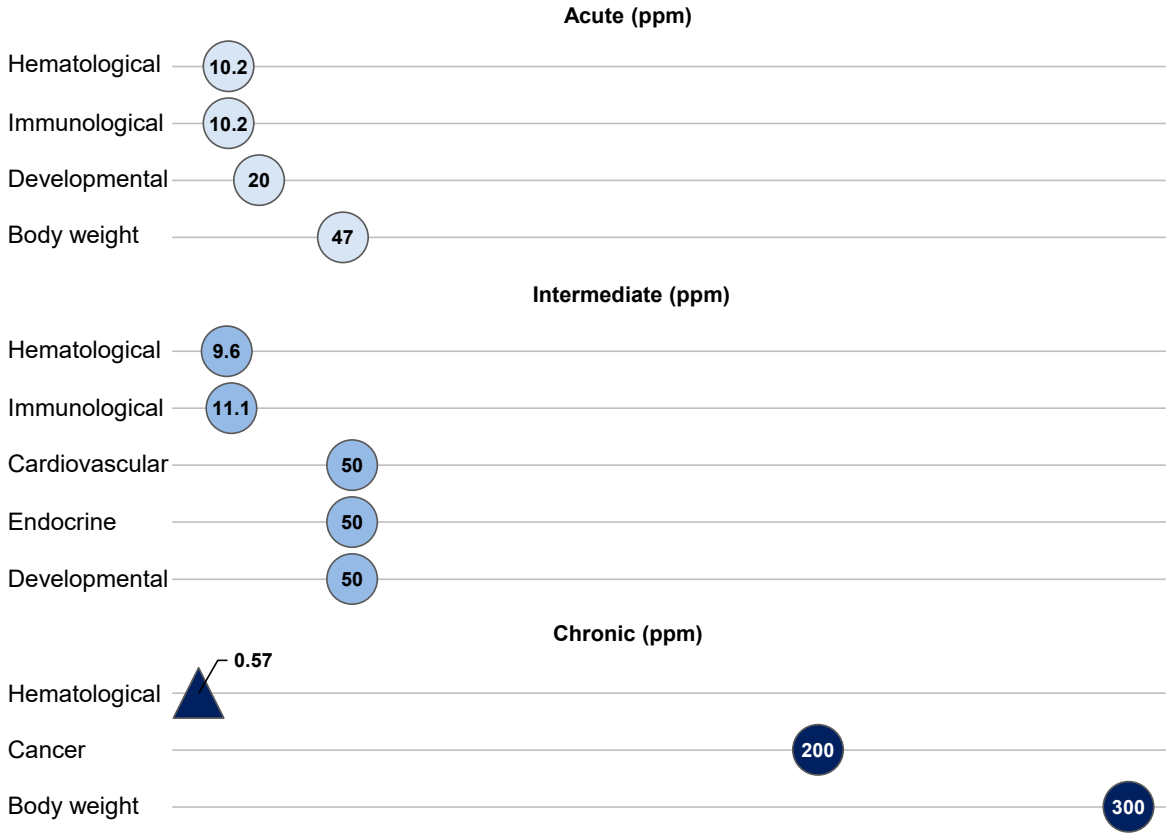


Figure 1-4. Summary of Sensitive Targets of Benzene – Oral

Available data indicate that the hematological system is the most sensitive target of benzene.
Numbers in circles are the lowest LOAELs (mg/kg/day) for all health effects in animals.
No reliable dose response data were available for humans.

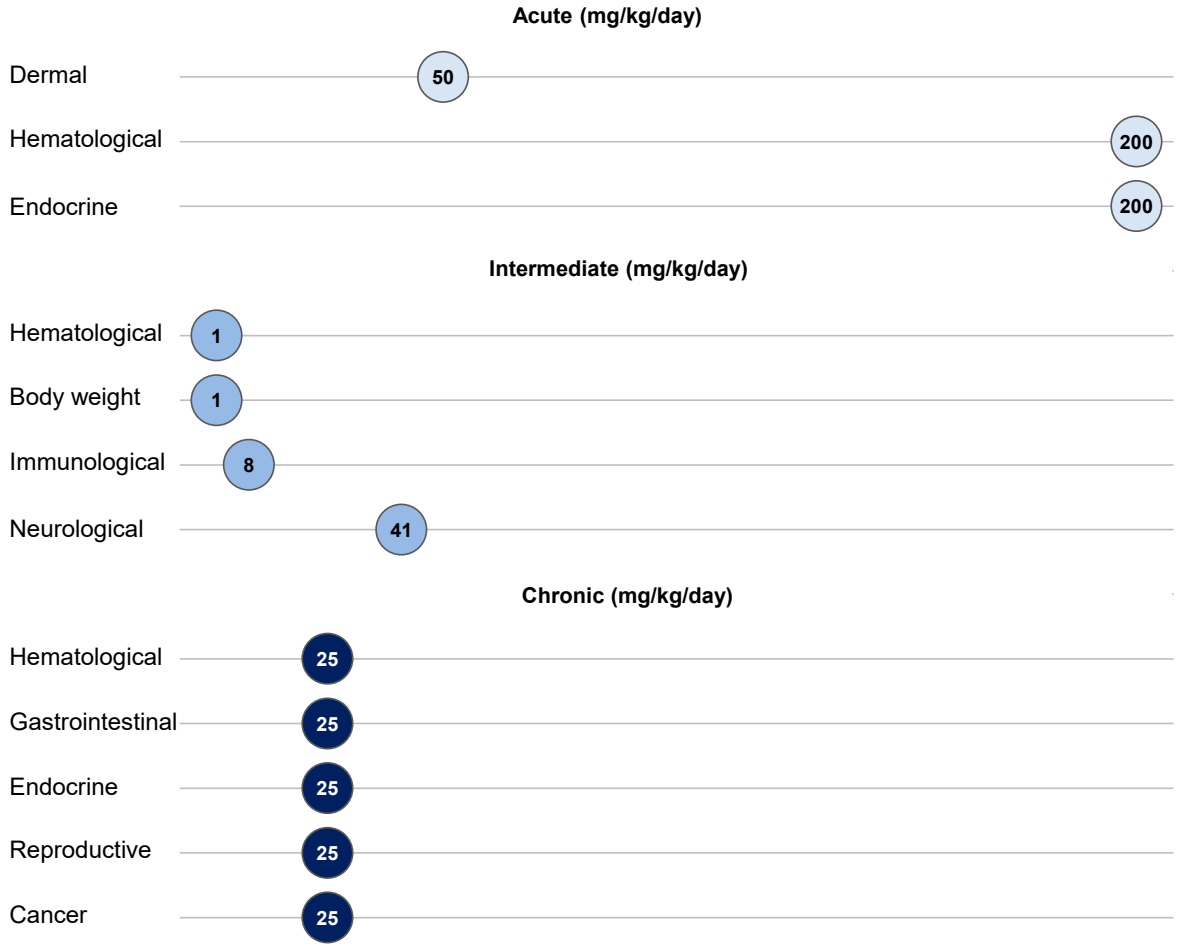


Table 1-1. Minimal Risk Levels (MRLs) for Benzene[a]

Exposure route	Exposure duration	Provisional MRL	Critical effect	POD type	POD value	Uncertainty/ modifying factor	Reference
Inhalation	Acute	**0.009 ppm** (0.03 mg/m^3)	Decreased number of peripheral lymphocytes and impaired function of marrow lymphocytes	$LOAEL_{HEC}$	2.55 ppm	UF: 300	Rozen et al. 1984
	Intermediate	**0.007 ppm** (0.02 mg/m^3)	Delayed splenic lymphocyte reaction to foreign antigens	$LOAEL_{HEC}$	1.98 ppm	UF: 300	Rosenthal and Snyder 1987
	Chronic	**0.002 ppm** (6x10^{-3} mg/m^3)	Decreased number of peripheral lymphocytes	$LOAEL_{ADJ}$	0.16 ppm	UF: 100	Lan et al. 2004a
Oral	Acute	**9x10^{-4} mg/kg/day**	Decreased peripheral WBC, lymphocyte, monocyte, and neutrophil counts	$NOAEL_{ADJ}$	0.09 mg/kg/day	UF: 100	Li et al. 2018
	Intermediate	**9x10^{-4} mg/kg/day**	Decreased peripheral WBC, lymphocyte, monocyte, and neutrophil counts	$NOAEL_{ADJ}$	0.09 mg/kg/day	UF: 100	Li et al. 2018
	Chronic	**3x10^{-4} mg/kg/day**	Decreased peripheral WBC, lymphocyte, monocyte, and neutrophil counts	$LOAEL_{ADJ}$	9.1x10^{-4} mg/kg/day[b]	MF: 3[c]	Lan et al. 2004a

[a]See Appendix A for additional information.
[b]Route-to-route extrapolation from the provisional chronic-duration inhalation MRL to equivalent oral exposure.
[c]An uncertainty factor for human variability was not applied in deriving the provisional chronic-duration oral MRL because an uncertainty factor of 10 for human variability was included in deriving the provisional chronic-duration inhalation MRL, which is the basis for the provisional chronic-duration oral MRL.

ADJ = adjusted for intermittent exposure; HEC = human equivalent concentration; LOAEL = lowest observed adverse effect level; MF = modifying factor; NOAEL = no-observed-adverse-effect level; POD = point of departure; UF = uncertainty factor; WBC = white blood cell

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.

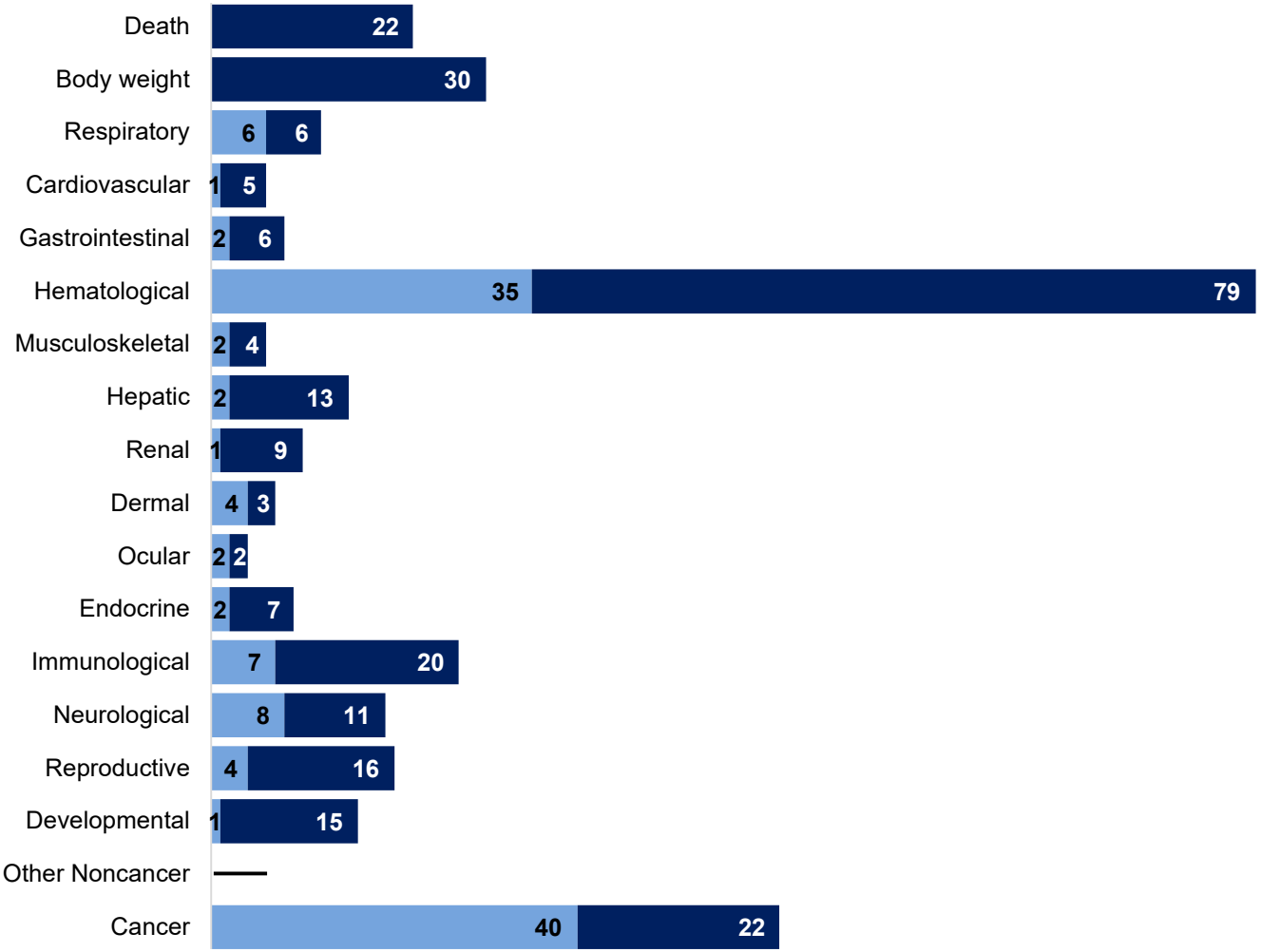
The toxicology of inhaled and oral benzene has also been studied extensively in mice and, to a lesser extent, in rats. These studies have confirmed the toxicity of benzene to hematopoietic tissues. Outcomes observed in animal studies include decreases in peripheral leukocytes and erythrocytes, decreases 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.

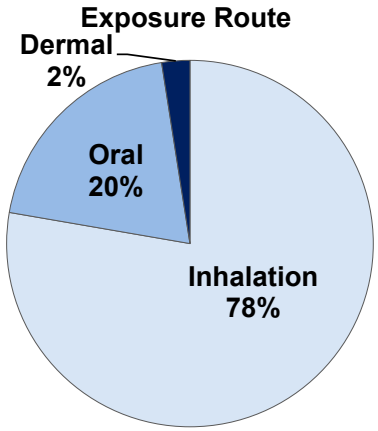
As illustrated in Figure 2-1, numerous human and animal studies evaluating adverse effects of benzene exposure were reviewed and included in this document. Most studies evaluated the effects of inhalation exposure, followed by oral exposure. The most studied endpoints include the hematological and immunological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

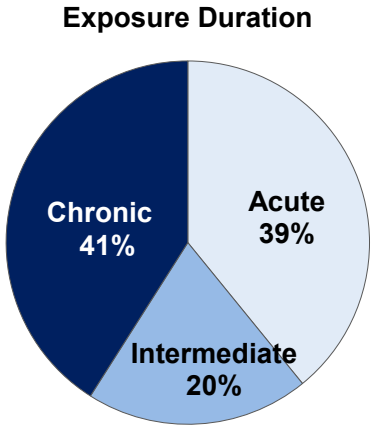
Figure 2-1. Overview of the Number of Studies Examining Benzene Health Effects*

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/6B NL) 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)

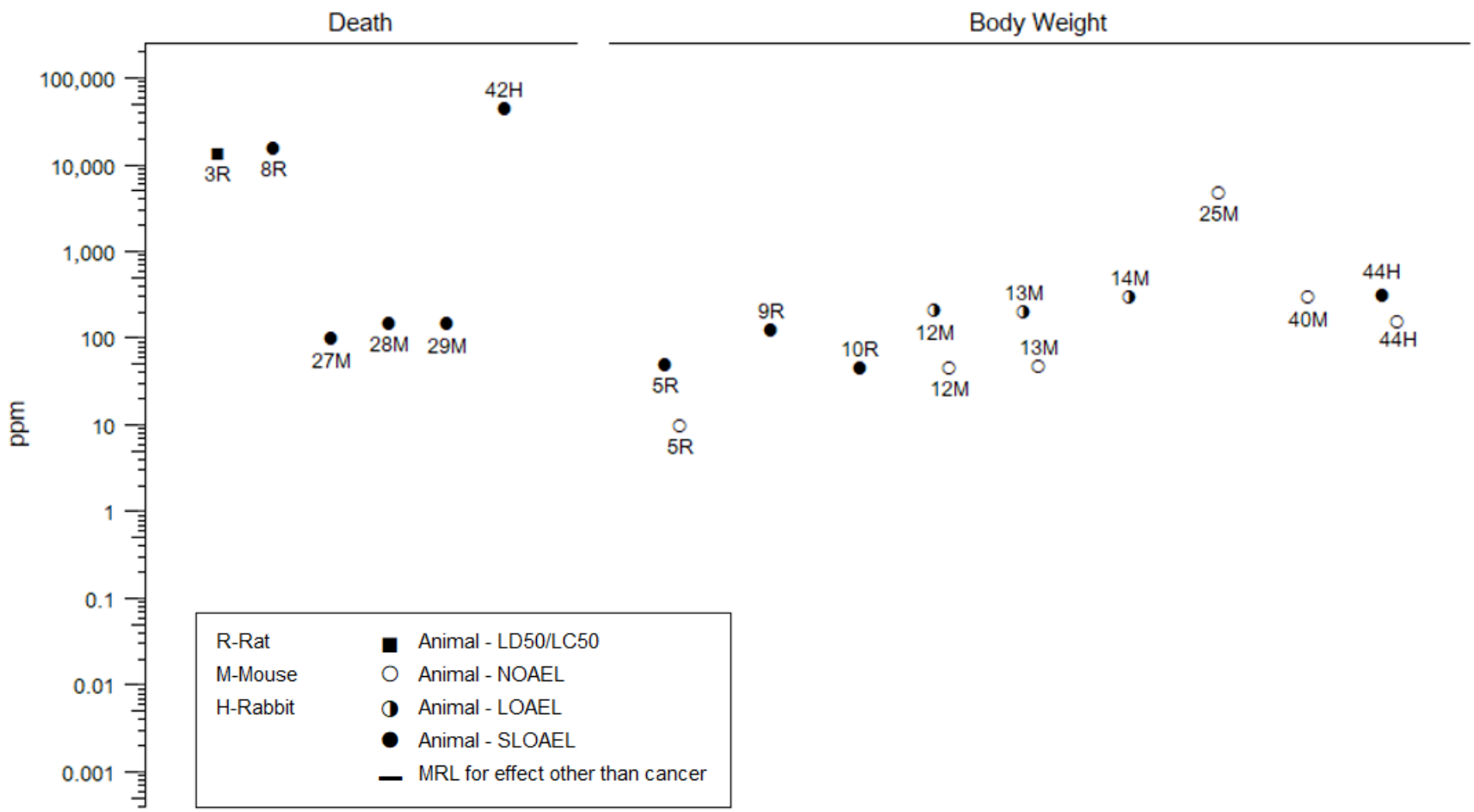


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Acute (≤14 days)

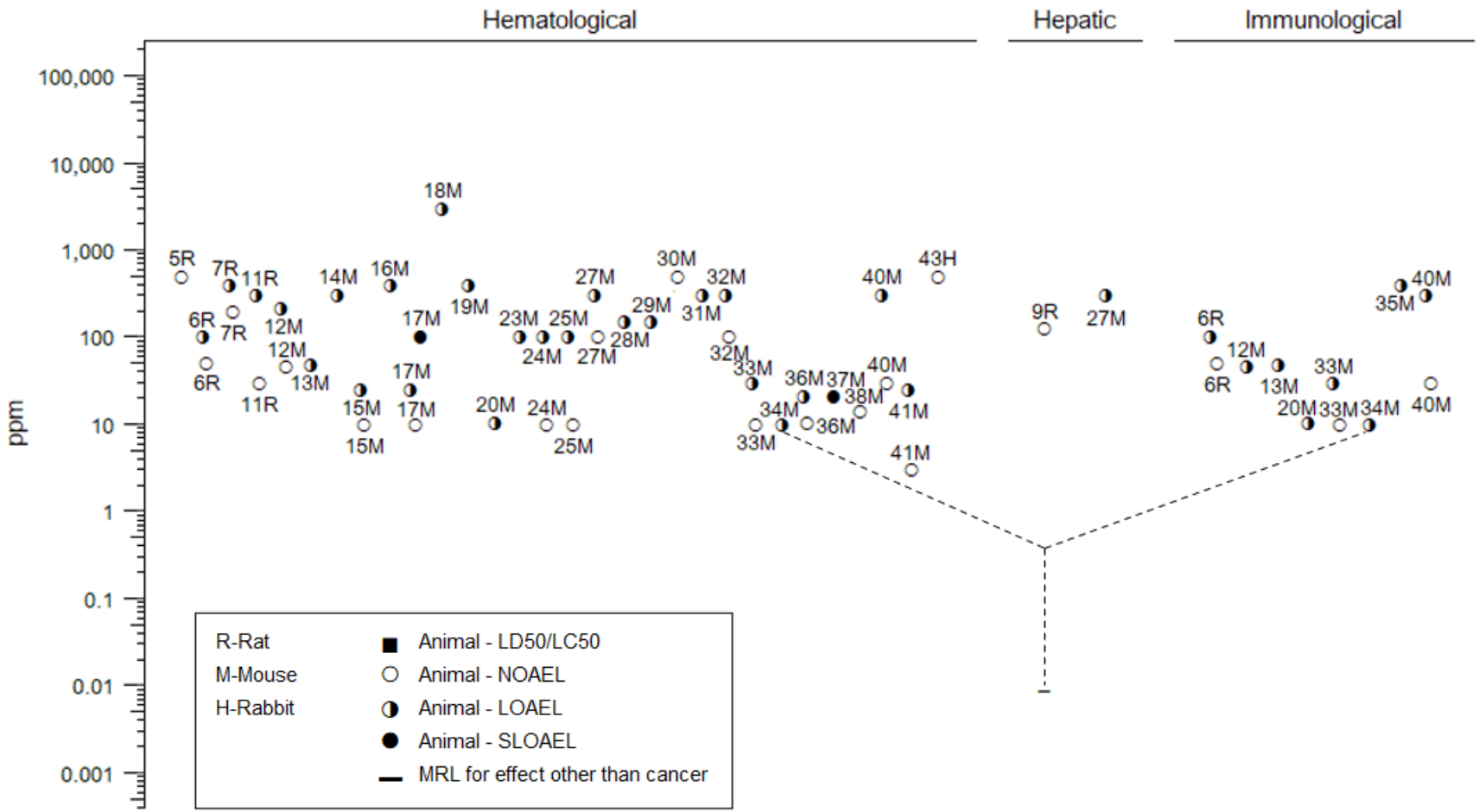


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Acute (≤14 days)

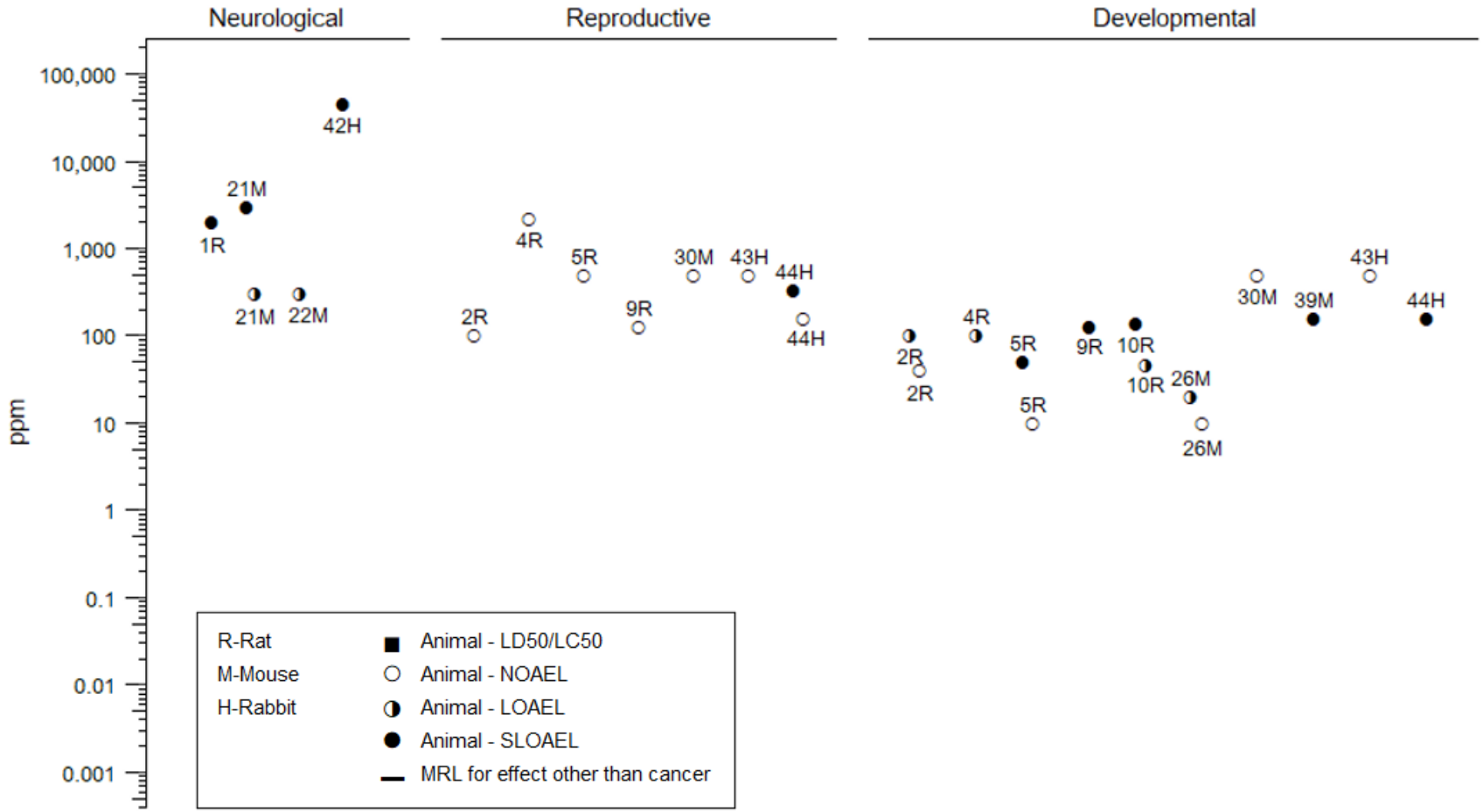


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Intermediate (15–364 days)

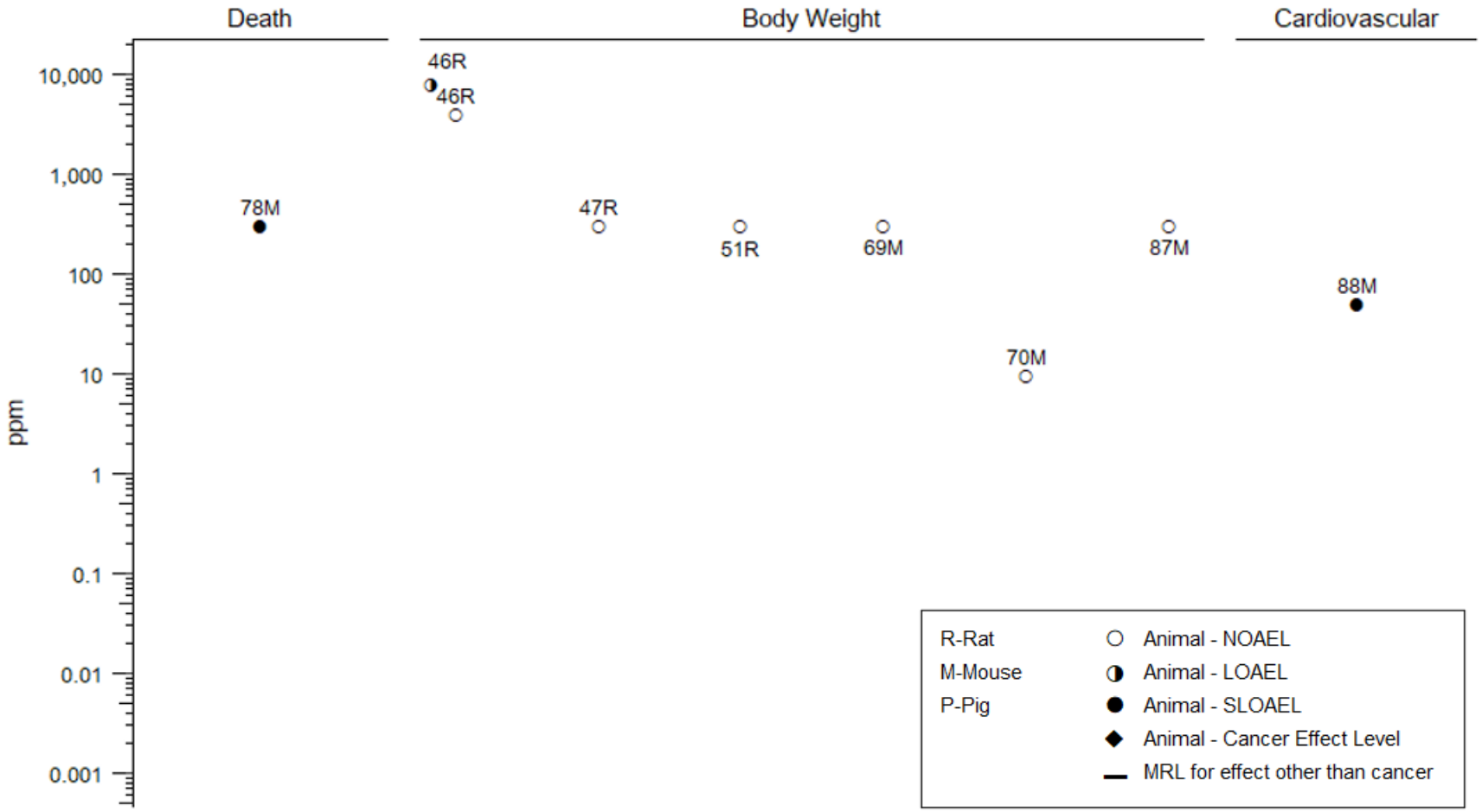


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Intermediate (15–364 days)

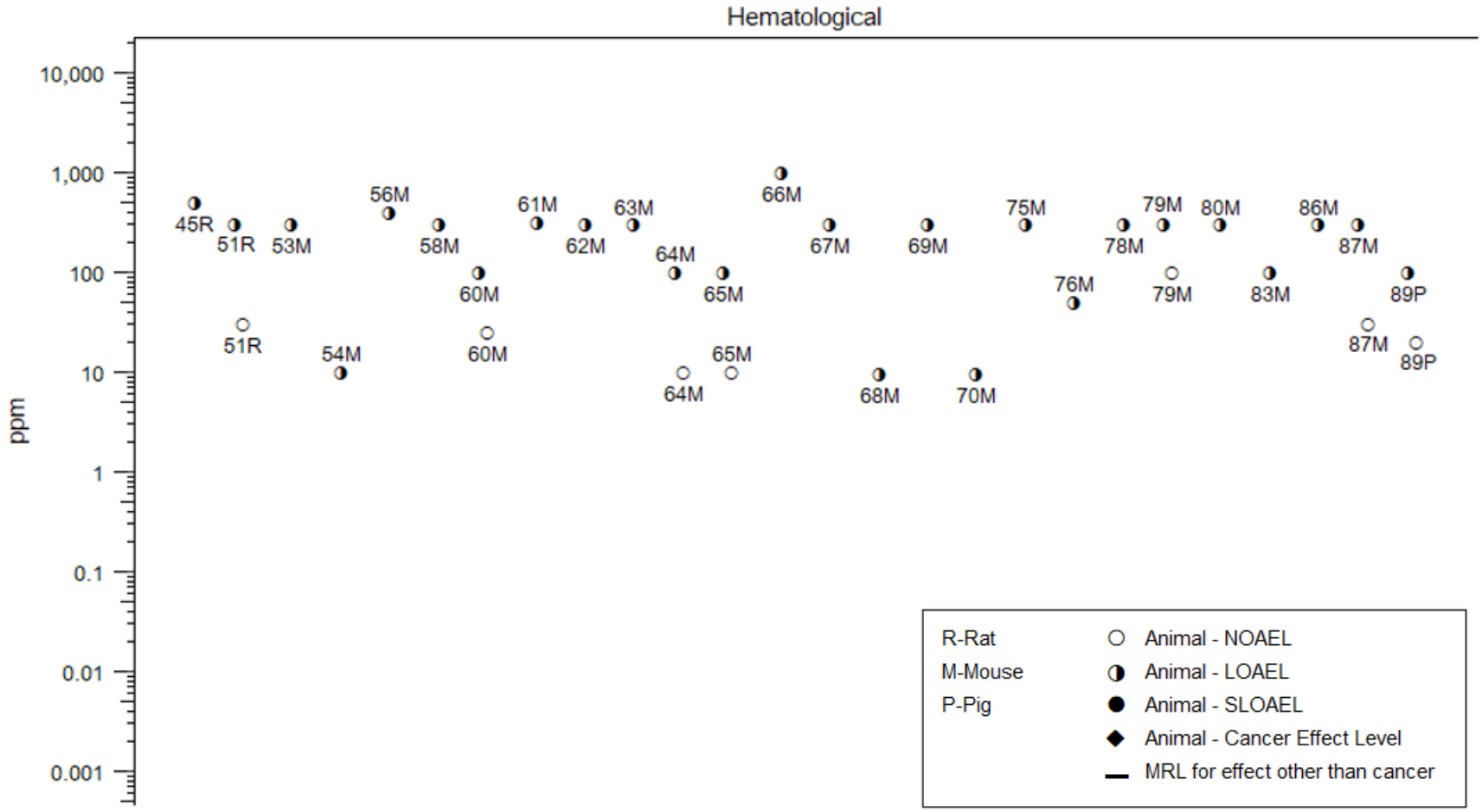


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Intermediate (15–364 days)

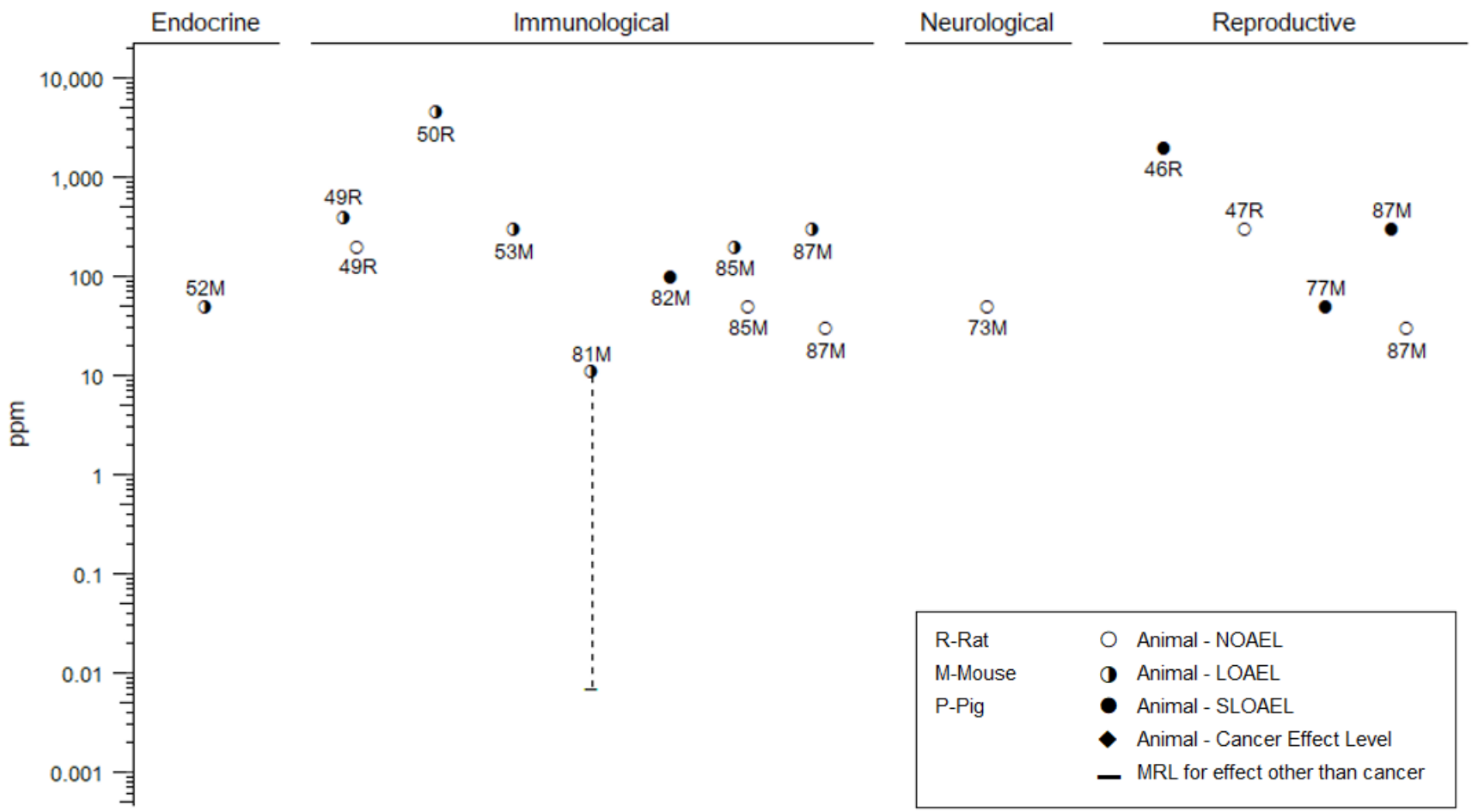


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Intermediate (15–364 days)

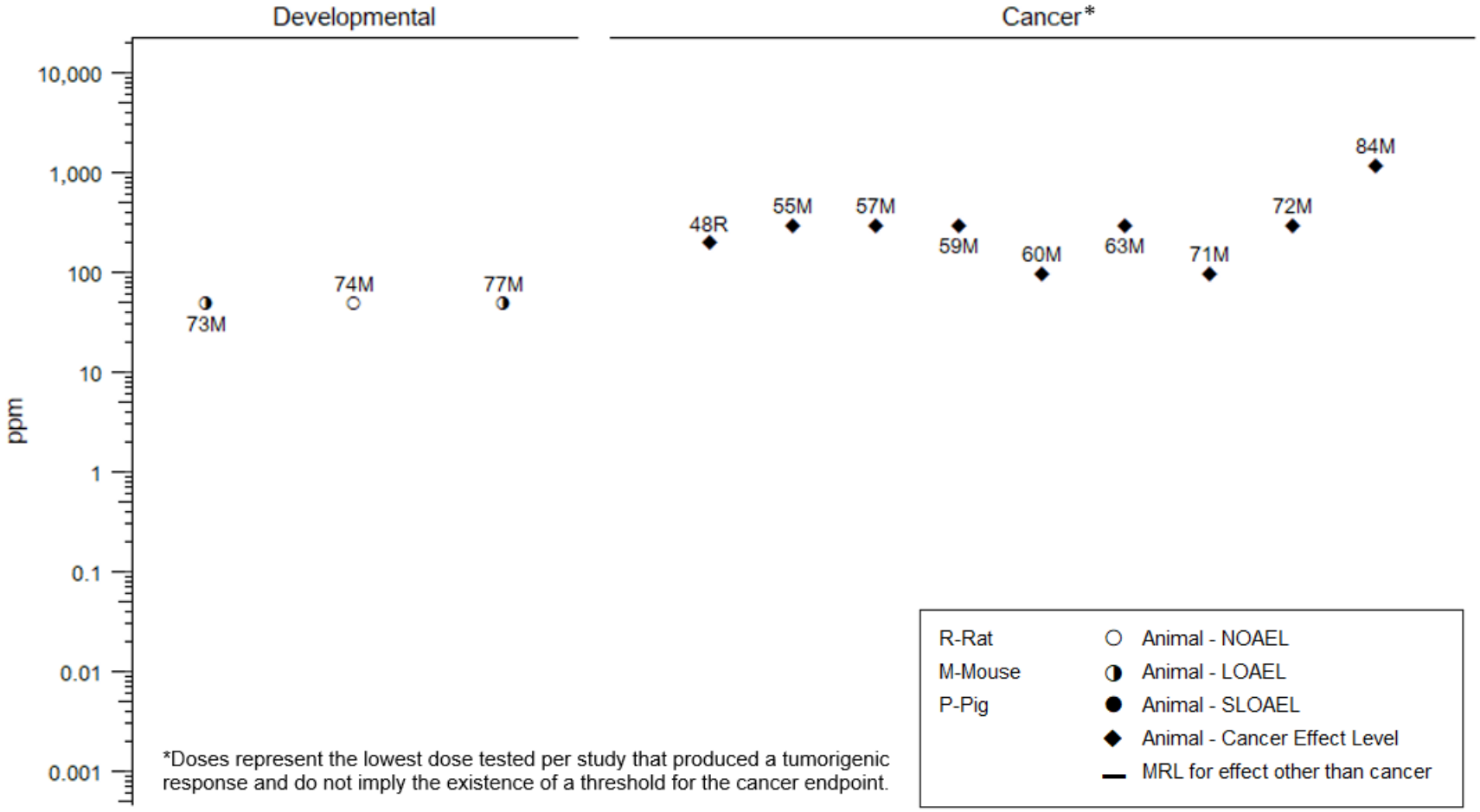


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Chronic (≥365 days)

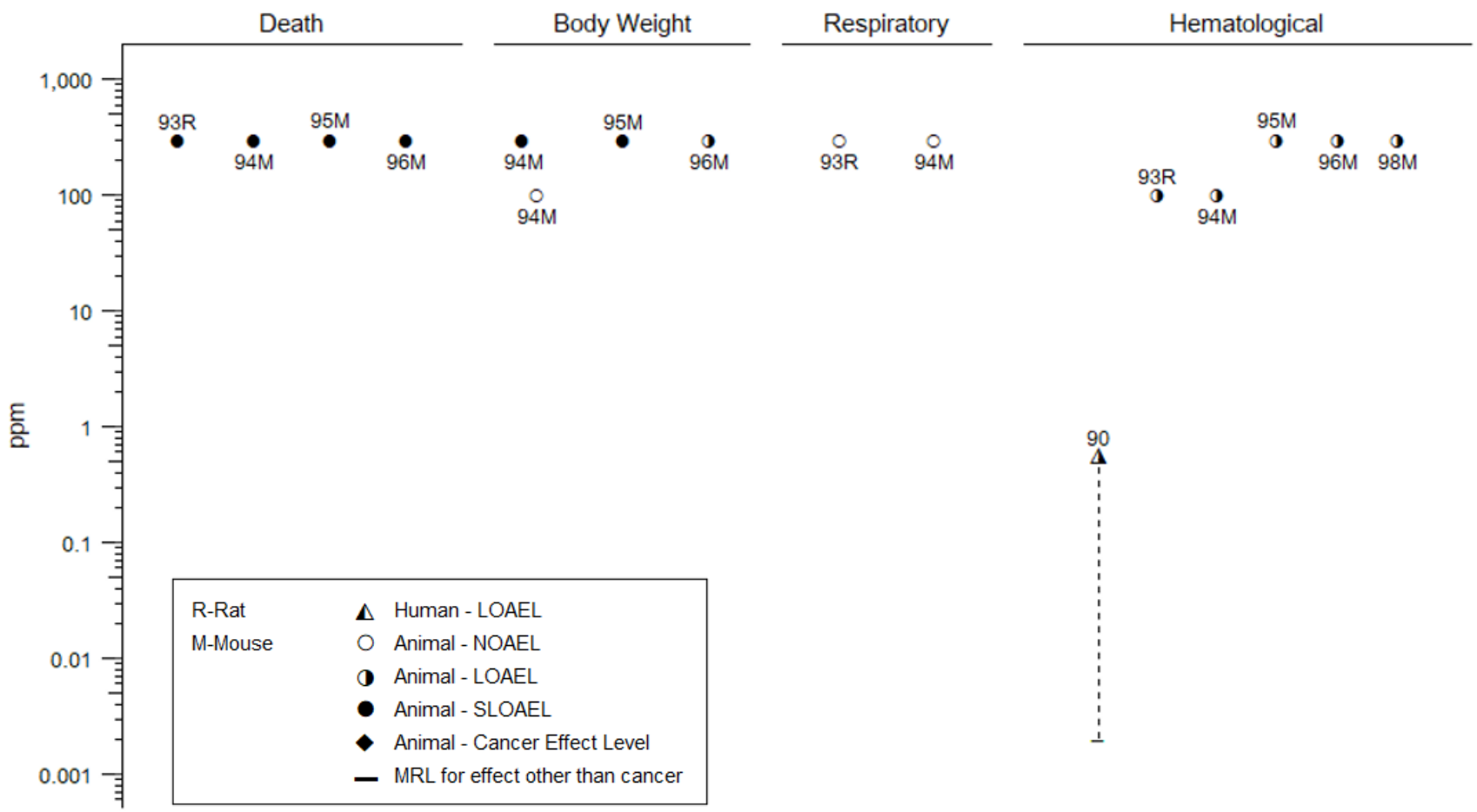
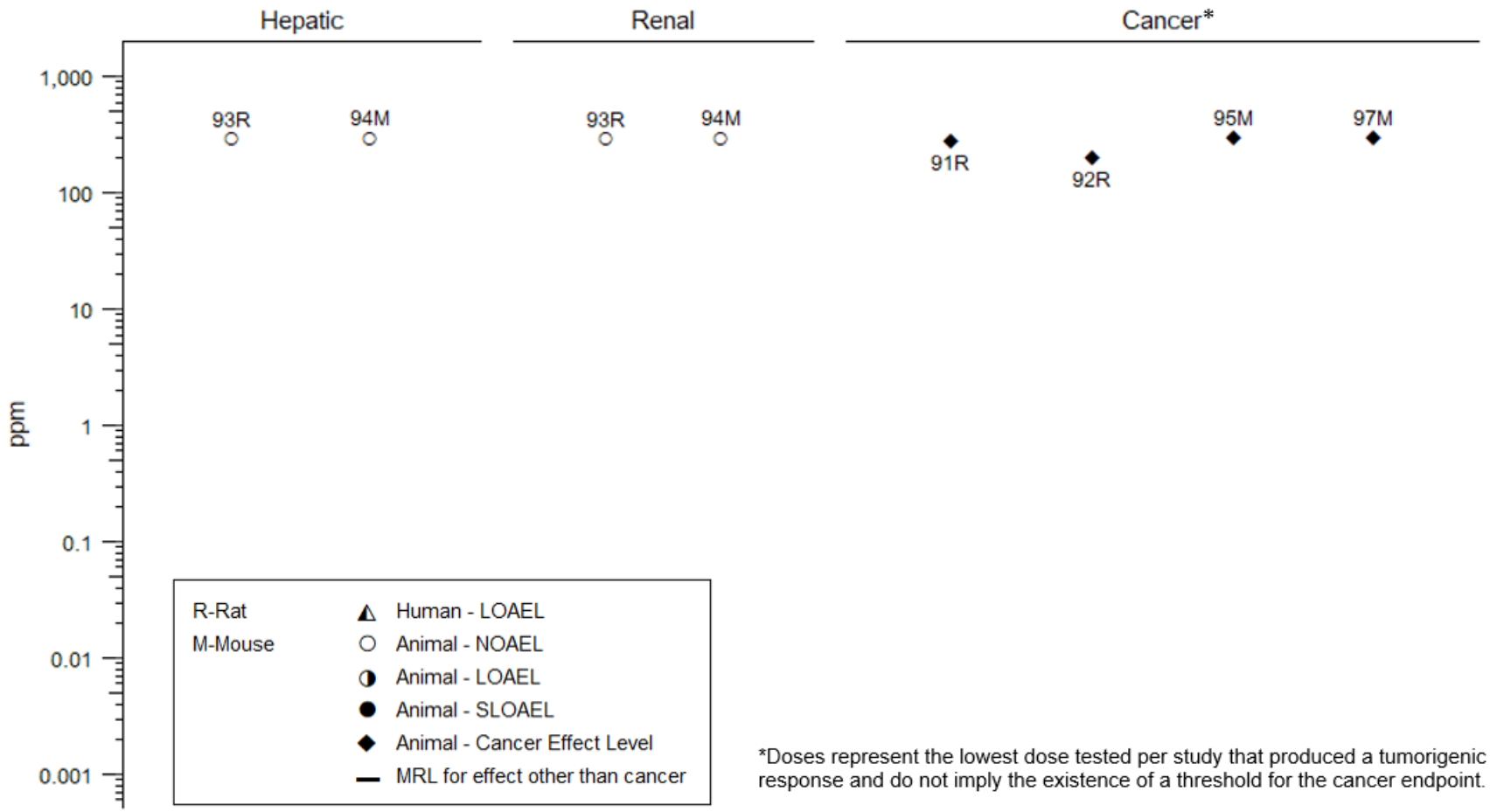


Figure 2-2. Levels of Significant Exposure to Benzene – Inhalation
Chronic (≥365 days)



*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)

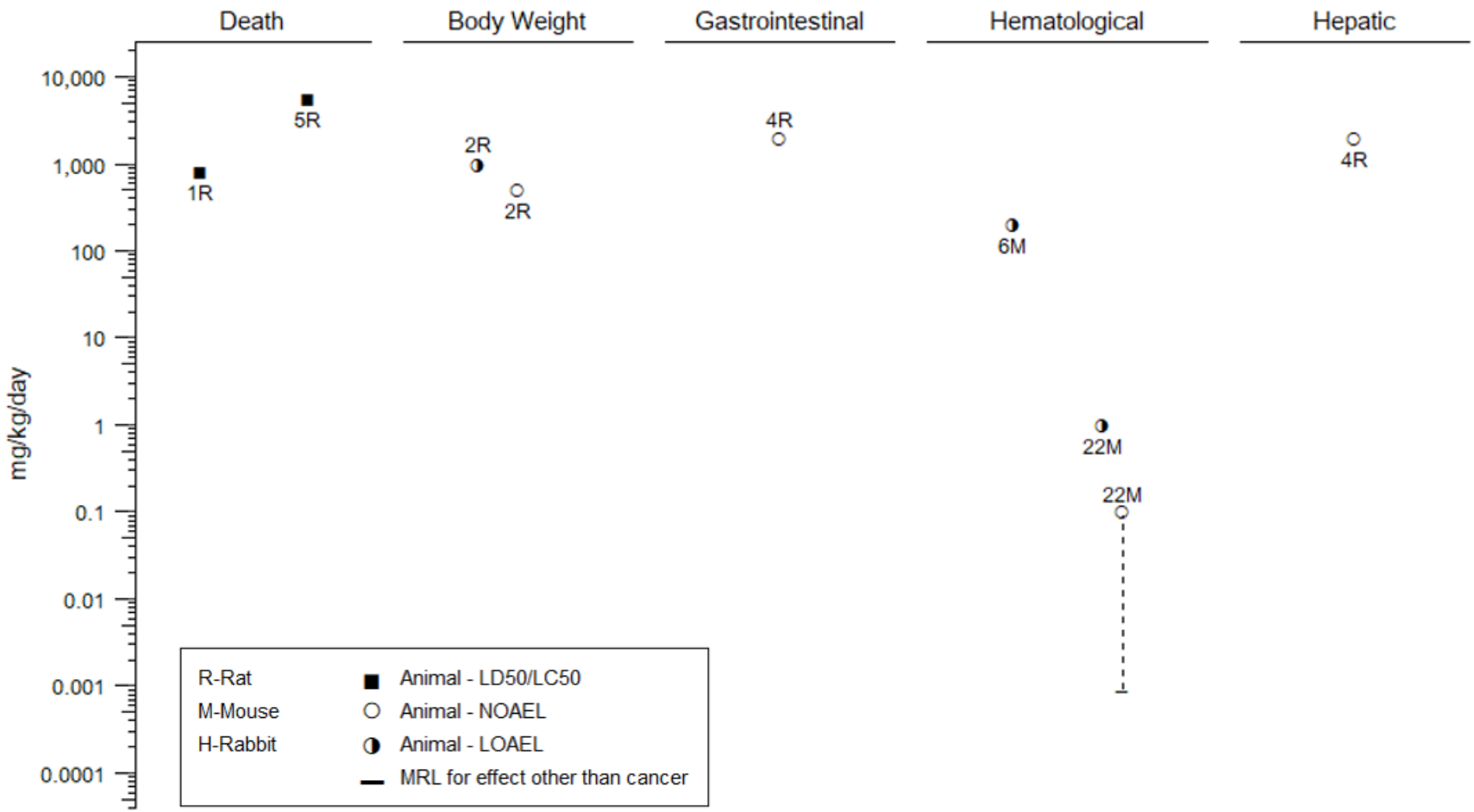


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Acute (≤14 days)

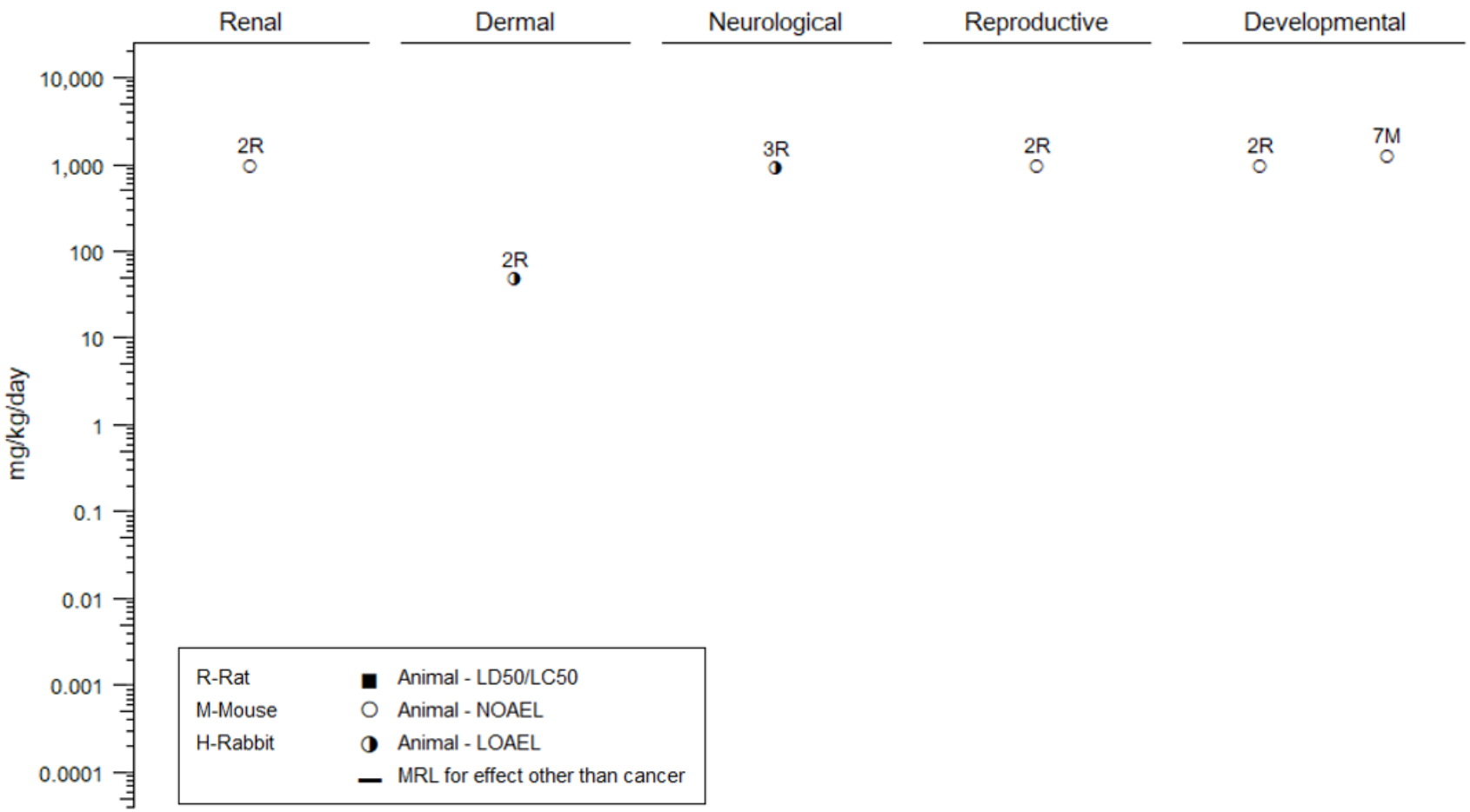


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Intermediate (15–364 days)

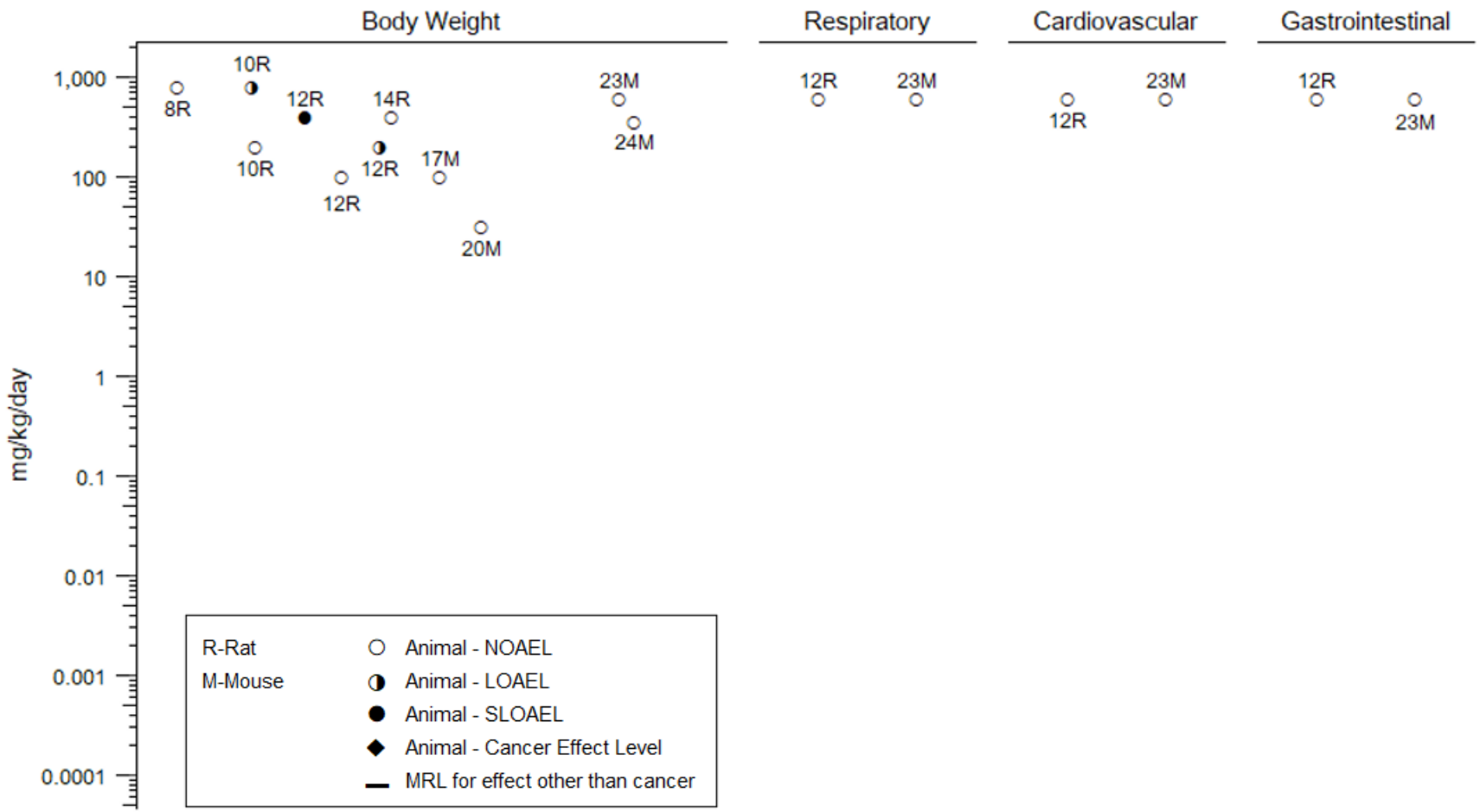


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Intermediate (15–364 days)

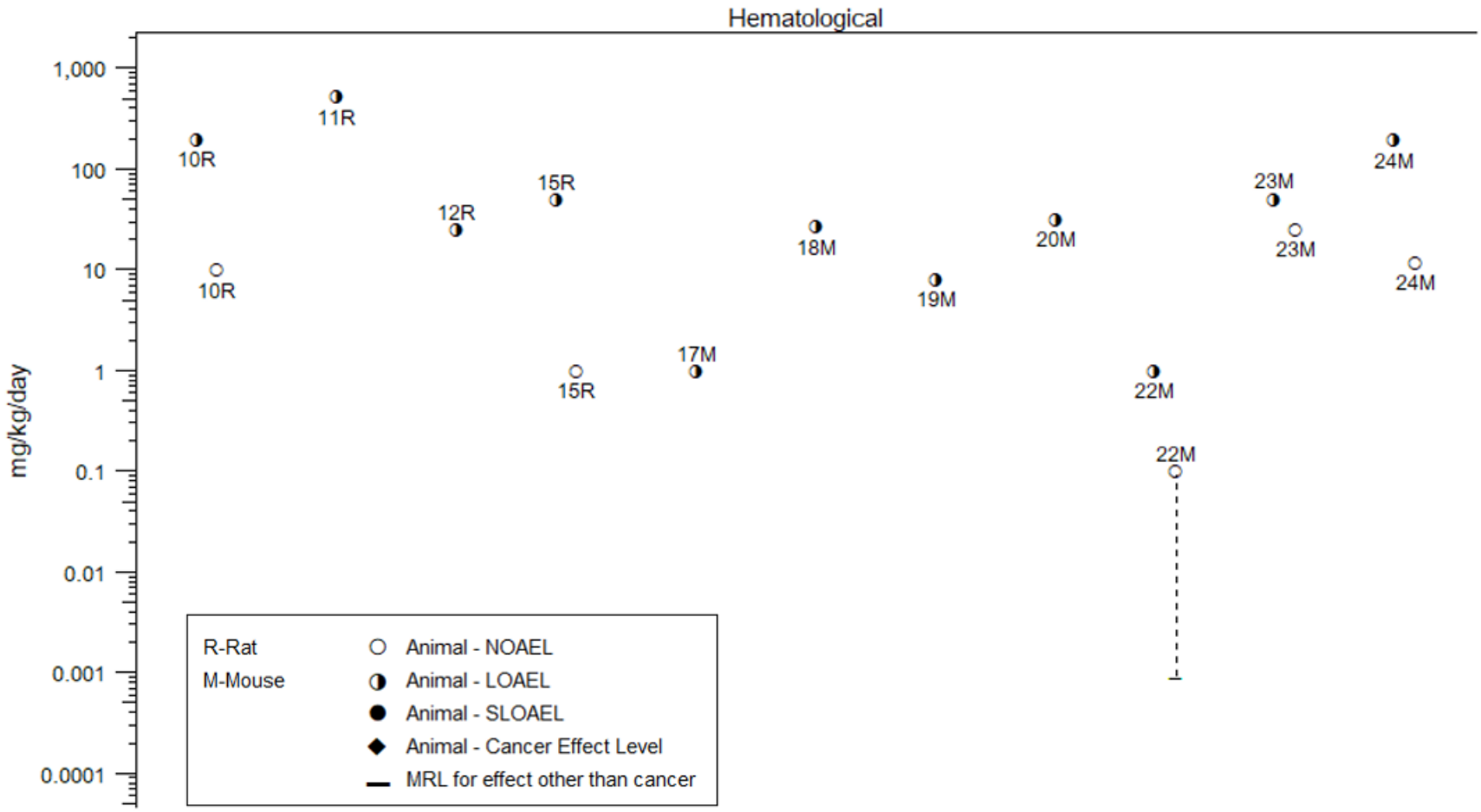


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Intermediate (15–364 days)

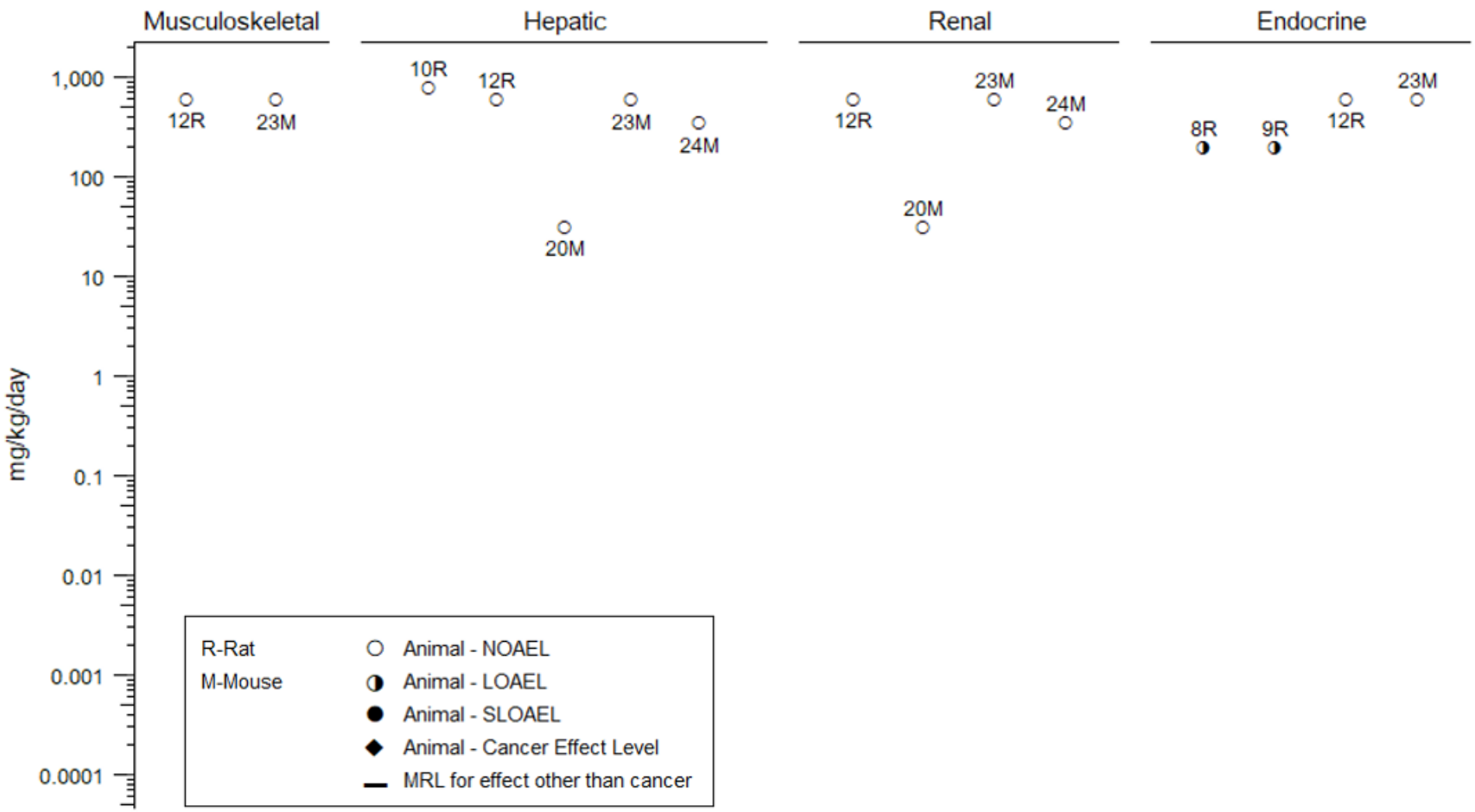


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Intermediate (15–364 days)

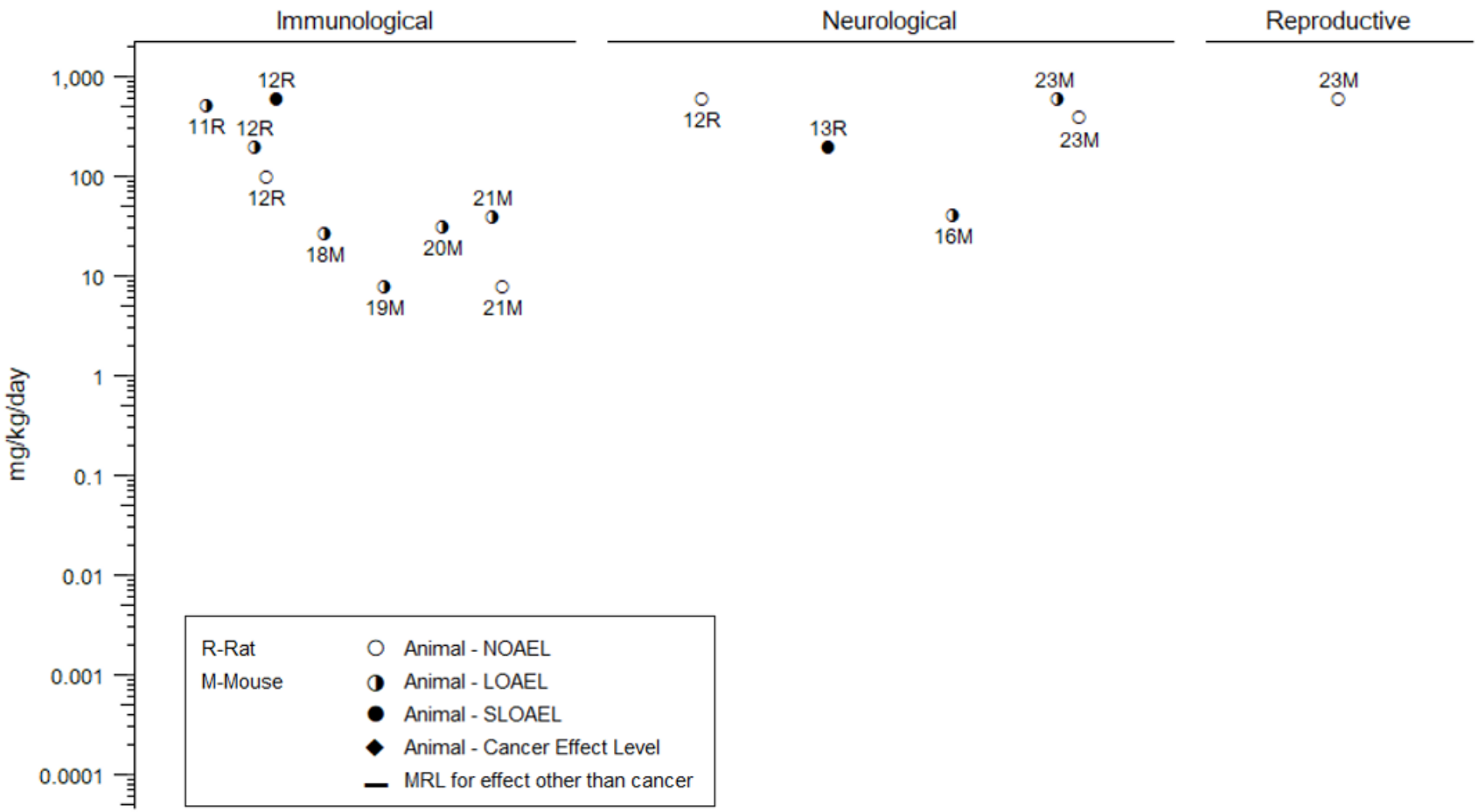


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Chronic (≥365 days)

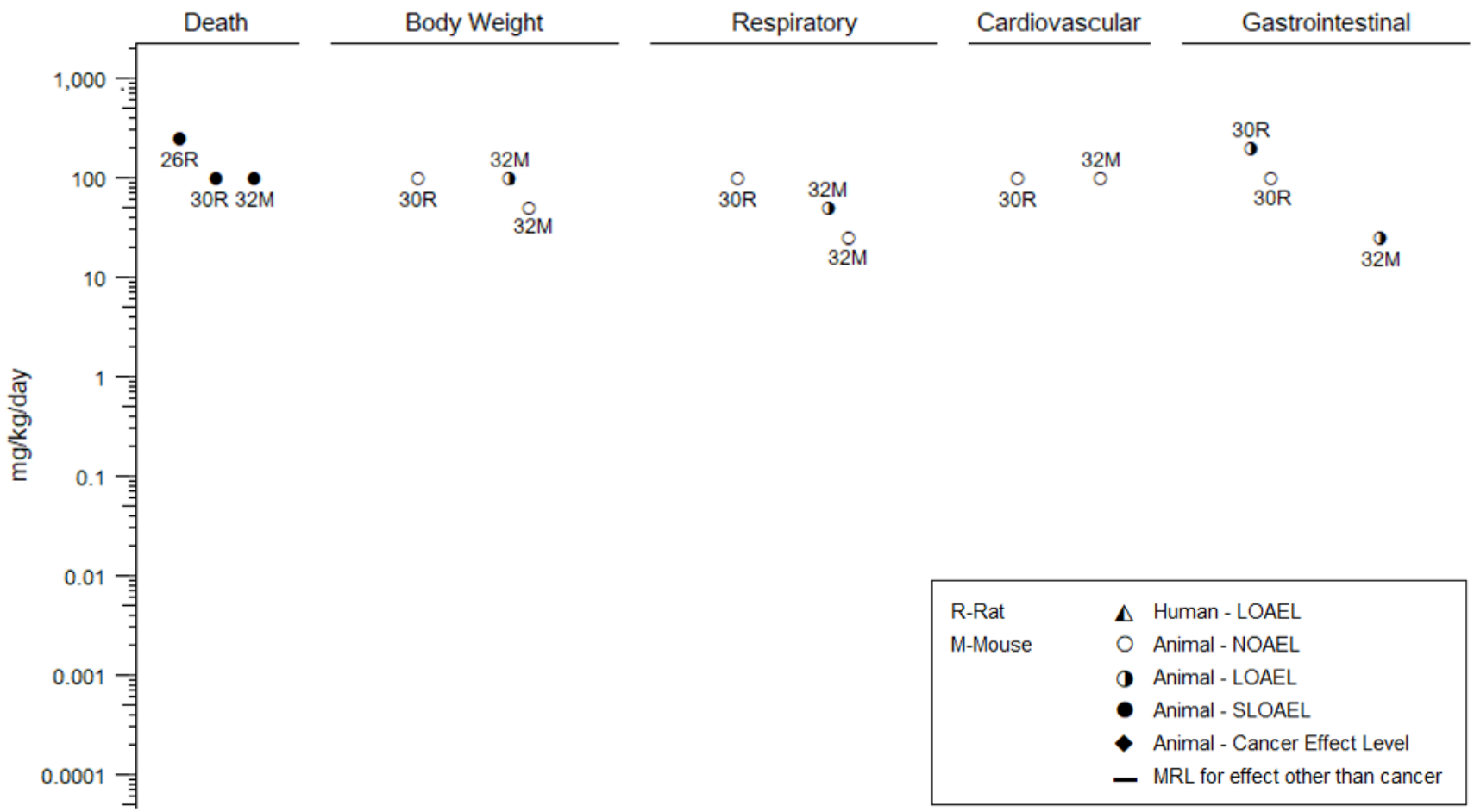


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Chronic (≥365 days)

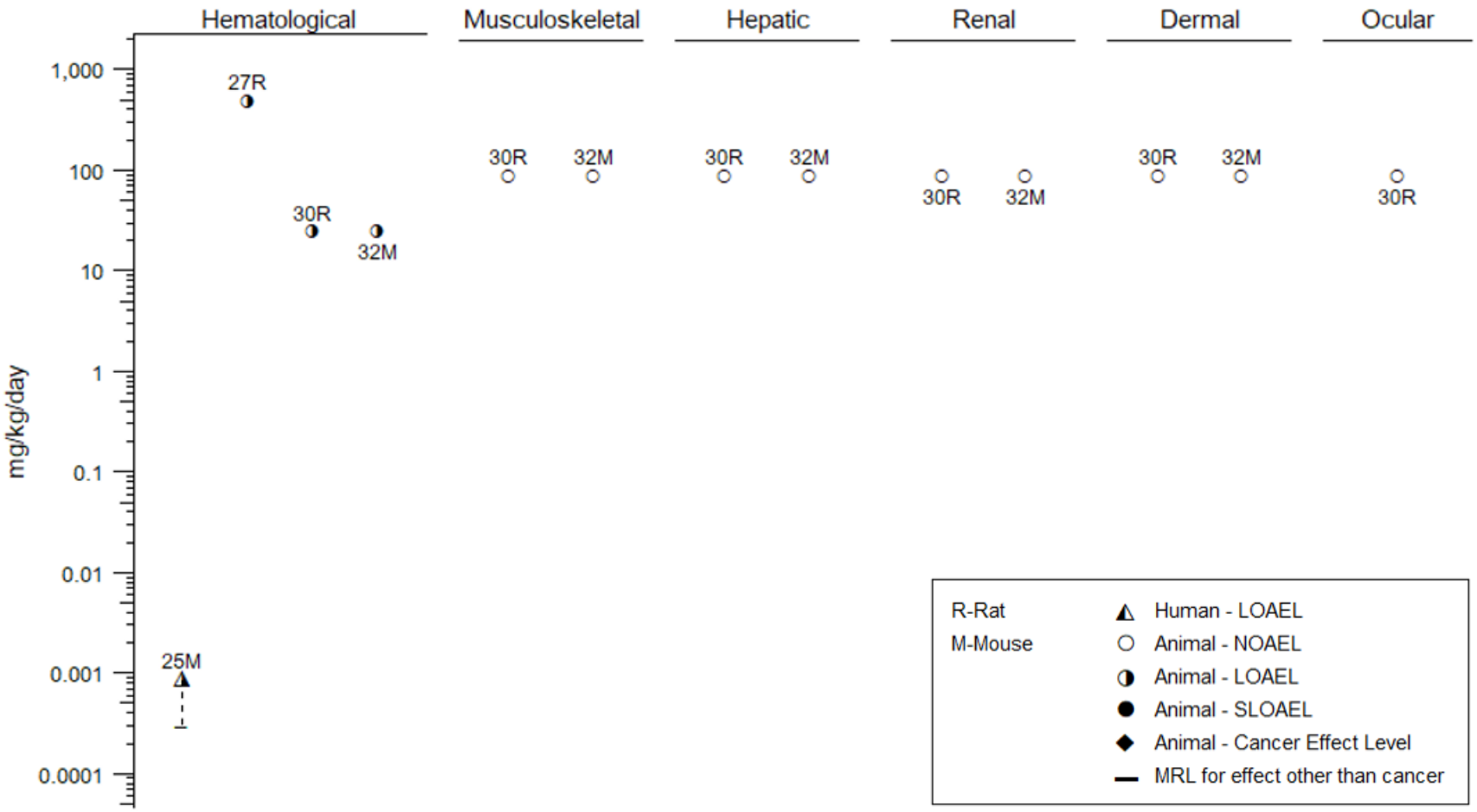


Figure 2-3. Levels of Significant Exposure to Benzene – Oral
Chronic (≥365 days)

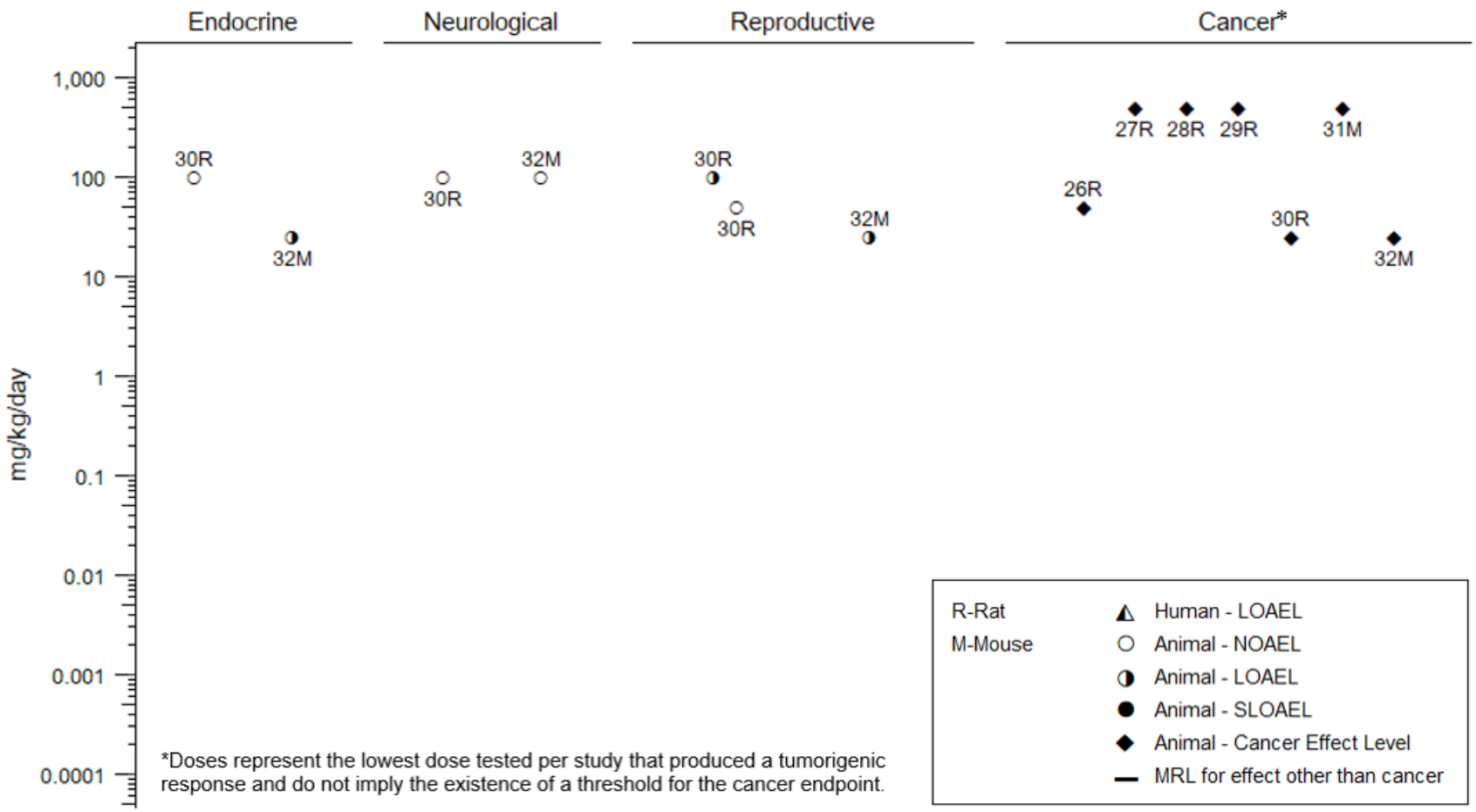


Table 2-3. Levels of Significant Exposure to Benzene – Dermal

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

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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

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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

			Cancer type										
Population (exposure)	Exposure (ppm)	Reference (n)	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: Males	Exposure-related tumor outcomes: Females	Reference
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: Males	Exposure-related tumor outcomes: Females	Reference
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: Males	Exposure-related tumor outcomes: Females	Reference
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: Males	Exposure-related tumor outcomes: Females	Reference
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: Males	Exposure-related tumor outcomes: Females	Reference
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: Males	Exposure-related tumor outcomes: Females	Reference
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

Species (sex, n)	Dose (mg/kg/day)	Duration	Exposure-related tumor outcomes: Males	Exposure-related tumor outcomes: 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

			Exposure-related tumor outcomes		
Species (sex, n)	Dose (mg/kg/day)	Duration	Males	Females	Reference
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

			Exposure-related tumor outcomes		
Species (sex, n)	Dose (mg/kg/day)	Duration	Males	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

			Exposure-related tumor outcomes		
Species (sex, n)	Dose (mg/kg/day)	Duration	Males	Females	Reference
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*

Species (test system)	Endpoint	Result		Reference
		With activation	Without activation	
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: With activation	Result: Without activation	Reference
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: With activation	Result: Without activation	Reference
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: With activation	Result: Without activation	Reference
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,

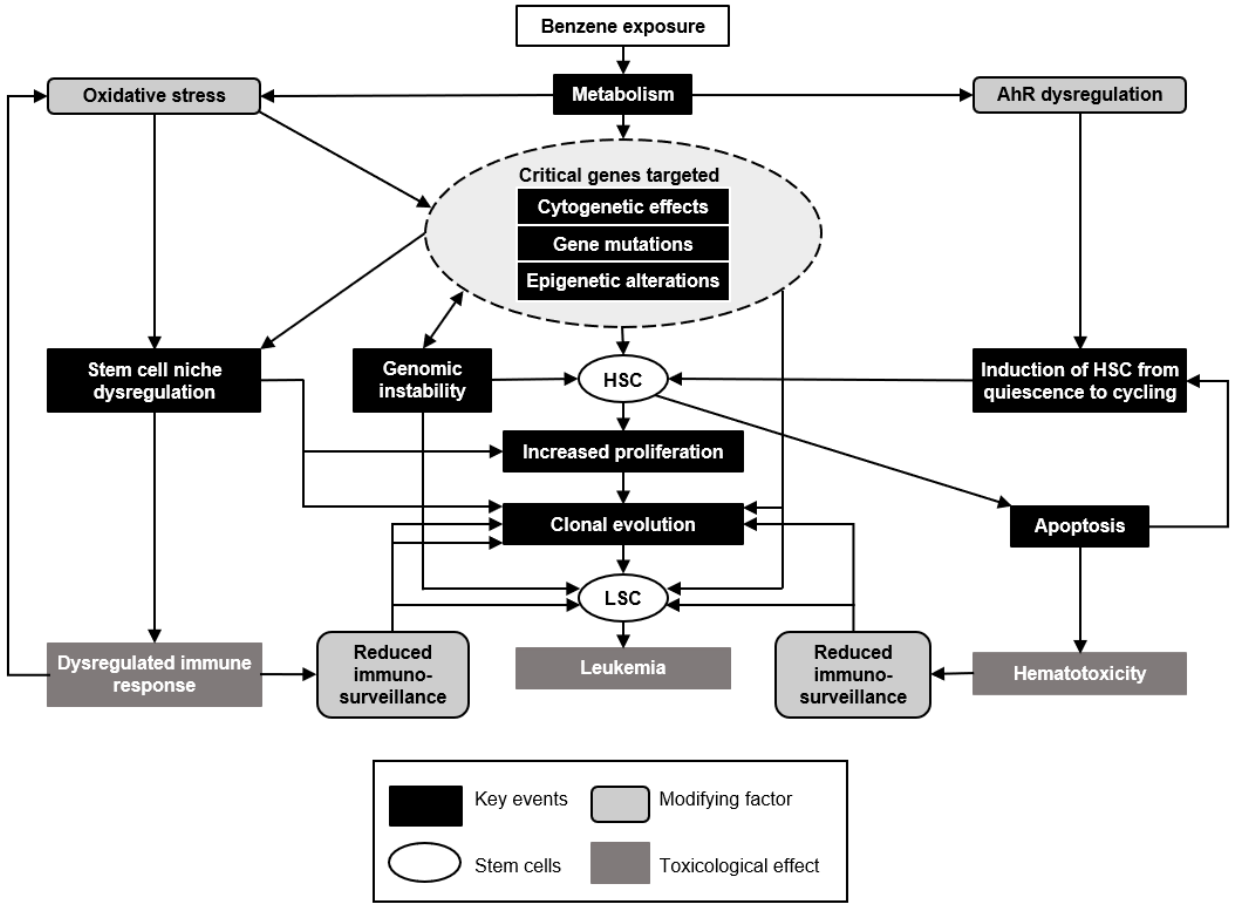
including NQO1, epoxide hydrolase, and MPO, may alter enzyme activity and thereby alter the levels of reactive metabolites. This is discussed in more detail in Section 3.2.

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.

CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Inhalation exposure is the major route of human exposure to benzene, although oral and dermal exposure can also occur.

- Benzene is readily absorbed following inhalation or oral exposure. Although benzene is also readily absorbed from the skin, much of the benzene applied to the skin evaporates from the skin surface.
- Absorbed benzene is rapidly distributed throughout the body and tends to accumulate in fatty tissues.
- Benzene is metabolized in liver and other tissues, including lymphocyte progenitor cells in bone marrow. Benzene metabolism results in the production of several reactive metabolites that are thought to contribute to benzene toxicity.
- At low exposure levels, benzene is rapidly metabolized and excreted predominantly as conjugated urinary metabolites. At higher exposure levels, metabolic pathways appear to become saturated and a large portion of an absorbed dose of benzene is excreted as parent compound in exhaled air.
- Benzene metabolism appears to be qualitatively similar among humans and various laboratory animal species. However, there are quantitative differences in the relative amounts of benzene metabolites.

3.1.1 Absorption

Inhalation exposure is probably the major route of human exposure to benzene. Several studies have estimated absorption from measurements of respiratory extraction of inhaled benzene (the difference between the concentration of benzene in inhaled and exhaled air (Laitinen et al. 1994; Lindstrom et al. 1994; Nomiyama and Nomiyama 1974a; Srbova et al. 1950; Yu and Weisel 1996). Existing evidence indicates that benzene is rapidly absorbed by humans following inhalation exposure. Results from a study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours showed that absorption was highest in the first few minutes of exposure, but decreased rapidly thereafter (Srbova et al. 1950). In the first 5 minutes of exposure, absorption was 70–80%, but by 1 hour, it was reduced to approximately 50% (range, 20–60%). Respiratory extraction (the net amount of benzene removed from inhaled air following inhalation of benzene) in six volunteers including males and females exposed to 52–62 ppm benzene for 4 hours was determined to be approximately 47% (Nomiyama and Nomiyama 1974a). In a similar study, three healthy nonsmoking volunteers were exposed to benzene at levels of 1.6 or 9.4 ppm for 4 hours

(Pekari et al. 1992). The amount of benzene absorbed was estimated from the difference between the concentration inhaled and the concentration exhaled. Respiratory extraction was 48% for the high dose and 52% for the low dose, supporting the evidence of Nomiyama and Nomiyama (1974a). Yu and Weisel (1996) measured the extraction of benzene from inhaled air by three female subjects exposed to benzene in smoke generated by burning cigarettes, which resulted in airborne benzene concentrations in the range of 32–69 ppm. The average extraction for exposure periods of 30 or 120 minutes was 64% and did not appear to be influenced by exposure duration.

In occupational exposure settings, benzene can be absorbed by inhalation and skin (Hostynek et al. 2012). Studies of occupational exposure to benzene suggest that absorption occurs both by inhalation and dermally in many workplace settings. In a study conducted in 1992 in Finland, car mechanics' exposure to benzene was evaluated (Laitinen et al. 1994). Different work phases were measured at five Finnish garages. Blood samples from car mechanics (eight nonsmokers) were taken 3–9 hours after exposure to benzene. The results were approximated to the time point of 16 hours after exposure. Fourteen air samples were taken from the breathing zone and five stationary samples were collected from the middle of the garage for background concentration levels. The average background concentration (stationary samples) of gasoline vapors was 6±7 cm^3/m^3 (2±2 ppm) and the concentration of benzene was under the detection limit of 0.2 cm^3/m^3 (0.1 ppm). The concentrations of benzene in the breathing zone varied from the detection limit of 0.2 cm^3/m^3 to 1.3 cm^3/m^3 (0.1–0.4 ppm) for unleaded gasoline and from the detection limit to 3.7 cm^3/m^3 (1.2 ppm) for leaded gasoline. The highest benzene exposure levels (2.4–3.7 cm^3/m^3 or 0.8–1.2 ppm) were measured when changing the filter to the fuel pump. The mechanics worked without protective gloves, and penetration through the skin was likely. During carburetor renewal and gathering, benzene concentrations were 0.5–1.1 cm^3/m^3 (0.2–0.3 ppm). During changing of the fuel filter to electronic fuel-injection system, benzene concentrations were 0.9–3.4 cm^3/m^3 (0.3–1.1 ppm). The approximated benzene concentrations in blood corresponding to the time point of 16 hours after the exposure showed much higher levels of exposure than could be expected according to the corresponding air measurements (8-hour TWA). The comparison of expected benzene concentrations in blood, if no dermal exposures were present, to the levels at the time point of 16 hours after the exposure showed that the dermal route can be the source of exposure (range 1.1–88.2%). Two of eight workers had minimal exposure through the skin (0–1.1%). The other six workers showed high dermal exposure (79.4%).

Exposure to benzene-contaminated water can also provide an opportunity for both inhalation and dermal absorption. Lindstrom et al. (1994) reported benzene exposures resulting from a showering scenario that occurred in a single-family residence whose water was contaminated with benzene. From 1986 to 1991,

benzene concentrations in water were between 33 and 673 μg/L (ppb). The exposure scenario involved 20-minute showers, air sampling within the bathroom and other rooms, personal breathing zone sampling of the monitoring team, and estimating the benzene doses of the person who showered. The shower scenario indicated exposure to benzene from the showerhead at 185–367 μg/L (ppb), while drain-level samples ranged from below the detectable limit (0.6 μg/L or ppb) to 198 μg/L (ppb). Benzene air concentrations were 758–1,670 μg/m^3 (235–518 ppb) in the shower stall, 366–498 μg/m^3 (113–154 ppb) in the bathroom, 81–146 μg/m^3 (25–45 ppb) in the bedroom, and 40–62 μg/m^3 (12–19 ppb) in the living room. The individual who took a 20-minute shower had estimated inhalation doses of 79.6, 105, and 103 μg (mean=95.9 μg) for 3 consecutive sampling days, which were 2.1–4.9 times higher than corresponding 20-minute bathroom exposures. Another inhalation dose estimate for the showered individual, based on syringe benzene levels, was 113 μg. The average dermal dose was estimated to be 168 μg. The total benzene dose resulting from the shower was estimated to be approximately 281 μg (40% via inhalation and 60% via dermal), suggesting a higher potential exposure to benzene via dermal contact from the water than through vaporization and inhalation. The estimated inhalation and dermal doses reported by Lindstrom et al. (1994) have not been validated by others and are therefore of questionable value for quantitative analysis.

Additional evidence of benzene absorption following inhalation exposure comes from data on cigarette smokers. Benzene levels were significantly higher in the venous blood of 14 smokers (median: 493 ng/L) than in a control group of 13 nonsmokers (median: 190 ng/L) (Hajimiragha et al. 1989). Cigarette smoke is known to contain benzene (Brunnemann et al. 1989; Byrd et al. 1990), and the subjects had no known exposure to other sources of benzene (Hajimiragha et al. 1989). Kok and Ong (1994) reported blood and urine levels of benzene as 110.9 and 116.4 ng/L, respectively, for nonsmokers, and 328.8 and 405.4 ng/L, respectively, for smokers. In a study based on workers at burning oil wells in Kuwait, benzene concentrations in blood were higher in smokers than in nonsmokers in the firefighter group (Etzel and Ashley 1994). Benzene-exposed ceramic workers who smoked had higher concentrations of benzene metabolites in their urine than nonsmokers (Ibrahim et al. 2014).

Benzene released from fires can also be absorbed. The National Association of Medical Examiners Pediatric Toxicology (PedTox) Registry reported blood benzene concentrations of 0.2–4.9 mg/L in eight children who died in fires and were dead at the scene, indicating absorption of benzene from burning materials (Hanzlick 1995). Blood benzene levels taken from U.S. engineers and firefighters working at burning oil wells in Kuwait were compared to blood benzene levels from non-exposed U.S. citizens (Etzel and Ashley 1994). The median concentrations of benzene in whole blood from engineers,

firefighters, and the U.S. reference group were 0.035 μg/L (range: not detected–0.055 μg/L), 0.18 μg/L (range: 0.063–1.1 μg/L), and 0.066 μg/L (range: not detected–0.54 μg/L), respectively. The median concentration in firefighters was generally higher than the median concentration in engineers.

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

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

In animals, benzene appears to be efficiently absorbed following oral dosing. Oral absorption of benzene was first demonstrated by Parke and Williams (1953). After radiolabeled (^{14}C) benzene was administered orally to rabbits (340–500 mg/kg), the total radioactivity eliminated in exhaled air and urine accounted for approximately 90% of the administered dose, indicating that at least this much of the administered dose was absorbed.

The amount of benzene absorbed in the gastrointestinal tract may vary by species, dose, and dosing vehicle (e.g., food, water, oil). Studies in rats and mice showed that gastrointestinal absorption was greater than 97% in both species when the animals were administered benzene by gavage (in corn oil) at doses of 0.5–150 mg/kg/day (Sabourin et al. 1987). In many animal studies, benzene is administered orally in oil to ensure predictable solubility and dose concentration control. This is unlike the predicted human oral exposure, which is likely to be in drinking water. There are a number of studies in which

benzene has been administered to animals in the drinking water, which more closely resembles predicted human oral exposure (Lindstrom et al. 1994). Although no information was located regarding the extent of oral absorption of benzene in aqueous solutions, it is reasonable to assume that oral absorption from water solutions would be nearly 100%.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats. Turkall et al. (1988) estimated bioavailability and kinetics of absorption of benzene in rats. Adult male rats received a gavage dose of ^{14}C-benzene, either as benzene alone or adsorbed to clay or sandy soil. Plasma concentrations of radiolabel were monitored for determining absorption half-times and area under the curve (AUC). Absorption half-times were estimated to be 7.1 minutes for benzene, 3.8 minutes for benzene adsorbed to sandy soil, and 6.1 minutes for benzene adsorbed to clay. Peak plasma concentrations and AUCs of radioactivity were higher following dosing with benzene adsorbed to sandy soil or clay, compared to benzene alone.

Studies conducted *in vivo* in humans and *in vitro* using human skin indicate that benzene can be absorbed dermally. Modjtahedi and Maibach (2008) estimated absorption of benzene after direct application of radiolabeled benzene to the forearm or palm of four adults. The mean fractions of applied dose absorbed were 0.13% when applied to the palm and 0.07% when applied to the forearm (estimated from measurements of radiolabel excreted in urine for 7 days following exposure). The above estimates were for benzene applied skin without occlusion to prevent evaporation. Occluding the application site increased absorption of benzene through excised human cadaver skin (Hui et al. 2009; Petty et al. 2011). Dermal absorption is influenced by a variety of factors including evaporation, deposition region, and solvents in which benzene is applied and is higher when applied in water relative to more volatile solvents (Blank and McAuliffe 1985; Gajjar and Kasting 2014; Lodén 1986; Petty et al. 2011).

In vivo experiments on four volunteers, to whom 0.0026 mg/cm^2 of ^{14}C-benzene was applied to forearm skin, indicated that approximately 0.05% of the applied dose was absorbed (Franz 1984). Absorption was rapid, with >80% of the total excretion of the absorbed dose occurring in the first 8 hours after application. Estimation of dermal absorption from radioactivity excreted in urine required an assumption about the fraction of the absorbed dose of radiolabel excreted in urine. A value of 45.3% was estimated from studies conducted in Rhesus monkeys that received a subcutaneous dose of radiolabeled benzene (Franz 1984). The estimate of 0.05% absorbed did not account for evaporation of the applied dose from the skin surface. In another study, 35–43 cm^2 of the forearm was exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25–2 hours (Hanke et al. 1961). The absorption was estimated from the amount of

phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be approximately 0.4 mg/cm^2/hour. In the same study, absorption from benzene in air was negligible. The estimate of absorption may be biased from sources other than benzene contributing to urinary phenol.

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

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

Adami et al. (2006) applied benzene to human skin *in vitro* and recovered 0.43% of the applied dose in the receptor fluid; a permeability coefficient of 0.000438 cm/hour was determined. Hui et al. (2009a, 2009b) observed dose-related increased absorption of radiolabeled benzene in cleaning solvents through human skin *in vitro* and reported that occlusion increased absorption.

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

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

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

Data indicate that soil adsorption decreases the dermal bioavailability of benzene. A study in which male rats were treated dermally with 0.004 mg/cm^2 ^{14}C-benzene, with or without 1 g of clay or sandy soil, reported benzene absorption half-lives of 3.1, 3.6, and 4.4 hours for pure benzene, sandy soil, and clay soil, respectively (Skowronski et al. 1988).

Benzene in air was rapidly absorbed through the skin of hairless mice that were attached to respirators to avoid pulmonary uptake of benzene vapors (Tsuruta 1989). The rate of benzene absorption through the skin increased linearly with dose. The skin absorption rate for 200 ppm was 4.11 nmol/cm^2/hour (0.31 μg/cm^2/hour); at 1,000 ppm, the rate was 24.2 nmol/cm^2/hour (1.89 μg/cm^2/hour), and at 3,000 ppm, the rate was 75.5 nmol/cm^2/hour (5.90 μg/cm^2/hour). The skin absorption coefficient was 0.619 cm/hour.

In an *in vitro* experiment using Fischer 344 rat skin, the partition coefficient for skin:air and skin permeability coefficient were determined at a benzene air concentration of 203 ppm (Mattie et al. 1994). The skin:air partition coefficient was estimated to be 34.5 and the permeability coefficient was estimated to be 1.52 mm/hour.

Based on data for skin absorption of benzene vapors in mice and occupational exposure data, Tsuruta (1989) estimated the ratio of skin absorption rate to pulmonary uptake for humans exposed to benzene to be 0.037. Dermal absorption could account for a relatively higher percentage of total benzene uptake in

occupational settings where personnel, using respirators but not protective clothing, are exposed to high concentrations of benzene vapor.

Modjtahedi and Maibach (2008) reported absorption of benzene through forearm skin and palm of volunteers. Absorption through the forearm skin and palm averaged 0.7 and 0.13%, respectively, of the applied dose, based on recovery in the urine. Fent et al. (2014) reported increased benzene levels in the breath of firefighters exposed to benzene and other substances during controlled structure burns, indicating that some degree of dermal absorption of benzene had occurred because the subjects wore their protective breathing systems during the burns.

3.1.2 Distribution

Information on the distribution of benzene in humans comes primarily from case studies. The data suggest that benzene is distributed throughout the body following absorption into blood. Since benzene is lipophilic, a high distribution to fatty tissue might be expected. Following inhalation exposure to benzene, the chemical has been detected in the biological fluids and tissues of the subjects (Pekari et al. 1992; Tauber 1970; Winek and Collom 1971; Winek et al. 1967). Fluid and tissue levels of benzene have been reported in cases of both accidental and intentional lethal exposures. Levels of 0.38 mg% in blood, 1.38 mg% in the brain, and 0.26 mg% in the liver were reported in a worker who died from exposure to very high air concentrations of benzene (Tauber 1970). Tauber (1970) did not report the exact units of the mg% (i.e., whether it was expressed as mg/100 mL or mg/100 g). An autopsy (time after death not indicated) performed on a youth who died while sniffing reagent-grade benzene revealed benzene concentrations of 2.0 mg% in blood, 3.9 mg% in brain, 1.6 mg% in liver, 1.9 mg% in kidney, 1 mg% in stomach, 1.1 mg% in bile, 2.23 mg% in abdominal fat, and 0.06 mg% in urine (Winek and Collom 1971). Winek and Collom (1971) did not report the exact units of the mg% (i.e., whether it was expressed as mg/100 mL or mg/100 g).

Results from animal studies indicate that absorbed benzene is distributed among several compartments. The parent compound is preferentially stored in the fat, although the relative uptake in tissues also appears to be dependent on the perfusion rate of tissues by blood. Because benzene distributes preferentially to fat, factors that affect body fat content (e.g., sex, lifestage) may affect body burdens and exposure-response relationships for health effects associated with exposure to benzene (Zhang et al. 2020).

Following a 10-minute inhalation exposure of pregnant mice to 2,000 ppm benzene, the parent compound and its metabolites were found to be present in lipid-rich tissues, such as brain and fat, and in well-perfused tissues, such as liver and kidney. Benzene was also found in placentas and fetuses immediately following inhalation of benzene (Ghantous and Danielsson 1986).

Rickert et al. (1979) measured the kinetics of distribution of inhaled benzene in rats. During inhalation exposure of rats to 500 ppm, steady-state concentrations were 11.5 μg/mL in blood, 164.4 μg/g in fat, 37.0 μg/g in bone marrow, 25.3 μg/g in kidney, 15.1 μg/g in lung 9.9 μg/g in liver, 6.5 μg/g in brain, and 4,9 μg/k in spleen. The half-time to steady state in blood was estimated to be 1.4 hours but was considerably less (not measurable) in bone marrow. Half-times to steady state ranged from 0.9 to 2.6 hours for other tissues. Elimination half-time from blood was 0.7 hours and ranged from 0.4 to 1.6 hours (fat) for other tissues. In this same study, benzene metabolites, phenol, catechol, and hydroquinone, were detected in blood and bone marrow following 6 hours of exposure to benzene, with levels in bone marrow exceeding the respective levels in blood. The levels of phenol in blood and bone marrow decreased much more rapidly after exposure ceased than did those of catechol or hydroquinone, suggesting the possibility of accumulation of the latter two compounds.

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

Benzene was detected in the liver, lung, and blood of rats and mice examined immediately following a 6-hour exposure to benzene vapors at a concentration of 50 ppm (Sabourin et al. 1988). Sabourin and coworkers (Sabourin et al. 1987, 1988) also examined effects, exposure concentration, exposure rate, and route of administration on the comparative metabolism of benzene in rats and mice. Results of these studies are summarized in Section 3.1.3, Metabolism.

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

Low et al. (1989) studied the kinetics of distribution of absorbed benzene in Sprague-Dawley rats. One hour after rats were dosed with 0.15 mg/kg of ^{14}C-benzene (gavage), tissue distribution of radiolabel was highest in liver and kidney, intermediate in blood, and lowest in the Zymbal gland, nasal cavity tissue, and mammary gland. One hour after exposure to 1.5 mg/kg benzene, the highest concentrations of radiolabel were found in bone marrow, kidney, and liver. Elimination of radiolabel from tissues was biphasic with rapid phase half-times ranging from 2.1 hours (blood) to 4.2 hours (kidney), following the 0.15 mg/kg dose. The elimination half-time for bone marrow was 3.2 hours. The slower-phase half-times following the 0.15 mg/kg dose ranged from 11 hours (bone marrow) to 29 hours (blood).

Low et al. (1989) also measured benzene metabolites in tissues. The highest tissue concentrations of benzene's metabolite hydroquinone 1 hour after administration of 15 mg/kg of ^{14}C-benzene (gavage) were in the liver, kidney, and blood, while the highest concentrations of the metabolite phenol were in the oral cavity, nasal cavity, and kidney. The major tissue sites of conjugated metabolites of benzene (phenyl sulphate and hydroquinone glucuronide) were blood, bone marrow, oral cavity, kidney, and liver. Muconic acid was also detected in these tissues. Additionally, the Zymbal gland and nasal cavity were depots for phenyl glucuronide, another conjugated metabolite of benzene. The Zymbal gland is a specialized sebaceous gland and a site for benzene-induced tumors. Therefore, it is reasonable to expect that lipophilic chemicals like benzene would partition readily into this gland. Radiolabel in the Zymbal gland constituted <0.0001% of the administered dose.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of ^{14}C-benzene alone, or adsorbed to clay or sandy soil. Stomach tissue then fat had the highest amounts of radioactivity 2 hours after exposure in all treatment groups. Tissue concentrations of radiolabel were not statistically different when benzene was administered alone or adsorbed to sandy soil or clay. Elimination half-times of radiolabel from plasma were 806 minutes for benzene alone, 648 minutes for benzene adsorbed to sandy soil, and 82.8 minutes for benzene adsorbed to clay.

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

A study of male rats treated dermally with 0.004 mg/cm^2 of ^{14}C-benzene, with and without 1 g of clay or sandy soil, revealed soil-related differences in tissue distribution following treatment. The ^{14}C activity (expressed as a percentage of initial dose per g of tissue) 48 hours after treatment with soil-adsorbed benzene was greatest in the treated skin (0.059–0.119%), followed by the kidney (0.024%) and liver

(0.013–0.015%), in both soil groups. In the pure benzene group, the kidney contained the largest amount of radioactivity (0.026%), followed by the liver (0.013%) and treated skin (0.011%) (Skowronski et al. 1988). In all three groups, <0.01% of the radioactivity was found in the following tissues: fat, bone marrow, esophagus, pancreas, lung, heart, spleen, blood, brain, thymus, thyroid, adrenal, testes, untreated skin, and remaining carcass.

3.1.3 Metabolism

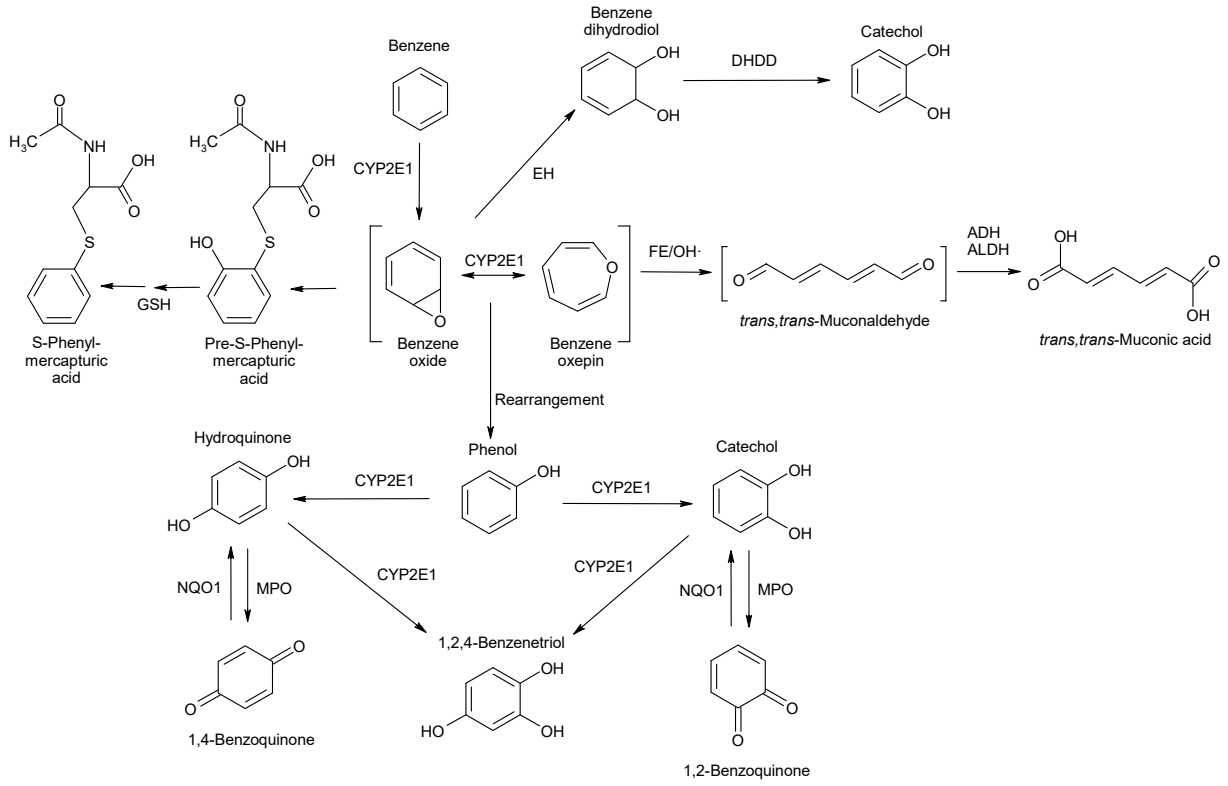
The metabolism of benzene has been studied extensively. It is generally understood that both cancer and noncancer effects are caused by one or more reactive metabolites of benzene. Available data indicate that metabolites produced in the liver are distributed to the bone marrow where benzene toxicity is expressed. Benzene metabolism also occurs in the bone marrow and other tissues.

Data regarding metabolism of benzene in humans are derived primarily from studies using inhalation exposures. Benzene is excreted both unchanged via the lungs and as metabolites (but also as parent compound in small amounts) in the urine. The rate and percentage of excretion via the lungs are dependent on exposure dose and route. Qualitatively, the metabolism and elimination of benzene appear to be similar in humans and laboratory animals (Henderson et al. 1989; Sabourin et al. 1988).

The metabolic scheme shown in Figure 3-1 is based on the results of numerous mechanistic studies of benzene metabolism (Henderson et al. 1989; Ross 1996, 2000). The first step is the CYP2E1-catalyzed oxidation of benzene to form benzene oxide (Lindstrom et al. 1997), which is in equilibrium with benzene oxepin (Vogel and Günther 1967). Several pathways are involved in the metabolism of benzene oxide. The predominant pathway involves nonenzymatic rearrangement to form phenol (Jerina et al. 1968), the major initial product of benzene metabolism (Parke and Williams 1953). Phenol is oxidized in the presence of CYP2E1 to catechol or hydroquinone, which are oxidized via MPO to the reactive metabolites, 1,2- and 1,4-benzoquinone, respectively (Nebert et al. 2002). The reverse reaction (reduction of 1,2- and 1,4-benzoquinone to catechol and hydroquinone, respectively) is catalyzed by NQO1 (Nebert et al. 2002). Both catechol and hydroquinone may be converted to the reactive metabolite, 1,2,4-benzenetriol, via CYP2E1 catalysis. Alternatively, benzene oxide may undergo epoxide hydrolase-catalyzed conversion to benzene dihydrodiol and subsequent dihydrodiol dehydrogenase-catalyzed conversion to catechol (Nebert et al. 2002; Snyder et al. 1993a, 1993b). Other pathways of benzene oxide metabolism that have been proposed include: (1) reaction with glutathione to form PhMA (Nebert et al. 2002; Sabourin et al. 1988; Schafer et al. 1993; Schlosser et al. 1993; Schrenk et al. 1992; van Sittert et al.

1993), and (2) iron-catalyzed ring-opening conversion to *trans*,*trans*-muconic acid, presumably via the reactive *trans*,*trans*-muconaldehyde intermediate (Bleasdale et al. 1996; Nebert et al. 2002; Ross 2000; Witz et al. 1990a, 1990b, 1996). PhMA is formed from the acid-catalyzed dehydration of the *in vivo* pre-phenylmercapturic acid (pre-PhMA) during sample preparation (Bowman et al. 2023; Sabourin et al. 1998).

Figure 3-1. Metabolic Pathways for Benzene



ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; CYP2E1 = cytochrome P450 2E1; DHDD = dihydrodiol dehydrogenase; EH = epoxide hydrolase; GSH = glutathione; MPO = myeloperoxidase; NQO1 = NAD(P)H:quinone oxidoreductase

Sources: Bowman et al. 2023; Nebert et al. 2002; Ross 2000; Sabourin et al. 1998

Each of the phenolic metabolites of benzene (phenol, catechol, hydroquinone, and 1,2,4-benzenetriol) can undergo sulfonic or glucuronic conjugation (Nebert et al. 2002; Schrenk and Bock 1990; not shown in Figure 3-1). The conjugates of phenol and hydroquinone are major urinary metabolites of benzene (Sabourin et al. 1989a; Wells and Nerland 1991).

Results of several studies provide strong evidence for the involvement of CYP2E1 in the oxidation of benzene. For example, no signs of benzene-induced toxicity were observed in transgenic CYP2E1 knockout mice (that do not express hepatic CYP2E1 activity) following exposure to benzene vapors

(200 ppm, 6 hours/day for 5 days) that caused severe genotoxicity and cytotoxicity in wild-type mice (Valentine et al. 1996a, 1996b). Pretreatment of mice with cytochrome P450 (CYP) inhibitors (toluene, propylene glycol, β-diethyl amino ethyl diphenyl propyl acetate hydrogen chloride [SKF-525A]) has been demonstrated to reduce both benzene metabolite formation (Andrews et al. 1977; Gill et al. 1979; Ikeda et al. 1972; Tuo et al. 1996) and resulting genotoxicity in mice (Tuo et al. 1996). Pretreatment with CYP inducers (3-methylcholanthrene and β-naphthoflavone) increased both benzene metabolism and benzene clastogenicity (Gad-El-Karim et al. 1986).

Other studies provided additional information about CYP2E1 involvement and suggest that other CYPs may metabolize benzene. Immunoinhibition studies in rat and rabbit hepatic microsomes provide additional support to the major role of CYP2E1 in benzene metabolism (Johansson and Ingelman-Sundberg 1988; Koop and Laethem 1992). Polymorphisms of CYP2E1 are associated with changes in urinary metabolites of benzene (Kim et al. 2007a). In human lymphocyte cultures, higher CYP2E1 expression was associated with increased numbers of DNA strand breaks (Zhang et al. 2011). Occupationally exposed workers with a phenotype corresponding to rapid CYP2E1 metabolism were more susceptible to benzene hematotoxicity than workers not expressing this phenotype (Rothman et al. 1997; Ye et al. 2015). *In vitro* studies using human liver microsomes demonstrate a positive correlation between benzene metabolism and CYP2E1 activity (Nedelcheva et al. 1999; Seaton et al. 1994). Although CYP2E1 appears to be the major catalyzing agent in initial benzene metabolism, other CYPs, such as CYP2B1 and CYP2F2, may also be involved (Gut et al. 1996a, 1996b; Powley and Carlson 2000, 2001; Sheets and Carlson 2004; Sheets et al. 2004; Snyder et al. 1993a, 1993b).

Although CYP2E1 is expressed in a variety of tissues, the liver is considered to be the primary site of production of toxic benzene metabolites. Partial hepatectomy diminished both the rate of metabolism of benzene and its toxicity in rats exposed to benzene via subcutaneous injection (Sammett et al. 1979).

Bone marrow of mice, rats, rabbits, and humans also expresses CYP2E1 (Bernauer et al. 1999, 2000; Schnier et al. 1989) and *in vitro* studies have shown that bone marrow obtained from mice, rabbits, and rats can metabolize benzene (Andrews et al. 1979; Ganousis et al. 1992; Gollmer et al. 1984; Irons et al. 1980; Schnier et al. 1989). Bone marrow expresses MPO, which can contribute to production of reactive metabolites in bone marrow, including quinones and semiquinone radicals (Ross et al. 1996; Smith 1999). Bone marrow fibroblasts and macrophages also express glutathione-S-transferase, uridine 5'-diphospho-glucuronosyltransferase (UDP-glucuronyltransferase), and peroxidase activities (Ganousis et al. 1992), which may contribute to metabolism of benzene in bone marrow.

Glutathione and quinone reductases play critical roles in modulating hydroquinone-induced toxicity. Bone marrow stromal cells obtained from rats had higher activities of glutathione reductase and quinone reductase than marrow stromal cells from mice (Zhu et al. 1995).

It remains unclear how much bone marrow, relative to the liver, contributes to the production of reactive intermediates of benzene metabolism in marrow. Irons et al. (1980) demonstrated that the isolated perfused rat femur was capable of metabolizing benzene (approximately 0.0002% of ^{14}C-benzene was recovered as metabolites); however, benzene oxide and phenol were not detected as metabolites of benzene in *in vitro* preparations of microsomes obtained from rat bone marrow (Lindstrom et al. 1999). An interpretation of this observation is that marrow may not be a major contributor to the initial steps in the oxidative metabolism of benzene. No studies were located regarding the potential for human bone marrow tissue to metabolize benzene.

In vitro studies have demonstrated that pulmonary microsomes of humans and laboratory animals are capable of metabolizing benzene, which appears to be catalyzed by both CYP2E1 and CYP2F2 (Powley and Carlson 1999, 2000; Sheets et al. 2004).

Mouse liver microsomes and cytosol have been shown to catalyze ring opening in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) *in vitro*, producing *trans*,*trans*-muconaldehyde, a six-carbon diene dialdehyde also referred to as muconic dialdehyde (Goon et al. 1993; Latriano et al. 1986), a known hematotoxin (Witz et al. 1985) and toxic metabolite of benzene (Henderson et al. 1989).

Metabolism of benzene and *trans,trans*-muconaldehyde in the isolated perfused rat liver indicated that benzene was metabolized to muconic acid, a ring-opened metabolite of benzene (Grotz et al. 1994). *trans,trans*-Muconaldehyde was metabolized to muconic acid and three other metabolites. Furthermore, mouse liver microsomes incubated with benzene produced the following metabolites: phenol, hydroquinone, *trans,trans*-muconaldehyde, *6*-oxo-*trans,trans*-2,4-hexadienoic acid, 6-hydroxy-*trans,trans*-2,4-hexadienal, and 6-hydroxy-*trans,trans*-2,4-hexadienoic acid (Zhang et al. 1995a). β-Hydroxymuconaldehyde, a new metabolite, was also identified.

The precursor of muconic acid is thought to be the precursor of muconic dialdehyde. Zhang et al. (1995b) suggested that *cis,cis*-muconaldehyde is formed first, followed by *cis,trans*-muconaldehyde, and finally

converted to *trans,trans*-muconaldehyde. Muconic dialdehyde has been shown to be metabolized *in vivo* in mice to muconic acid (Witz et al. 1990b).

Small amounts of muconic acid were found in the urine of rabbits and mice that received oral doses of ^{14}C-benzene (Gad-El-Karim et al. 1985; Parke and Williams 1953). The percentage of this metabolite formed varied with the administered benzene dose and was quite high at low doses (17.6% of 0.5 mg/kg benzene administered to C57BL/6 mice) (Witz et al. 1990b). Other studies in animals support these results (Brondeau et al. 1992; Ducos et al. 1990; McMahon and Birnbaum 1991; Sabourin et al. 1989a; Schad et al. 1992).

The muconic acid pathway also appears to be active in humans (Bechtold and Henderson 1993; Ducos et al. 1990, 1992; Lee et al. 1993; Melikian et al. 1993, 1994). Muconic acid has been detected in urine from male and female smokers and nonsmokers (Melikian et al. 1994). Melikian et al. (1994) also found that the amount of muconic acid produced varied by sex, pregnancy status, and smoking level. Because of its relative importance in benzene toxicity, additional modeling studies have been conducted to further describe how *trans,trans*-muconaldehyde is transformed to muconic acid (Bock et al. 1994).
A study of 131 participants showed that the median *trans,trans*-muconic acid concentration among smokers was approximately 2.5 times higher compared to nonsmokers, and participants who smoked >20 cigarettes per day had a higher median *trans,trans*-muconaldehyde concentration compared to participants who smoked no more than 10 or 11–20 cigarettes per day (Buratti et al. 1996). A study of 136 smokers showed that urinary *trans,trans*-muconaldehyde concentrations were correlated with cigarettes smoked per day (Taniguchi et al. 1999). In a study of 177 participants in Iran's Golestan Province with a high incidence of cancer, cigarette and hookah smokers had significantly higher levels of *trans,trans*-muconic acid, with median creatinine-adjusted levels of 65.1 and 82.4µg/g, respectively, than nonsmokers who had a median level of 30.2 µg/g (Bhandari et al. 2023).

Kenyon et al. (1995) compared urinary metabolites in B6C3F1 mice after oral dosing with phenol to results of Sabourin et al. (1989a) who administered a comparable oral dose of benzene to B6C3F1 mice. Phenol administration resulted in lower urinary levels of hydroquinone glucuronide and higher levels of phenol sulfate and phenol glucuronide (Kenyon et al. 1995) compared to benzene administration (Sabourin et al. 1989a). Kenyon et al. (1995) hypothesized that the differences in the urinary metabolite profiles between phenol and benzene after oral dosing were due to zonal differences in the distribution of metabolizing enzymes within the liver.

Conjugating enzymes are more concentrated in the periportal area of the liver, the first region to absorb the compound, whereas oxidizing enzymes are more concentrated in the pericentral region of the liver. Based on this hypothesis, during an initial pass through the liver after oral administration, phenol would have a greater opportunity to be conjugated as it was absorbed from the gastrointestinal tract into the periportal region of the liver, thus resulting in less free phenol being delivered into the pericentral region of the liver to be oxidized. With less free phenol available for oxidation, less hydroquinone would be produced, relative to conjugated phenol metabolites.

In contrast to phenol metabolism, benzene must be oxidized before it can be conjugated. Therefore, metabolism of benzene would be minimal in the periportal region of the liver, with most of the benzene reaching the pericentral region to be oxidized to hydroquinone. Based on this scheme, the study authors suggested that benzene administration would result in more free phenol being delivered to oxidizing enzymes in the pericentral region of the liver than administration of phenol itself (Kenyon et al. 1995).

Benzene has been found to stimulate its own metabolism, thereby increasing the rate of toxic metabolite formation. Pretreatment of mice, rats, and rabbits subcutaneously with benzene increased benzene metabolism *in vitro* without increasing CYP2E1 concentrations (Arinc et al. 1991; Gonasun et al. 1973; Saito et al. 1973). In contrast, there was no significant effect on the metabolism of benzene when Fischer 344 rats and B6C3F1 mice, pretreated with repeated inhalation exposure to 600 ppm of benzene, were again exposed to 600 ppm benzene (Sabourin et al. 1990).

The rate of benzene metabolism can be altered by pretreatment with various compounds. Benzene is a preferential substrate of CYP2E1, which also metabolizes ethanol. CYP2E1 can be induced by these substrates and is associated with the generation of hydroxyl radicals, probably via cycling of the cytochrome (Chepiga et al. 1991; Parke 1989; Snyder et al. 1993a, 1993b). It is possible that hydroxy radical formation by CYP2E1 may play a role in the benzene ring-opening pathway, leading to the formation of *trans,trans*-muconaldehyde. Phenol, hydroquinone, benzoquinone, and catechol have also been shown to induce CYPs in human hematopoietic stem cells (Henschler and Glatt 1995). Therefore, exposure to chemicals that stimulate the activity of this enzyme system prior to exposure to benzene could increase the rate of benzene metabolism.

Both NADPH-linked and ascorbate-induced lipid peroxidation activities induced *in vitro* were lowered 5.5 and 26%, respectively, in rats following oral administration of 1,400 mg/kg/day of benzene for 3 days, followed by intraperitoneal injection of phenobarbital. These results suggest that benzene alters hepatic

drug metabolism and lipid peroxidation. The decrease in lipid peroxidation could be due to the antioxidant property of the metabolites (Pawar and Mungikar 1975).

The ultimate disposition and metabolic fate of benzene depends on animal species, dose, and route of exposure. The dose of benzene affects both the total metabolism and the concentrations of individual metabolites formed. In mice, the percentage of hydroquinone glucuronide decreased as the dose increased. In both rats and mice, the percentage of muconic acid decreased as the dose increased. The shift in metabolism may affect the dose-response relationship for toxicity and has been observed in all animal species studies thus far (Sabourin et al. 1989a, 1992; Witz et al. 1990a, 1990b).

Species differences in benzene metabolism have been observed. Mice have a higher minute volume per kg body weight than rats (1.5 times higher) when benzene is inhaled. As a result, equilibrium between the concentration of benzene in inhaled air and blood was more rapid in mice than rats, although the steady-state level in blood was not influenced (Sabourin et al. 1987). Benzene metabolism is saturable in mice and rats; however, rats have a higher capacity to metabolize benzene than mice (Sabourin et al. 1987). In this study, complete saturation of benzene metabolism occurred in mice at oral doses >50 mg/kg, whereas rats continued to metabolize benzene at oral doses >50 mg/kg.

Species differences in benzene metabolism following oral exposure were observed in rats and mice administered benzene by gavage at doses of 0.5–150 mg/kg/day (Sabourin et al. 1987). Metabolism was dose dependent. For rats and mice, doses <15 mg/kg, >90% of the benzene was metabolized, while at doses >15 mg/kg, an increasing percentage of orally administered benzene was exhaled unmetabolized. Additionally, total metabolites per unit body weight were equal in rats and mice at doses up to 50 mg/kg/day. However, total metabolites in mice did not increase at doses >50 mg/kg/day, suggesting saturation of metabolic pathways (Sabourin et al. 1987).

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

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

There are qualitative and quantitative differences in rodent benzene metabolism. Benzene metabolism has been studied in isolated hepatocytes (Orzechowski et al. 1995). In this study, mouse hepatocytes incubated with benzene produced two metabolites (1,2,4-trihydroxybenzene sulfate and hydroquinone sulfate) that were not found in rat hepatocyte incubations. These sulfate metabolites were also produced by mouse hepatocytes incubated with the benzene metabolites, hydroquinone and 1,2,4-benzenetriol. Mouse hepatocytes were almost 3 times more effective in metabolizing benzene, compared to rat hepatocytes. This difference was accounted for in the formation of hydroquinone, hydroquinone sulfate, and 1,2,4-trihydroxybenzene sulfate.

Sabourin et al. (1988) compared metabolites of benzene in tissues of Fischer 344 rats and B6CF1 mice following exposure to 50 ppm benzene. In rats, phenol, catechol, and hydroquinone were not detected in in the liver, lungs, and blood. The major water-soluble metabolites in rat tissues were muconic acid, phenyl sulfate, prephenyl mercapturic acid, and an unknown metabolite. The unknown was present in amounts equal to the amounts of phenyl sulfate in the liver; phenyl sulfate and the unknown were the major metabolites in the liver. In contrast to rats, phenol and hydroquinone were detected in the liver, lungs, and blood of mice, and catechol was detected in the liver, but not in the lungs or blood. As in the rat, the unknown was present in amounts equal to the amounts of phenyl sulfate in the liver. Mice had more muconic acid in the liver indicating a greater risk for toxicity than from phenyl conjugated metabolites, which are less toxic and water soluble (Sabourin et al. 1988).

The effect of exposure rate on benzene metabolism was studied in Fischer 344 rats and B6C3F1 mice that were exposed to several different combinations of concentrations and durations that resulted in the same total amount of benzene (Sabourin et al. 1989a). The benzene inhalation exposures evaluated in the study were 600 ppm for 0.5 hour, 150 ppm for 2 hours, or 50 ppm for 6 hours. In rats, the area under the curve

(AUC) for water-soluble metabolites in tissues (blood, liver, lung) was not affected by the benzene exposure rate. However, in mice, exposure rate effects were observed that indicated a shift from markers of toxicity (hydroquinone glucuronide, muconic acid, phenylglucuronide, and prephenylmercapturic acid) to less toxic metabolites. As compared with exposures with lower benzene concentrations but longer durations, the fastest exposure rate (0.5 hour times 600 ppm) had lower AUCs for muconic acid and hydroquinone glucuronide in the blood, liver, and lungs. In blood and lung tissues there were decreased AUC ratios for muconic acid relative to phenylsulfate and decreased AUC for hydroquinone glucuronide relative to phenylsulfate. These changes indicate that at the faster exposure rate, mice tended to shift a greater portion of their benzene metabolism toward phenyl conjugation, which produces less toxic metabolites.

The detoxification pathways for benzene appear to be low-affinity, high-capacity pathways, whereas pathways leading to the putative toxic metabolites appear to be high-affinity, low-capacity systems (Henderson et al. 1989). Accordingly, if the exposure dose regimen, via inhalation, extends beyond the range of linear metabolism rates of benzene (200 ppm by inhalation) (Sabourin et al. 1989b), then the fraction of toxic metabolites formed relative to the amount administered will be reduced.

Bois and Paxman (1992) used a PBPK model to assess effects of dose rate on the disposition of benzene metabolites. Simulations were performed for rats exposed either for 15 minutes to 32 ppm or for 8 hours to 1 ppm (equivalent 8-hour TWAs). The amount of metabolites (hydroquinone, catechol, and muconaldehyde) formed was 20% higher after the 15-minute exposure at the higher level than after the 8-hour exposure at the lower level. Differences between the model predictions (Bois and Paxman 1992) and the empirical data of Sabourin et al. (1989a, 1989b) may be related, at least in part, to the higher benzene exposure levels (50, 150, and 600 ppm) used by Sabourin and coworkers. The pattern of urinary metabolites observed in exposed workers has provided evidence of a high- and low-affinity pathway for metabolite production (Kim et al. 2006a, 2006b; Rappaport et al. 2009, 2010). In theory, a high-affinity pathway could result in nonlinear metabolic clearance of benzene, with faster clearance at lower exposures (<0.1 ppm). Evidence of nonlinear metabolic clearance at low exposure concentrations remains equivocal, with some studies finding non-linearities and other studies not observing non-linearity (Cox et al. 2017; McNally et al. 2017; Price et al. 2012).

Covalent binding of benzene metabolites to cellular macromolecules is thought to be related to benzene's mechanism of toxicity, although the relationship between adduct formation and toxicity is not clear. Benzene metabolites have been found to form covalent adducts with proteins from blood in humans

(Bechtold et al. 1992b). Benzene metabolites form covalent adducts with nucleic acids and proteins in rats and mice (Norpoth et al. 1988; Rappaport et al. 1996). Covalent binding of benzene metabolites to proteins has been observed in mouse or rat liver, bone marrow, kidney, spleen, blood, and muscle *in vivo* (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Creek et al. 1997; Longacre et al. 1981a, 1981b; Sun et al. 1990). Metabolite binding to proteins has also been observed in perfused bone marrow preparations (Irons et al. 1980) and in rat and mouse liver DNA *in vivo* (Creek et al. 1997; Lutz and Schlatter 1977). Binding of benzene metabolites to DNA also has been observed in *in vitro* preparations of rabbit and rat bone marrow mitochondria (Rushmore et al. 1984). Exposure-related increases in blood levels of albumin adducts of benzene oxide and 1,4-benzoquinone were noted among workers occupationally exposed to benzene air concentrations of 0.07–46.6 ppm (Rappaport et al. 2002a, 2002b). Several reactive metabolites of benzene have been proposed as agents of benzene hematotoxic and leukemogenic effects. These metabolites include benzene oxide, reactive products of the phenol pathway (catechol, hydroquinone, and 1,4-benzoquinone), and *trans*,*trans*-muconaldehyde.

3.1.4 Excretion

Available human data indicate that following inhalation exposure to benzene, the major route for elimination of unmetabolized benzene is via exhalation. Benzene has also been detected in samples of human breast milk (Fabietti et al. 2004). Absorbed benzene is also excreted in humans via metabolism to phenol and muconic acid followed by urinary excretion of conjugated derivatives (sulfates and glucuronides). In six male and female volunteers exposed to 52–62 ppm benzene for 4 hours, respiratory excretion (the amount of absorbed benzene excreted via the lungs) was approximately 17%; no gender-related differences were observed (Nomiyama and Nomiyama 1974a, 1974b). Results from a study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours showed that 16.4–41.6% of the retained benzene was excreted by the lungs within 5–7 hours (Srbova et al. 1950). The rate of excretion of benzene was the greatest during the first hour.

Results of a study involving a single human experimental subject exposed to concentrations of benzene of 6.4 and 99 ppm for 8 hours and 1 hour, respectively, suggested that excretion of benzene in breath has three phases and could possibly have four phases. The initial phase is rapid and is followed by two (or three) slower phases (Sherwood 1988). The initial phase with a high exposure concentration (99 ppm) and a short-term exposure duration (1 hour) had a more rapid excretion rate (half-life=42 minutes) and a greater percentage of the total dose excreted (17%) than did the initial phase with a low exposure concentration (6.4 ppm) and longer exposure duration (8 hours) (half-life=1.2 hours, percentage of total

dose excreted=9.3%). Subsequent phases showed an increase in the half-lives. Benzene metabolites were excreted in urine. This limited study indicated a greater proportion of the total dose was excreted in urine than in breath (Sherwood 1988).

Absorbed benzene is also eliminated in humans by metabolism to phenol and muconic acid followed by urinary excretion of conjugated derivatives (sulfates and glucuronides). Srbova et al. (1950) found that only 0.07–0.2% of benzene extracted from inhaled air was excreted in the urine as benzene. Sherwood (1988) showed that urinary excretion of phenol conjugate was biphasic, with an initial rapid excretion phase, followed by a slower excretion phase. Ghittori et al. (1993) stated that benzene in urine may be a useful biomarker of occupational exposure.

The urinary excretion of phenol in workers was measured following a 7-hour work shift exposure to 1–200 ppm benzene. A correlation of 0.881 between exposure level and urinary phenol excretion was found (Inoue et al. 1986). Urine samples were collected from randomly chosen subjects not exposed to known sources of benzene, from subjects exposed to side stream cigarette smoke, or from supermarket workers presumed exposed to benzene from unknown sources (Bartczak et al. 1994). Samples analyzed for muconic acid found concentrations ranging from 8 to 550 ng/mL.

The primary excretory pathway for benzene and its metabolites is the urine. Excretory products that are known to correlate with blood benzene levels include muconic acid, PhMA, and 8-hydroxy-deoxyguanosine. Blood benzene and urinary benzene levels have been correlated ($r=0.61$, $p<0.001$) in a smoker and nonsmoker group (Kok and Ong 1994). Furthermore, urinary muconic acid and PhMA levels have been correlated (0.40–0.81) with benzene blood levels (mean=3.3 μg/L) and benzene air levels, which reached a maximum of 13 mg/m^3 (Popp et al. 1994).

Smoking tobacco can increase the concentrations of benzene or benzene-correlated biomarkers excreted into blood and urine. Blood and urine levels of benzene were 110.9 and 116.4 ng/L, respectively, in nonsmokers, and 328.8 and 405.4 ng/L, respectively, in smokers (Kok and Ong 1994). Melikian et al. (1994) compared urinary muonic acid levels in smokers and nonsmokers and in pregnant and nonpregnant woman. The mean urinary levels of muconic acid in groups of male, female-nonpregnant, and female-pregnant smokers were 3–5 times higher than urinary levels in the corresponding nonsmoking groups (Melikian et al. 1994). Similar mean urinary concentrations of muconic acid were observed in males who smoke and nonpregnant female smokers. On a creatinine basis, urinary muconic acid levels were similar in pregnant and nonpregnant smokers.

Animal data show that exhalation is the main route for excretion of unmetabolized benzene and that metabolized benzene is excreted primarily in urine. Only a small amount of an absorbed dose is eliminated in feces.

A biphasic pattern of excretion of unmetabolized benzene in expired air was observed in rats exposed to 500 ppm for 6 hours, with half-times for expiration of 0.7 hour for the rapid phase and 13.1 hours for the slow phase (Rickert et al. 1979). Excretion of exhaled benzene was also biphasic in mice following an intraperitoneal dose of 0.13 mg/kg (Zhang et al. 2017). The half-life for the slow phase of benzene elimination suggests the accumulation of benzene.

The major route of excretion following a 6-hour, nose-only inhalation exposure of rats and mice to various concentrations of ^{14}C-benzene (10–1,000 ppm) appeared to be dependent on the inhaled concentration (Sabourin et al. 1987). When exposed to the same concentrations, the inhaled dose per kg body weight was 150–200% higher in mice compared to rats. At all concentrations, fecal excretion accounted for <3.5% of the radioactivity for rats and <9% for mice. At lower exposure concentrations (i.e., 13–130 ppm in rats and 11–130 ppm in mice), <6% of the radioactivity was excreted in expired air.

At the highest exposure concentrations (rats, 870 ppm; mice, 990 ppm), the percentages of exhaled unmetabolized benzene were 48 and 14% in rats and mice, respectively, following termination of the exposure. Most of the benzene-associated radioactivity that was not exhaled was found in the urine and in the carcass 56 hours after the end of exposure to these high concentrations. The radioactivity in the carcass was associated with the pelt of the animals. The study authors assumed that this was due to contamination of the pelt with urine since the inhalation exposure had been nose-only. Further investigation confirmed that the radioactivity was associated with the fur of the animals. Accordingly, the percentage of the total radioactivity excreted by these animals (urine and urine-contaminated pelt) that was not exhaled or associated with feces was 47–92% for rats and 80–94% for mice. At exposures of 260 ppm in rats, 85–92% of the radioactivity was excreted as urinary metabolites, while at exposures of 130 ppm in mice, 88–94% of the radioactivity was excreted as urinary metabolites. The total urinary metabolite formation was 5–37% higher in mice than in rats at all doses. This may be explained by the greater amount of benzene inhaled by mice per kg of body weight (Sabourin et al. 1987).

In mice exposed to 50 ppm benzene (6 hours/day for 8 days), the following metabolites were detected in urine above levels in unexposed mice: phenol, *trans,trans*-muconic acid, hydroquinone, and

s-phenylmercapturic acid (Bird et al. 2010). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

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

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

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

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

also affected the metabolite profile in the urine. At low doses, a greater fraction of the benzene was converted to putative toxic metabolites than at high doses, as reflected in urinary metabolites.

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

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

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

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

Results of a study in which male rats were dermally treated with 0.004 mg/cm^2 of ^{14}C-benzene, with or without 1g of clay or sandy soil, showed that for all treatment groups, the major routes of excretion were the urine and, to a lesser extent, the expired air (Skowronski et al. 1988). The highest amount of radioactivity in urine appeared in the first 12–24 hours after treatment (58.8, 31.3, and 25.1% of the absorbed dose, respectively, for pure benzene, sandy soil–adsorbed benzene, and clay soil-adsorbed benzene). In the group treated with pure benzene, 86.2% of the absorbed dose was excreted in the urine. Sandy soil and clay soil significantly decreased urinary excretion to 64.0 and 45.4%, respectively, of the absorbed dose during the same time period. Rats receiving pure benzene excreted 12.8% of the absorbed dose in expired air within 48 hours. Only 5.9% of the radioactivity was collected in expired air 48 hours after treatment with sandy soil–adsorbed benzene, while experiments with clay soil–adsorbed benzene revealed that 10.1% of the radioactivity was located in expired air. Less than 1% of the absorbed dose was expired as $^{14}CO_2$ in all groups. The ^{14}C activity in the feces was small (<0.5% of the applied radioactivity) in all groups 48 hours after treatment. Phenol was the major urinary metabolite detected in the 0–12-hour urine samples of all treatment groups. The percentage of total urinary radioactivity associated with phenol was 37.7% for benzene alone, 44.2% for benzene adsorbed to sandy soil, and 45.5% for benzene adsorbed to clay soil. Smaller quantities of hydroquinone, catechol, and benzenetriol were also detected (Skowronski et al. 1988).

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

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

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

Models are simplified representations of a system with the intent of reproducing or simulating its structure, function, and behavior. PBPK models are more firmly grounded in principles of biology and biochemistry. They use mathematical descriptions of the processes determining uptake and disposition of chemical substances as a function of their physicochemical, biochemical, and physiological characteristics (Andersen and Krishnan 1994; Clewell 1995; Mumtaz et al. 2012a; Sweeney and Gearhart 2020). PBPK models have been developed for both organic and inorganic pollutants (Ruiz et al. 2011) and are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Mumtaz et al. 2012b; Ruiz et al. 2011; Sweeney and Gearhart 2020; Tan et al. 2020). PBPK models can also be used to more accurately extrapolate from animal to human, high dose to low dose, route to route, and various exposure scenarios and to study pollutant mixtures (El-Masri et al. 2004). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints (Clewell 1995).

Several PBPK models have been developed that simulate the disposition of benzene in humans (Bois et al. 1996; Brown et al. 1998; Fisher et al. 1997; Knutsen et al. 2013a, 2013b; Majumdar et al. 2016; Medinsky et al. 1989c; Ruiz et al. 2020; Sinclair et al. 1999; Travis et al. 1990), mice (Cole et al. 2001; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990), and rats (Bois et al. 1991a; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990). A comparative summary of the models is provided in Table 3-1. All of the models have the same general structure (Figure 3-2). Most of the models simulate inhalation and oral exposures; one model provides a simulation of dermal absorption (Sinclair et al. 1999). Physiological parameters and partition coefficients for simulating benzene biokinetics of human females were reported for the Brown et al. (1998) and Fisher et al. (1997) models. Flow-limited exchange of benzene between blood and tissues is assumed in all models, with excretion of benzene in exhaled air and, in one case, to breast milk (Fisher et al. 1997). All models include simulations of blood, fat, liver, lung, and lumped compartments representing other slowly-perfused tissues (e.g., skeletal muscle) and rapidly-perfused tissues (e.g., kidneys, other viscera). Simulation of bone marrow, the primary target for benzene toxicity, is included in the models reported by Bois et al. (1991a, 1996), Knutsen et al. (2013a, 2013b), Sinclair et al. (1999), and Travis et al. (1990). The Knutsen et al. (2013a, 2013b) model includes a bladder compartment that accumulates benzene metabolites circulating in blood.

Table 3-1. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

	Simulated areas					
Reference	Species	Absorption pathways	Tissues[a]	Metabolic pathways[b]	Excretion pathways[c]	Comment
Bois et al. 1991a	Rat	Inhalation, oral	BL, BM, FA, LI, LU, RP, SP	BM, LI: BZ→BO(c) BO→BG(c) BO→PH(f) BO→GSH(c) BG→DI(c) PH→HQ(c) PH→CA(c) BM, LI, LU, GI: PH→PHCO(c)	EH: BZ UR: PH	Simulates metabolic pathways in bone marrow, and phenol conjugation in lung and gastrointestinal tract

Table 3-1. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

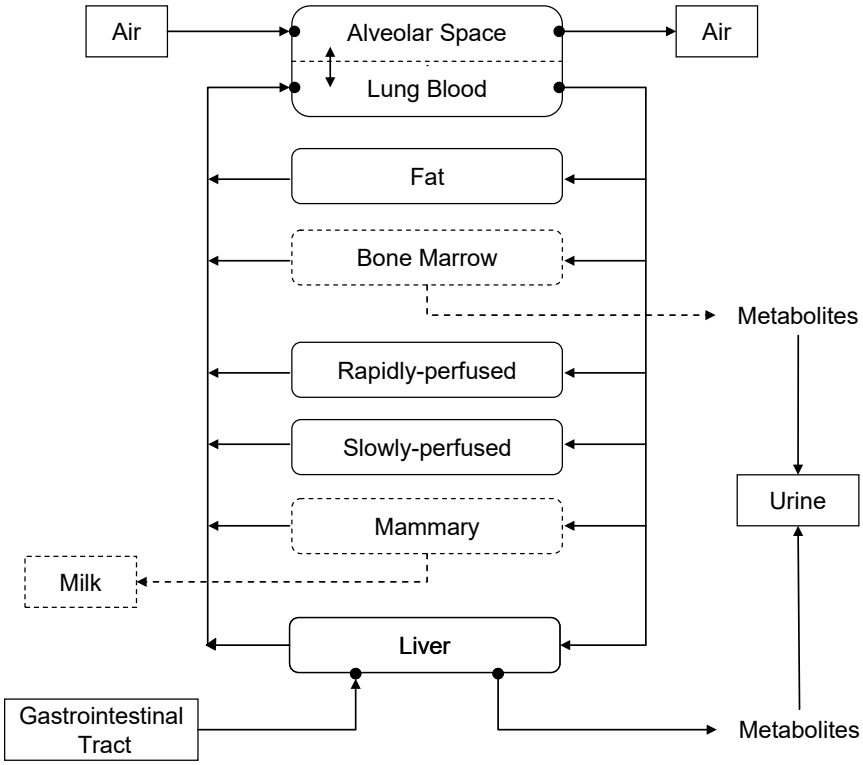
	Simulated areas					
Reference	Species	Absorption pathways	Tissues[a]	Metabolic pathways[b]	Excretion pathways[c]	Comment
Bois et al. 1996	Human	Inhalation	BL, BM, FA, LI, LU, RP, SP	BM, LI: $BZ \rightarrow M_{tot}(c)$ LI: $PHX_{end} \rightarrow PH(z)$	EH: BZ UR: M_{tot} PH	Simulates metabolic pathways in bone marrow, and endogenous production of phenolic metabolites
Brown et al. 1998	Human (males, females)	Inhalation	BL, FA, LI, LU, RP, SP	LI: $BZ \rightarrow M_{tot}(f)$	EH: BZ	Simulates males or females
Cole et al. 2001	Mouse	Inhalation, oral	BL, FA, LI, LU, RP, SP	LI: $BZ \rightarrow BO(c)$ $BO \rightarrow PH(f)$ $BO \rightarrow PMA(f)$ $BO \rightarrow MA(f)$ $PH \rightarrow HQ(c)$ $PH \rightarrow PHCO(c)$ $PH \rightarrow CA(c)$ $CA \rightarrow THB(c)$ $HQ \rightarrow HQCO(c)$	EH: BZ UR: CA MA PHCO PMA HQCO THB	All metabolism is assigned to the liver
Fisher et al. 1997	Human	Inhalation	FA, LI, LU, RP, SP, MI	LI: $BZ \rightarrow M_{tot}(c)$	EH: BZ MI: BZ	Simulates transfer of benzene to breast milk
Knutsen et al. 2013a, 2013b	Human, mouse	Inhalation	BL, BM, FA, LI, LU, RP, SP, UB	LI, BM: $BZ \rightarrow BO(c)$ $BO \rightarrow PH(f)$ $BO \rightarrow PMA(f)$ $BO \rightarrow MA(f)$ $PH \rightarrow HQ(c)$ $PH \rightarrow PHCO(c)$ $PH \rightarrow CA(c)$ $CA \rightarrow THB(c)$ $HQ \rightarrow HQCO(c)$	EH: BZ UR: CA HQ MA PHCO PMA	Metabolism assigned to liver and marrow (4% of liver)
Majumdar et al. 2016	Human	Inhalation	FA, LI, LU, RP, SP	LI: $BZ \rightarrow M_{tot}(c)$	UR: M_{tot} MA	All metabolism is assigned to the liver
Medinsky et al. 1989a, 1989b, 1989c	Human, mouse, rat	Inhalation, oral	FA, LI, LU, RP, SP	LI: $BZ \rightarrow BO(c)$ $BO \rightarrow PHCO(c)$ $BO \rightarrow PMA(c)$ $BO \rightarrow HQCO(c)$ $BO \rightarrow MA(c)$	EH: BZ	All metabolism is assigned to the liver

Table 3-1. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

	Simulated areas					
Reference	Species	Absorption pathways	Tissues[a]	Metabolic pathways[b]	Excretion pathways[c]	Comment
Sinclair et al. 1999	Human	Inhalation, oral, dermal	BL, BM, LI, LU, MU, RP	BM, LI: BZ→M_{tot}(c)	EH: BZ UR: M_{tot} PH	Simulates dermal exposure and absorption
Sun et al. 1990	Mouse, rat	Inhalation, oral	BL, FA, LI, LU, RBC, RP, SP	LI: BZ→BO(c) BO→PHCO(c) BO→PMA(c) BO→HQCO(c) BO→MA(c) RBC: BO→HBA(c,f)	EH: BZ	Simulates formation of hemoglobin adducts in RBCs derived from benzene oxide
Travis et al. 1990	Human, mouse, rat	Inhalation, oral	BL, BM, FA, LI, LU, MU, RP	BM, LI: BZ→M_{tot}(c)	EH: BZ	Total metabolism of benzene in the bone marrow and liver

[a]Tissues simulated: BL = blood; BM = bone marrow; FA = fat; GI = gastrointestinal; LI = liver; LU = lung; MU = muscle; RBC = red blood cells; RP = other rapidly-perfused tissues; SP = other slowly-perfused tissues; UB = urinary bladder.
[b]Metabolic pathways simulated: BG = benzene glycol; BO = benzene oxide; BZ = benzene; CA = catechol; DI = diols; GSH = glutathione; HBA = hemoglobin adduct; HQ = hydroquinone; HQCO = hydroquinone conjugates; MA = muconic acid; M_{tot} = total metabolites; PH = phenol; PHCO = phenol conjugates; PMA = phenylmercapturic acid; PHX_{end} = endogenous phenolic metabolites; THB = trihydroxybenzene; (c) = capacity-limited; (f) = first-order; (z) = zero-order.
[c]Excretion pathways simulated: EH = exhalation; MI = breast milk; UR = urine.

Figure 3-2. General Structure of Physiologically Based Pharmacokinetic Models of Benzene*



*Tissues shown with dashed lines are not simulated in all models. Flow-limited exchange of benzene between blood and tissues is assumed. Metabolism is simulated to varying degrees of complexity (Table 3-1).

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

for formation and excretion of metabolites is simulated, allowing predictions of metabolite levels in tissues. All of the models use typical parameters and values for species-specific blood flows and tissue volumes.

Brief summaries of the models presented in Table 3-1 are provided below, with emphasis on unique features that are applicable to risk assessment.

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

Description of the Model. The Medinsky et al. (1989a, 1989b, 1989c) model simulates absorption and disposition of benzene in the human, mouse, and rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process; absorption and excretion of benzene in the lung are assumed to be flow-limited. Exchange of benzene between blood and tissues is assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of benzene to benzene oxide as a function of the concentration of benzene in liver. Conversion of benzene oxide to phenol conjugates, phenylmercapturic acid, hydroquinone conjugates, and muconic acid are simulated as parallel, capacity-limited reactions in liver. The model simulates rates of formation of metabolites, but not the disposition (e.g., excretion) of metabolites. Metabolism parameter values (V_{max}, K_m) for the mouse and rat models were estimated by optimization of the model to observations of total metabolites formed in mice and rats exposed by inhalation or oral routes to benzene (Medinsky et al. 1989b; Sabourin et al. 1987). Human metabolism parameter values were derived from allometric scaling of the values for mice (Medinsky et al. 1989c).

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

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

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

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

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

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

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

Sun et al. 1990

Description of the Model. The Sun et al. (1990) model is an extension of the mouse and rat models developed by Medinsky et al. (1989a, 1989b, 1989c). The Sun et al. (1990) model includes a simulation of the formation of hemoglobin adducts derived from benzene oxide. Adduct formation is represented as

the sum of capacity-limited and first-order functions of the concentration of benzene oxide in the liver. Parameter values were estimated by optimization to measurements of hemoglobin adduct formations in rats and mice exposed to single gavage doses of benzene (Sun et al. 1990).

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

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

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

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

High-low Dose Extrapolation. The model was calibrated with observations made in mice and rats exposed to single gavage doses of 0.1–10,000 μmol/kg (0.008–800 mg/kg) and evaluated for predicting observations in mice and rats exposed by inhalation to 600 ppm benzene.

Inter-route Extrapolation. The model examined two routes of exposure: oral and inhalation. The model was found to be useful in predicting the concentrations of hemoglobin adducts in blood in rodents after oral and inhalation exposure.

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

Travis et al. 1990

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

Risk Assessment. This model has been used to predict the amounts of benzene in expired air, concentrations of benzene in blood, and total amount of benzene metabolized following inhalation exposures to humans and inhalation, intraperitoneal, gavage, or subcutaneous exposures in mice or rats (Travis et al. 1990). Cox (1996) applied the model to derive internal dose-response relationships for benzene in humans.

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

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

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

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

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

Strengths and Limitations. Strengths of the model are that it simulates: (1) disposition of inhaled and ingested (single dose) benzene in mice, rats, and humans; and (2) concentrations of benzene, and rates and amount of benzene metabolized in bone marrow, a target tissue for benzene metabolites. Limitations of the model include: (1) the model simulates metabolic elimination of benzene, but not the rates of formation of major metabolites; and (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues.

Fisher et al. 1997

Description of the Model. The Fisher et al. (1997) model extends the model reported by Travis et al. (1990) to include a simulation of lactational transfer of benzene to breast milk in humans. Other tissues simulated include blood, fat, liver, lung, other slowly-perfused tissues, and rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited, as is excretion of benzene in breast milk. The lactational transfer model includes simulations of breast milk production and loss from nursing; the latter is represented as a first-order process. Estimates of blood:air and blood:milk partition coefficients during lactation (from which the milk:blood partition coefficient could be calculated) were measured in nine lactating subjects (Fisher

et al. 1997). The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites and their disposition (e.g., excretion) are not simulated. Metabolism parameter values (K_m,V_{max}) and tissue:blood partition coefficients were derived from Travis et al. (1990).

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

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

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

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

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

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

Strengths and Limitations. Strengths of the model are that it simulates the disposition of inhaled benzene in females during lactation, including transfers of benzene to breast milk and nursing infants; concentrations of benzene in blood and tissues; and rates of elimination of benzene metabolites.

Limitations of the model include that the model does not simulate rates of formation of major metabolites and that the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Sinclair et al. 1999

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

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

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

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

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

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

Inter-route Extrapolation. The model simulates inhalation, oral, and dermal exposures.

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

Bois et al. 1991a

Description of the Model. The Bois et al. (1991a) model simulates absorption and disposition of benzene and the benzene metabolite, phenol, in the rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene and phenol are simulated as a first-order function for dose. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene and phenol between blood and tissues. Excretion of phenol is simulated as a first-order transfer to urine. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in bone marrow, liver, gastrointestinal tract, and respiratory tract (see Table 3-1). All pathways are assumed to be capacity-limited reactions, except for the spontaneous hydrolysis of benzene oxide to form phenol, which is simulated as a first-order process. The model simulates rates of formation of metabolites and first-order excretion of phenol; however, disposition (e.g., excretion) of other metabolites is not simulated. Parameter values, including metabolism parameter values, were optimized to a reference set of observations of metabolites formed in rats exposed by inhalation or to single gavage doses of benzene (see below).

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

Validation of the Model. The model was calibrated (Bois and Paxman 1992; Bois et al. 1991a) with observations made in rats exposed to single gavage doses of benzene, or to inhalation exposures of 13–870 ppm (Sabourin et al. 1987, 1989b), in rats administered single parenteral doses of phenol (Cassidy

and Houston 1984), and in *in vitro* metabolism studies (Sawahata and Neal 1983). Further evaluations against data not used in the calibration were not reported.

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

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

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

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

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

Bois et al. 1996

Description of the Model. The Bois et al. (1996) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates metabolic elimination of benzene as a single capacity-limited (i.e., Michaelis-Menten) reaction, occurring in bone marrow and liver. Endogenous formation of phenolic metabolites is also simulated as a zero-

order process occurring in liver. The model simulates first-order excretion of total metabolites and the phenol fraction (approximately 80% of total). Parameter values (physiological and chemical) were estimated by Bayesian optimization techniques (Markov Chain Monte Carlo analysis) using reference observations of benzene concentration in blood and urinary excretion of phenol in human subjects who were exposed to benzene in air (Pekari et al. 1992).

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

Validation of the Model. The model was calibrated with observations of benzene concentrations in blood and urinary phenol levels, made in three human subjects who were exposed to 1.7 or 10 ppm benzene for 4 hours (Pekari et al. 1992). Further evaluations against data not used in the calibration have not been reported.

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

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

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

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

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

Brown et al. 1998

Description of the Model. The Brown et al. (1998) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites, or their disposition (e.g., excretion), are not simulated. For the purpose of comparing model predictions to observations, 80% of the total metabolites formed and excreted in urine (i.e., amount of benzene eliminated by metabolism) in 24 hours was assumed to be phenol. The K_m parameter for metabolism was derived from Travis et al. 1990; the V_{max} was estimated by optimization of the model to observations of blood concentrations of benzene and benzene in exhaled breath of female and male subjects who were exposed to 25 ppm benzene for 2 hours (Sato et al. 1975). Partition coefficients for males and females were derived from vial equilibrium studies conducted on blood and/or tissues from males and females (Fisher et al. 1997; Paterson and Mackay 1989).

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

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

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

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

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

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

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

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled benzene in female and male humans as well as the concentrations of benzene and rates of elimination of benzene metabolites. Limitations of the model include: (1) the model does not simulate rates of formation of major benzene metabolites; (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues; and (3) the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Cole et al. 2001

Description of the Model. The Cole et al. (2001) model simulates absorption and disposition of benzene in the mouse. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in liver (see Table 3-1). Capacity-limited reactions in bone marrow and liver include benzene to benzene oxide, phenol to hydroquinone, phenol to catechol, catechol to trihydroxybenzene, and conjugation of phenol and hydroquinone. First-order reactions in liver include conversion of benzene oxide to phenol, muconic acid, and phenylmercapturic acid. The model simulates rates of formation of metabolites, tissue distribution of benzene oxide, phenol, and hydroquinone; and first-order excretion of metabolites in urine. Capacity-limited metabolism

parameter values were estimated from *in vitro* studies of mouse liver (Lovern et al. 1999; Nedelcheva et al. 1999; Seaton et al. 1995); first-order parameters were estimated by optimization of model output to observations of metabolites formed in mice exposed by inhalation or to single gavage doses (Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988). Blood:tissue partition coefficients for benzene and metabolites were derived from Medinsky et al. (1989a) or estimated based on the n-octanol-water partition coefficient (Poulin and Krishnan 1995).

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

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

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

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

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

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

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested benzene, including rates and amounts of major metabolites. Most of the metabolism parameter

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

Three studies have expanded or enhanced the Cole et al. (2001) mouse PBPK model (Knutsen et al. 2013a, 2013b; Manning et al. 2010; Yokley et al. 2006). Yokley et al. (2006) estimated parameter values for humans, including human population distributions for several metabolism parameters. Knutsen et al. (2013a, 2013b) expanded the Yokley et al. (2006) human model to include two additional compartments representing bone marrow and urinary bladder. This enabled dosimetry predictions for benzene and metabolites in bone marrow and provided a compartment for simulating background levels (e.g., pre-exposure) of benzene metabolite conjugates in urine. Manning et al. (2010) extended the Cole et al. (2001) mouse model to include a kidney compartment and subdivided the liver compartment into three zones to represent heterogeneous distribution of enzymes that participate in the production of benzene metabolites.

Yokley et al. (2006)

Yokley et al. (2006) estimated human population distributions of metabolism parameters for the Cole et al. (2001) model. Parameters evaluated included the specific activity of CYP2E1 in liver (V2E1), maximum rates of conjugation of phenol (VPH1, VPH2) and hydroquinone (VHQ), and first-order clearances for formation of phenylmercapturic acid (k3) and muconic acid (k4) from benzene oxide. Data from *in vitro* studies of human liver tissue were used to establish prior log-normal distributions for V2E1, VPH1, VPH2, and VHQ. Parameters k3 and k4 were assigned log-normal prior distributions with means equal to the mouse model values and SDs of 0.1 and 2, respectively. Posterior distributions were computed from Markov Chain Monte Carlo simulations with calibration data from human subjects. These data included a clinical study in which three subjects were exposed to 1.7 or 10 ppm benzene for 4 hours and benzene in blood and exhaled air were measured (Pekari et al. 1992), and an occupational study in which benzene metabolites in urine were monitored in 35 workers who were exposed to 25 ppm benzene during their work shifts (Rothman et al. 1998; Waidyanatha et al. 2004). The population model predicted the observed variability in blood benzene and exhaled benzene and urinary levels of muconic acid, phenylmercapturic acid, phenol, and hydroquinone; however, it underpredicted urinary catechol and benzenetriol levels.

Manning et al. (2010)

Manning et al. (2010) developed a PBPK model of benzene and its major metabolites benzene oxide, phenol, and hydroquinone. The model is an extension of the Cole et al. (2001) model, with the addition of a kidney compartment, and expansion of the liver compartment to include three sub-compartments. The three liver compartments were included in the model to simulate the heterogeneous distribution of CYP2E1 and sulfotransferases. CYP2E1 is more strongly expressed in the pericentral region of the liver and sulfotransferases are more strongly expressed in the periportal region of the liver (Ingelman-Sundberg et al. 1988; Tsutsumi et al. 1989). This heterogeneous, or zonal, distribution of enzymes is thought to give rise to different metabolic patterns following an external dose of benzene or phenol (Hoffmann et al. 1999; Koop et al. 1989). Following an external dose of phenol, sulfotransferases in the periportal region of the liver convert a large fraction of the absorbed dose to sulfate esters before it can be delivered to the pericentral region of the liver where it can be metabolized to benzene oxide through the CYP2E1 pathway and to downstream metabolites, including hydroquinone. Following an external dose of benzene, phenol is formed in the pericentral region of the liver and, as a result, a larger fraction of the benzene dose is converted to hydroquinone.

The three-compartment liver model has flow-limited transfer of chemical from blood to liver compartment 1, representing the periportal region, through an intermediate compartment 2, to compartment 3, representing the pericentral region. Each compartment is assumed to comprise one-third of the total volume of the liver. Activities of hepatic sulfotransferase and glucuronyltransferase are assigned to compartment 1, whereas CYP2E1, epoxide hydrolase, and GST activities are assigned to compartment 3. Non-enzymatic conversion of benzene oxide to phenol is assumed to occur in all three compartments. Sulfate and glucuronic acid conjugates of phenol and hydroquinone are formed in liver compartment 1. Metabolites formed in compartment 3 include the CYP2E1 metabolites benzene oxide (from phenol), hydroquinone and catechol (from phenol), and benzenetriol (from hydroquinone), the epoxide hydrolase metabolite of benzene oxide (muconic acid), and the GST metabolite of benzene oxide (phenyl-mercapturic acid). The kidney compartment is assigned 10% of the CYP2E1 activity relative to the liver. Formation of phenylmercapturic acid is assumed to occur in blood, kidney, fat, slowly perfused tissue, and rapidly perfused tissues.

Tissue/blood partition coefficients for benzene metabolites, phenol and hydroquinone, were estimated from physical-chemical properties (Poulin and Krishnan 1995). Phenol and hydroquinone were assumed to bind in all tissues. Binding was represented in the model with first-order clearance terms, which were

optimized. Information regarding tissue/blood partition coefficients for benzene was taken from the literature (Medinsky et al. 1989a). The partition coefficients for benzene were used for benzene oxide. Parameters governing CYP2E1 and conjugation rates (Km, Vmax) were scaled to the whole liver from estimates made in *in vitro* studies (Lovern et al. 1999; Seaton et al. 1995). First-order clearances for GST-mediated formation of phenylmercapturic acid and epoxide hydrolase-mediated formation of muconic acid were optimized.

Data used in optimizing the model were derived from benzene oral dosing studies (Henderson et al. 1989; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1987) and inhalation studies (Sabourin et al. 1988) conducted in mice. The introduction of three liver compartments to account for zonal distribution of metabolism improved some aspects of performance of the model at predicting dose-dependent metabolism of benzene. For example, it improved agreement between observed and predicted benzene concentrations in liver and phenol concentrations in blood following inhalation of benzene, and predictions of formation of phenol and hydroquinone conjugates following oral dosing with benzene.

Knutsen et al. (2013a, 2013b)

Knutsen et al. (2013a, 2013b) expanded the Yokley et al. (2006) human model to include two additional compartments representing bone marrow and urinary bladder. Saturable metabolism was assumed for the formation of benzene oxide from benzene, hydroquinone and catechol from phenol, benzenetriol from catechol and hydroquinone, and conjugates. Bone marrow is assumed to oxidize to benzene oxide at approximately 4% of the hepatic maximal rate. Maximal rates for all other saturable conversions in liver and bone marrow (per mg tissue protein) were assumed to be proportional to tissue masses. First-order metabolic clearance was assumed for formation of muconic acid and phenylmercapturic acid from benzene oxide and phenol from benzene oxide, with first-order clearance rates identical in liver and bone marrow. The bladder compartment receives conjugated metabolites resulting from exposure to benzene and was assigned values for background levels of metabolites expected in the absence of exposure to benzene. Background levels of urinary metabolites were assigned values based on measurements made in humans who were not exposed to benzene (Waidyanatha et al. 2004).

Metabolism parameters for CYP2E1 were assigned initial values from quantitative studies of human liver CYP2E1 (Lipscomb et al. 2003a, 2003b) and all metabolism parameters were calibrated to achieve agreement with measurements of urinary benzene metabolites in workers exposed to benzene during the work shift (Waidyanatha et al. 2004). The calibrated model was validated by comparing observed levels

of benzene in blood and exhaled air in three subjects who inhaled benzene (1.9 and 9.4 ppm) for 4 hours (Pekari et al. 1992) and observed and predicted urinary metabolite levels measured in workers exposed to benzene (Kim et al. 2006a).

In comparison to the Yokley et al. (2006) human model, calibration of the Knutsen et al. (2013a, 2013b) model resulted in lower values for the maximal rate of metabolism of phenol and first-order clearance of benzene to benzene oxide and higher values for first-order clearance of phenol to catechol and hydroquinone to benzenetriol. Good agreement was achieved between observed and predicted levels of benzene in blood and exhaled air, and benzene metabolites in urine (Kim et al. 2006a; Pekari et al. 1992). Knutsen et al. (2013a, 2013b) did not report the sensitivity of these predictions (exhaled benzene or urinary metabolites) to model parameters that govern metabolite doses to marrow. Therefore, it is possible that the model could reliably simulate exhaled benzene and urinary metabolites while not reliably predicting metabolite doses to marrow. A contributor to this uncertainty is the relatively small contribution of marrow to benzene metabolism (marrow metabolism is assumed to be 4% of liver metabolism). The model was used to compare predicted blood and bone marrow metabolite exposures resulting from an 8-hour exposure to air concentrations of benzene of 5–100 ppm. The total metabolites formed (24-hour AUC) were higher in blood compared to bone marrow. Both compartments exhibited saturation kinetics, with saturation in bone marrow predicted at lower exposures. The model is configured to simulate kinetics following an inhalation exposure; there is no gastrointestinal tract compartment for simulating oral exposure.

Majumdar et al. (2016)

Majumdar et al. (2016) modified a PBPK model of tetrachloroethylene (Bernillon and Bois 2000) to create a human PBPK model for benzene. The compartment structure is identical to the Cole et al. (2001) model and includes lung, liver, adipose, and lumped compartments representing other slowly perfused tissue and rapidly perfused tissues. The model simulates capacity-limited metabolism of benzene (V_{max}, K_m) in liver. Excretion of total metabolites in urine is simulated as a fist-order function (minute^{-1}) of the concentration of total metabolites formed in liver. Urinary *trans*,*trans*-muconic acid is calculated as a fraction of total amount of metabolites excreted in urine. The sources for the benzene parameters (e.g., partition coefficients and metabolism) are reported in Table 1 of Majumdar et al. (2016). Comparisons of model predictions to observations were not reported. Majumdar et al. (2016) applied the model to predict the benzene body burden associated with measured levels of exposure and urinary *trans*,*trans*-muconic acid levels, and corresponding cancer risks, in a groups of petrol pump attendants and automobile drivers.

Pech et al. (2023a, 2023b) applied the Majumdrar et al. (2016) model to estimate benzene exposures from urinary *trans*,*trans*-muconic acid measurements, and corresponding cancer and noncancer risks, in children who resided in homes that also served as shoe-making workshops.

3.1.6 Animal-to-Human Extrapolations

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

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

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

PBPK models have been explored for applications of animal-to-human extrapolations of benzene dosimetry (Bois et al. 1991a, 1996; Cole et al. 2001; Medinsky 1995; Medinsky et al. 1989a, 1989b, 1989c; Travis et al. 1990). Each model simulates benzene metabolism in multiple compartments, including production of hydroquinone and muconaldehyde in the liver, with further metabolism in the

bone marrow. However, the models are not sufficiently refined to allow confident predictions of the kinetics of benzene metabolism in humans.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to benzene are discussed in Section 5.7, Populations with Potentially High Exposures.

Several factors may contribute to alterations in the toxicity of benzene. These include age-related differences, genetic polymorphisms, and underlying conditions, as discussed below.

Age-Related Differences. The adverse health effects of benzene are due to reactive metabolites. At early stages of human development, metabolic pathways may not be fully functional, which might result in a lower level of susceptibility to benzene. In the elderly, metabolic pathways become less functional, which may lead to lower susceptibility. No clear evidence of age-related differences in susceptibility to benzene toxicity was located. Fetuses may be exposed as benzene crosses the placenta and is found in cord blood at concentrations that equal or exceed those of maternal blood (Dowty et al. 1976). In a study of rats exposed to 20 ppm benzene on GD 1–15, circulating erythroid precursors decreased and granulocytic precursor cells increased in neonates and 6-week-old pups (Keller and Snyder 1988). However, no information is available on effects in offspring of humans exposed to benzene *in utero*. In addition, nursing infants can be exposed to benzene in the breast milk (Fabietti et al. 2004).

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

As discussed in Section 2.17, studies in animals have identified several developmental effects following gestational exposure. These effects include decreased fetal weight, increased skeletal variations, alterations in hematological parameters, neurodevelopmental effects, and altered glucose homeostasis. Due to the very limited data in humans, 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.

Sex-related Differences. Studies in humans found that the elimination of benzene is slower in women than in men, likely due to the higher percentage and distribution of body fat tissue (Sato et al. 1975). On the other hand, an association between urinary benzene metabolite levels and insulin resistance in elderly adults demonstrated a stronger relationship in men than in women (Choi et al. 2014). Furthermore, sex differences were observed upon occupational benzene exposure, particularly effects related to biotransformation of benzene to *trans*,*trans*-muconic acid and hematological parameters (Moro et al. 2017).

Genetic Polymorphisms. More recent studies of workers indicate that susceptibility to benzene-induced toxicity and genetic damage may be associated with polymorphisms in multiple genes. Information in workers was identified for polymorphisms in genes encoding for enzymes involved in benzene metabolism, DNA repair enzymes, and cytokines. The studies discussed below have evaluated associations between genetic polymorphisms and effects in workers. Results indicate that various subpopulations based on polymorphisms may be more susceptible to benzene-induced toxicity.

Polymorphisms of enzymes involved in benzene metabolism. Genetic polymorphisms exist for several genes encoding for enzymes involved in the metabolism of benzene. As discussed below, studies in workers have assessed the effects of polymorphisms on benzene toxicity for genes encoding for NQO1, GST, epoxide hydrolase, and MPO (De Palma and Manno, 2014).

The flavoenzyme, NQO1, catalyzes the reduction of 1,2- and 1,4-benzoquinone (reactive metabolites of benzene) to catechol and hydroquinone, respectively (Nebert et al. 2002), thus protecting cells from oxidative damage by preventing redox cycling. The NQO1*1 (wild-type) allele codes for normal NQO1 enzyme and activity. An NQO1*2 allele encodes a nonsynonymous mutation that has negligible NQO1 activity (NQO1 null). Approximately 5% of Caucasians and African Americans, 16% of Mexican-Americans, and 18–20% of Asians are homozygous for the NQO1*2 allele (Kelsey et al. 1997; Smith and Zhang 1998). Rothman et al. (1997) evaluated the relationship of NQO1 polymorphism on hematotoxicity in a case-control study of 50 benzene-exposed workers and 50 controls in China. Results indicate that workers with the NQO1*2 allele were at an increased risk of hematotoxicity. Similar results were observed in a cross-sectional study of 250 shoe factory workers in China, with lower leukocyte counts observed in NQO1 null workers compared to NQO1 wild-type workers (Lan et al. 2004a). In a study of a population of Bulgarian petrochemical workers (i.e., 158 and 50 controls; 208 total individuals), benzene exposure in workers with the NQO1-null allele showed an increased frequency of DNA single-strand breaks, compared to controls (Garte et al. 2008).

Glutathione-S-transferases (GSTs), which are involved in benzene oxide metabolism to the less toxic form PhMA, have several genotypes. In a cross-sectional study, Nourozi et al. (2018) assessed how positive (e.g., wild-type) and null polymorphisms of glutathione genotypes GSTP1, GSTT1, and GSTM1 may alter the hematological effect of benzene in a cross-sectional study of 124 workers and 184 controls at a petrochemical plant in Iran. Results showed that workers with GSTT1-null genotype and combined GSTT1-null and GSTM1-null genotypes had an increased risk of hematological effects. Garte et al. (2008) reported an increased frequency of DNA single-strand breaks in 158 Bulgarian petrochemical workers with GSTT1 and GSMT1 variants.

Epoxide hydrolase (EH) plays an important role in benzene metabolism by converting benzene derived epoxides to more water-soluble derivatives that are less toxic. Thus, EH-null phenotypes that result in decreased conversion of toxic epoxides have the potential to cause increased hematotoxicity. A case-control study of 268 workers with benzene hematotoxicity and 268 without hematotoxicity evaluated associations with epoxide hydrolase polymorphisms (Sun et al. 2007). EH-null haplotypes 2 (with a

AGAC substitution), 4 (with a GAGT substitution), and 6 (with a GGGT substitution) were associated with an increased risk of hematotoxicity.

In the benzene metabolism scheme, MPO catalyzes the oxidation of phenol to the reactive metabolites, catechol and hydroquinone. Polymorphisms of MPO that result in a decrease in this reaction could decrease the toxicity of benzene. In a study of 250 shoe factory workers in China, workers expressing the MPO-null gene had an increased risk of hematotoxicity (Lan et al. 2004a). Lower leukocyte counts were observed in MPO-null workers compared to MPO wild-type workers.

Cytokines. Several cytokines, chemokines, and cellular adhesion molecules are involved in hematopoiesis. Thus, polymorphisms for the genes encoding for these molecules have the potential to alter hematotoxicity of benzene. Lan et al. (2005) evaluated associations between several of these molecules, including several interleukins (IL) and vascular cell adhesion molecule 1 (VCAM1), and cell counts for granulocytes, lymphocytes, and $CD4^+$ T-cells in a cross-sectional study of 250 exposed shoe workers and 140 controls in China. The following inverse associations were observed between cell counts and polymorphisms: total lymphocyte counts and IL-4, IL-12A, and VCAM1; granulocyte counts and IL-1A, IL-4, IL-10, CSF3 $CD4^+$ and $CD8^+$, T-cells, and VCAM1.

DNA repair enzymes. Several enzymes are involved in the repair of oxidative damage to DNA, producing increased formation of the damaged DNA product, 7,8-dihydro-8-oxoguanine (8-oxoG). In humans, three enzymes are involved in the repair of this damage: hMTH1, hOGG1, and hMYH genes. Thus, genetic polymorphisms of these enzymes could alter susceptibility to the effects of benzene. A cross-sectional study evaluated polymorphisms of the genes for three repair enzymes, hMTH1, hOGG1, and hMYH in 152 chronic benzene poisoning patients and 152 healthy workers exposed to benzene (Wu et al. 2008). In the population, polymorphisms of hMTH1 and hMYH were associated with an increased risk of toxicity (depression of peripheral leukocyte counts).

Underlying Health Conditions. Individuals with medical conditions that include reduced bone marrow function, decreased blood factors, or low blood cell counts would be at increased risk for benzene toxicity. Treatments for certain medical conditions might result in decreases in blood cell counts, which could lead to increased susceptibility to benzene poisoning. Specific studies evaluating how benzene may affect patients with underlying hematopoietic diseases or conditions were not identified. However, it is hypothesized that individuals with underlying hematopoietic conditions or diseases would be at increased risk. For example, suppression of immune cells is a well-established effect of numerous cancer

treatments. Benzene exposure in immunosuppressed patients would likely increase susceptibility for benzene-induced hematotoxicity. In addition, individuals with anemia may be more susceptible to the hematopoietic effects of benzene.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 2006).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for benzene from this report are discussed in Section 5.6, General Population Exposure.

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 2006). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to benzene are discussed in Section 3.3.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 2006). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by benzene are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the

biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Several biomarkers have been identified to demonstrate exposure to benzene. Unmetabolized benzene can be detected in the expired air and urine of humans exposed to benzene vapors (Farmer et al. 2005; Fustinoni et al. 2005; Sherwood 1988; Waidyanatha et al. 2001). In addition to unmetabolized benzene, urinary metabolites of benzene, including phenol, *trans,trans*-muconic acid (or urinary *trans,trans*-muconic acid, also reported as t,t-MA), and S-phenyl mercapturic acid (PhMA or urinary PhMA, also reported as SPMA), are commonly used as biomarkers of exposure (Boogard et al. 2022; Chaiklieng et al. 2021; Daugheri et al. 2022; Lovreglio et al. 2011). Urinary pre-PhMA has recently been assessed as a biomarker for benzene exposure (Bowman et al. 2023). Historically, urinary phenol was most often used to monitor benzene exposure; however, it is not specific for exposure to benzene and is a metabolic product of other chemicals (Astier 1992; Inoue et al. 1986, 1988; Karacic et al. 1987; Pekari et al. 1992). Urinary benzene and PhMA are specific biomarkers for benzene exposure. *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). The Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) reports benzene levels in blood (CDC 2022a).

According to IARC (2018), current practice is to use urinary markers that are more specific for benzene (unmetabolized benzene, *trans,trans*-muconic acid (t.t-MA), and PhMA) than phenol, although they are typically at lower urinary concentrations. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends using urinary *trans,trans*-muconic acid and PhMA to monitor benzene exposure to workers (ACGIH 2019). Urinary levels of PhMA have been correlated with occupational exposure to benzene (Boogaard and van Sittert 1996; Farmer et al. 2005; Inoue et al. 2000; Qu et al. 2005). Significant exposure-response trends for urinary *trans,trans*-muconic acid and PhMA levels have been demonstrated in occupationally exposed subjects at exposure levels of ≤1 ppm (Qu et al. 2005).

3.3.2 Biomarkers of Effect

The most sensitive effects of benzene exposures are hematotoxicity, immunotoxicity, and leukemia. While these effects are considered hallmark effects of benzene poisoning and occupational exposure, they

are not unique to benzene, as exposure to other chemicals (e.g., toluene) and medical conditions can produce similar effects. Therefore, the occurrence of these effects should not be interpreted as confirmatory evidence for benzene exposure. However, these effects taken in conjunction with known benzene exposure may be considered as biomarkers of effect.

In addition to using levels of urinary benzene and benzene metabolites for monitoring purposes (discussed in Section 3.3.1), various biological indices might also be helpful in characterizing the effects of exposure to benzene. The monitoring for benzene exposure may best be accomplished by using a series of biomarkers of effect with correlation of the results. Decreases in leukocyte counts have been used as an indicator of occupational benzene exposures. DNA adducts with benzene metabolites, chromosomal aberrations in bone marrow and peripheral blood lymphocytes, and sister chromatid exchanges could be used to monitor for benzene effects (IARC 2018; McHale et al. 2012). However, other than the formation of DNA adducts with benzene metabolites, these biomarkers are not specific to benzene exposure.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Studies have been conducted on the interaction of benzene with other chemicals, both *in vivo* and in the environment. Benzene metabolism is complex, and numerous xenobiotics can induce or inhibit specific routes of detoxification and/or activation in addition to altering the rate of benzene metabolism and clearance from the body. Phenol, hydroquinone, benzoquinone, and catechol have been shown to induce CYPs in human hematopoietic stem cells (Henschler and Glatt 1995). Therefore, exposure to chemicals that stimulate the activity of this enzyme system prior to exposure to benzene could increase the rate of benzene metabolism. As discussed below, toluene, Aroclor 1254, phenobarbital, acetone, and ethanol are known to alter the metabolism and toxicity of benzene. Interactions reported in *in vivo* studies occurred at benzene exposure levels higher than those likely be encountered near hazardous waste sites.

Benzene, toluene, ethylbenzene, and xylenes (BTEX) frequently occur together at hazardous waste sites; therefore, ATSDR (2004) evaluated potential interactions of this common mixture. Based on predictions from PBPK models and data from binary mixtures, joint neurotoxic action is expected for BTEX mixtures (or ternary or binary mixtures therein). Data were not adequate to predict interactions within this mixture for other health effects.

Pretreatment of mice with CYP inhibitors (toluene, propylene glycol, β-diethyl amino ethyl diphenyl propyl acetate hydrogen chloride [SKF-525A]) has been demonstrated to reduce both benzene metabolite

formation (Andrews et al. 1977; Gill et al. 1979; Ikeda et al. 1972; Tuo et al. 1996) and resulting genotoxicity in mice (Tuo et al. 1996). Pretreatment with CYP inducers (3-methylcholanthrene and β-naphthoflavone) increased both benzene metabolism and benzene clastogenicity (Gad-El-Karim et al. 1986).

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

Other chemicals that induce specific isoenzymes of CYP can increase the rate of benzene metabolism and may alter metabolism pathways favoring one over another. Ikeda and Ohtsuji (1971) presented evidence that benzene hydroxylation was stimulated when rats were pretreated with phenobarbital and then exposed to 1,000 ppm of benzene vapor for 8 hours/day, 6 days/week for 2 weeks. Rats exposed to phenobarbital showed no effects on the metabolism of micromolar amounts (35–112.8 μmol) of benzene *in vitro* (Nakajima et al. 1985). Phenobarbital pretreatment of the rats alleviated the suppressive effect of toluene on benzene hydroxylation by the induction of oxidative activities in the liver (Nakajima et al. 1985).

Co-administration of toluene inhibited the biotransformation of benzene to phenol in rats (Ikeda et al. 1972; Inoue et al. 1988). This was due to competitive inhibition of the oxidation mechanisms involved in the metabolism of benzene. Coexposure of mice to benzene and toluene resulted in higher frequency of

micronuclei in polychromatic erythrocytes compared to exposure to benzene or toluene alone (Bird et al. 2010; Wetmore et al. 2008).

Mathematical models of benzene and phenol metabolism suggest that the inhibition by benzene of phenol metabolism, and by phenol on benzene metabolism, occurs through competition for a common reaction site, which can also bind catechol and hydroquinone (Purcell et al. 1990; Schlosser et al. 1993). Flavonoids have been shown to inhibit phenol hydroxylase or increase phenol hydroxylase activity in a dose-dependent manner, dependent on the oxidation potential of the flavonoid (Hendrickson et al. 1994).

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

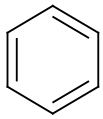
Li et al. (2009b) subjected groups of mice to intratracheal instillation of either benzene or carbon nanotubes or combined instillation of benzene and carbon nanotubes. Combined instillation resulted in considerably more severe histopathological pulmonary toxicity than that observed in mice exposed to benzene or carbon nanotubes alone.

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of benzene is presented in Table 4-1. Although the term benzol is found in older literature and in Europe for the commercial product (Folkins 2012), benzene is the name presently used by the International Union of Pure and Applied Chemistry (IUPAC) and the American Society for Testing and Materials (ASTM) for the pure product (Fruscella 2002; NLM 2023).

Table 4-1. Chemical Identity of Benzene

Characteristic	Information	Reference
Chemical name	Benzene	NLM 2023
Synonym(s)	[6] Annulene, benzeen (Dutch), benzen (Polish), benzol, benzole; benzolo (Italian), coal naphtha, cyclohexatriene, fenzen (Czech), phene, phenyl hydride, pyrobenzol, pyrobenzole	NLM 2023
Registered trade name(s)	Polystream	IARC 1982
Chemical formula	C_6H_6	NLM 2023
SMILES	C1=CC=CC=C1	NLM 2023
Chemical structure		NLM 2023
CAS Registry Number	71-43-2	NLM 2023

CAS = Chemical Abstracts Service; SMILES = simplified molecular-input line-entry system

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Benzene is a flammable organic compound with a petroleum-like odor. It is formed from human activities and by natural processes. Benzene is slightly soluble in water and evaporates rapidly into air. Information regarding the physical and chemical properties of benzene is presented in Table 4-2. The major impurities found in commercial products are toluene, xylene, phenol, thiophene, carbon disulfide, acetylnitrile, and pyridine (NIOSH 1974). Commercially refined benzene-535 is free of hydrogen sulfide and sulfur dioxide but contains a maximum of 1 ppm thiophene and a maximum of 0.15% nonaromatics (Fruscella 2002). Benzene is also commercially available as nitration-grade (99% pure), thiophene-free, 99 mole%, 99.94 mole%, and nanograde quality (NLM 2023).

Table 4-2. Physical and Chemical Properties of Benzene

Property	Information	Reference
Molecular weight	78.11 g/mol	Budavari et al. 2001
Color	Clear, colorless liquid	Budavari et al. 2001
Physical state	Colorless to light yellow liquid	NLM 2023
Melting point	5.558°C	NLM 2023
Boiling point	80.08°C	NLM 2023
Density at 20°C, g/cm^3	0.8756	NLM 2023
Odor	Aromatic (petroleum-like)	NFPA 1994; NLM 2023
Odor threshold:		
Water	2.0 mg/L	NLM 2023
Air[a]	Detection range: 34–119 ppm (geometric mean: 61 ppm) Recognition: 97 ppm	AIHA 1989
Taste threshold	0.5–4.5 mg/L	NLM 2023
Solubility:		
Water at 25°C	w/w: 1,790 mg/L	NLM 2023
Organic solvent(s)	Alcohol, chloroform, ether, carbon disulfide, acetone, oils, carbon, tetrachloride, glacial acetic acid	Budavari et al. 2001
Partition coefficients:		NLM 2023; Karickhoff 1981; Kenaga 1980
Log K_{ow}	2.13	
Log K_{oc}	1.8–1.9	
Vapor pressure at 25°C	94.8 mm Hg	NLM 2023
Henry's law constant at 25°C	5.5x10^{-3} atm-m^3/mol	Mackay and Leinonen 1975
Autoignition temperature	498°C	NFPA 1994
Flashpoint	-11°C (closed cup)	Budavari et al. 2001
NFPA hazard classification:		OSHA 2021
Health	2	
Flammability	3	
Reactivity	0	
Flammability limits in air	1.2% (lower limit); 7.8% (upper limit)	OSHA 2021
Conversion factors	1 ppm=3.26 mg/m^3 at 20°C and 1 atm pressure; 1 mg/m^3=0.31 ppm	NLM 2023
Explosive limits	1.4% (lower limit); 8% (upper limit)	NLM 2023

[a]Odor threshold values considered by AIHA (1989) to be acceptable based on review of peer-reviewed reports of odor thresholds for benzene (range 0.78–100 ppm).

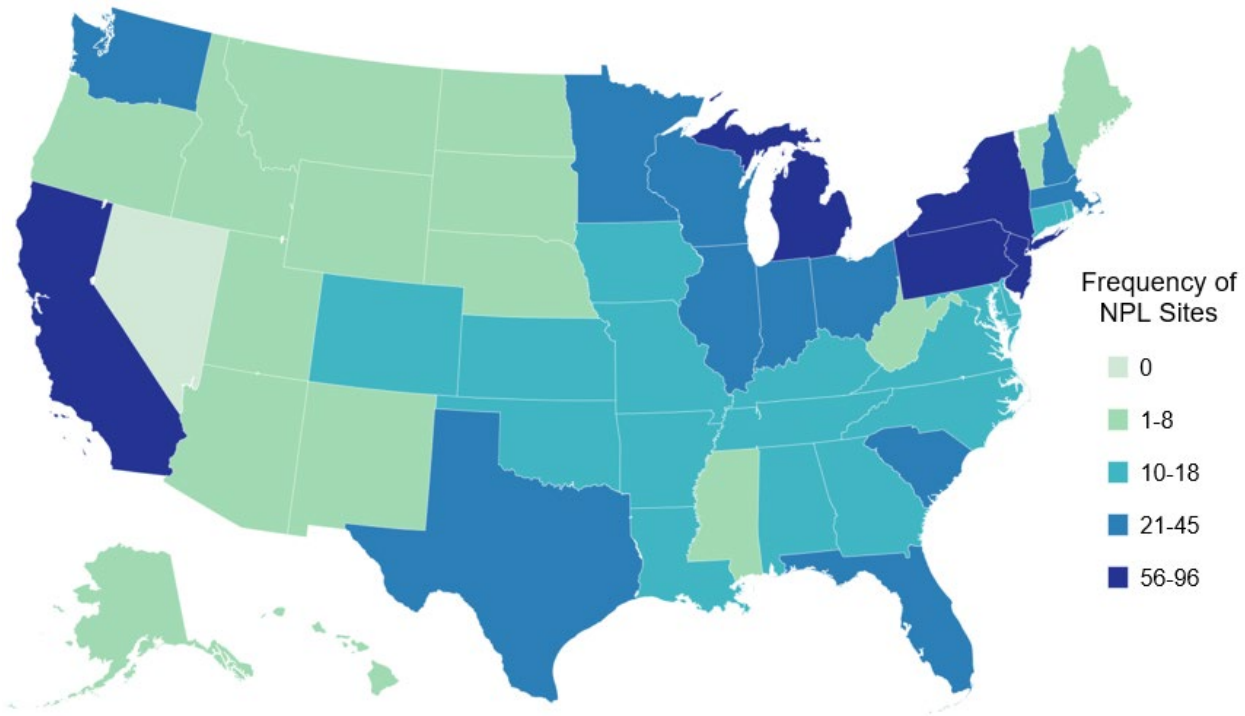
NFPA = National Fire Protection Association. Level 3 flammability classification is highly flammable. A level 2 health classification means the material is hazardous.

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Benzene has been identified in at least 982 of the 1,868 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2022a). However, the number of sites in which benzene has been evaluated is not known. The number of sites in each state is shown in Figure 5-1. Of these sites, 977 are located within the United States, 2 are located in the Virgin Islands, and 3 are located in Puerto Rico (not shown).

Figure 5-1. Number of NPL Sites with Benzene Contamination



Source: ATSDR 2022a

- The general population is most likely to be exposed to trace levels of benzene in ambient air and, typically at higher concentrations, in indoor air. Benzene is ubiquitous in the atmosphere from anthropogenic and natural sources, but concentrations have decreased in the past decades. Indoor air sources include evaporative emissions from cars in attached garages and cooking on gas stoves. Benzene has also been detected in surface and groundwater.

- Activities such as pumping gasoline and smoking increase inhalation benzene exposure.

- Benzene has been detected in the parts per billion range in some foods from cooking processes or formation from added preservatives, and rarely (<1%) in municipal water. These are not expected to be major exposure pathways.

- Environmental exposure from air, surface water, and drinking water may be increased for people living near hazardous waste sites.

- Benzene readily volatilizes to air from water and is mobile in soils and will migrate to groundwater. Bioaccumulation in biota is not expected to occur to a significant degree.

- Residence times in the atmosphere ranged from hours to days based on indirect photolysis. Indirect photolysis may also be a transformation mechanism in surface water. Benzene is readily biodegraded in aerobic conditions (e.g., surface water and soil) and is not readily degraded under anerobic conditions (e.g., groundwater and subsurface sediments).

Benzene is released to the environment by both natural and industrial sources, although the anthropogenic emissions are undoubtedly the most important. Emissions of benzene to the atmosphere result from gasoline vapors, auto exhaust, and chemical production and user facilities. EPA's estimate of nationwide benzene atmospheric emissions from various point and non-point sources was approximately 152,000 metric tons in 2020 (EPA 2020a). Benzene is released to water and soil from industrial discharges, landfill leachate, and gasoline leaks from underground storage tanks.

Chemical degradation reactions, primarily reaction with hydroxyl radicals, limit the atmospheric residence time of benzene to only a few days. Under certain conditions, atmospheric residence time may be as brief as a few hours. Benzene released to soil or waterways is subject to volatilization, photooxidation, and biodegradation. Biodegradation, principally under aerobic conditions, is an important environmental fate process for water- and soil-associated benzene.

Benzene is ubiquitous in the atmosphere. It has been identified in air samples of both rural and urban environments and in indoor air. Although a large volume of benzene is released to the environment, environmental levels are low due to degradation processes. Benzene partitions mainly into air (99.9%) and inhalation is the dominant pathway of human exposure accounting for >99% of the total daily intake of benzene (Hattemer-Frey et al. 1990; MacLeod and MacKay 1999).

The general population is exposed to benzene primarily by tobacco smoke (both active and passive smoking) and by inhaling contaminated air, particularly in areas with heavy motor vehicle traffic and around filling stations, and in some cases, poorly ventilated indoor air. Indoor air benzene pollution sources include gas stoves and ovens, evaporative emissions from cars in attached garages, and fuel or

wood-based heat sources (e.g., fireplaces). Use of contaminated tap water for cooking, showering, etc., can also be a source of inhalation exposure since benzene can volatilize from water. Air around manufacturing plants that produce or use benzene and air around landfills and hazardous waste sites that contain benzene are additional sources of exposure.

Another source is from smoking, which was found to be the largest anthropogenic source of direct human exposure to benzene (Duarte-Davidson et al. 2001; Hattemer-Frey et al. 1990). In the United States, tobacco cigarette smoking was found to be the predominant source of increased blood benzene concentrations in which people who smoked at least one cigarette per day had a mean level of 0.140 μg/L benzene compared to people who smoked less than one cigarette per day, which includes nonsmokers, and had a mean level below detection (<0.024 μg/L) (Chambers et al. 2011).

Exposure to benzene can also result from ingestion of contaminated food or water but this is not expected to be a major exposure pathway. Compared to inhalation, dermal exposure accounts for a minor portion of the total exposure of the general population. Dermal exposure may occur in the general population from direct contact with gasoline (e.g., spillage while filling gas tank). Individuals occupationally exposed to benzene tend to have higher dermal doses than the general population.

5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.2.1 Production

Table 5-1 summarizes information on companies that reported the production, import, or use of benzene for the Toxics Release Inventory (TRI) in 2022 (TRI22 2023). TRI data should be used with caution since only certain types of industrial facilities are required to report. This is not an exhaustive list.

Table 5-1. Facilities that Produce, Process, or Use Benzene

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AK	18	1,000	49,999,999	1, 3, 4, 5, 7, 9, 12, 13, 14
AL	18	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
AR	8	1,000	9,999,999	1, 2, 3, 5, 6, 9, 12
AZ	17	1,000	9,999,999	1, 5, 9, 12
CA	53	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 9, 12, 13, 14
CO	26	0	99,999,999	1, 2, 3, 5, 8, 9, 12, 13, 14

Table 5-1. Facilities that Produce, Process, or Use Benzene

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
CT	3	100,000	9,999,999	1, 5, 7, 9, 12
DE	1	100,000	999,999	1, 2, 3, 4, 9
FL	24	0	9,999,999	1, 2, 4, 5, 7, 9, 12, 13, 14
GA	7	10,000	9,999,999	1, 5, 8, 9, 12
GU	2	1,000	999,999	7, 9
HI	10	0	9,999,999	1, 2, 3, 4, 5, 6, 9, 12, 13, 14
IA	42	1,000	9,999,999	1, 4, 5, 7, 8, 9, 12
ID	3	1,000	999,999	1, 5, 9, 14
IL	36	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
IN	34	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
KS	22	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14
KY	22	1,000	49,999,999	1, 3, 5, 6, 7, 8, 9, 10, 12, 13, 14
LA	79	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MA	8	1,000	9,999,999	2, 4, 7, 8, 9, 12
MD	6	1,000,000	9,999,999	9
ME	7	0	9,999,999	2, 3, 4, 7, 9, 12
MI	32	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
MN	19	1,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14
MO	14	0	9,999,999	1, 5, 7, 8, 9, 10, 12
MP	2	10,000	999,999	1, 2, 3, 5, 7, 9
MS	14	10,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
MT	9	100,000	9,999,999	1, 2, 3, 4, 5, 6, 9, 12, 13, 14
NC	11	0	9,999,999	1, 5, 9, 12
ND	30	0	9,999,999	1, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14
NE	21	1,000	9,999,999	1, 2, 3, 5, 7, 8, 9, 12, 14
NH	5	0	99	12
NJ	10	1,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 13, 14
NM	24	100	9,999,999	1, 3, 5, 6, 7, 8, 9, 11, 12, 13, 14
NV	3	1,000,000	9,999,999	9
NY	17	1,000	9,999,999	1, 2, 4, 5, 7, 8, 9, 12
OH	37	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
OK	32	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
OR	4	100,000	9,999,999	1, 5, 7, 9
PA	21	100	9,999,999	1, 2, 4, 5, 6, 7, 8, 9, 12, 13, 14
PR	7	100	49,999,999	2, 3, 4, 8, 9, 12, 13, 14
RI	3	100,000	9,999,999	1, 5, 7, 9, 12
SC	5	100	99,999	1, 5, 8, 12
SD	16	1,000	99,999	1, 5, 7, 9, 12, 13, 14

Table 5-1. Facilities that Produce, Process, or Use Benzene

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
TN	17	0	49,999,999	1, 3, 5, 7, 8, 9, 11, 12, 13, 14
TX	264	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
UT	16	10,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
VA	6	1,000	999,999	1, 5, 9
VI	3	100,000	9,999,999	1, 5, 7, 9
WA	16	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 12, 13, 14
WI	19	0	9,999,999	1, 5, 7, 9, 12, 14
WV	11	1,000	9,999,999	1, 3, 5, 6, 7, 8, 9, 12, 13, 14
WY	17	100	9,999,999	1, 2, 3, 4, 6, 8, 9, 12, 13, 14

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/uses:

1. Produce
2. Import
3. Used Processing
4. Sale/Distribution
5. Byproduct
6. Reactant
7. Formulation Component
8. Article Component
9. Repackaging
10. Chemical Processing Aid
11. Manufacture Aid
12. Ancillary
13. Manufacture Impurity
14. Process Impurity

Source: TRI22 2023 (Data are from 2022)

In 1825, Faraday first isolated benzene from a liquid condensed by compressing oil gas. Benzene was first synthesized by Mitscherlich in 1833 by distilling benzoic acid with lime. Benzene was first commercially recovered from light oil derived from coal tar in 1849 and from petroleum in the 1940s (Fruscella 2002). Several years after the end of World War II, the rapidly expanding chemical industry created an increased demand for benzene that the coal carbonization industry could not fulfill. To meet this demand, about 95% of commercial production of benzene shifted to the petroleum and petrochemical industries via recovery from petroleum sources (Fruscella 2002). These sources include refinery streams (catalytic reformates), pyrolysis gasoline, and toluene hydrodealkylation. Catalytic reformate is the major source of benzene, accounting for about 30% of worldwide production (Fruscella 2002).

During catalytic reforming, cycloparaffins are converted to benzene by isomerization, dehydrogenation, and dealkylation, and paraffins are converted to benzene by cyclodehydrogenation (Fruscella 2002). The type of catalyst used and process conditions determine which reaction will predominate. The benzene is recovered by solvent extraction (e.g., with tetramethylene sulfone).

Pyrolysis gasoline is a liquid byproduct produced by the steam cracking of lower paraffins (gas oil) or heavier hydrocarbons (heavy naphtha) and contains ~65% aromatics, about half of which is benzene (Fruscella 2002). Benzene is recovered from pyrolysis gasoline through hydrogenation to remove olefinic constituents, solvent extraction, and distillation for the optimization of benzene yield and the recovery of benzene.

In the toluene hydrodealkylation process, toluene or toluene/xylene mixtures are reacted with hydrogen at temperatures of 500–595°C with usual pressures of 4–6 mPa (40–60 atm), and demethylated to produce benzene and methane. Another process whereby toluene is converted to benzene and xylenes by transalkylation or disproportionation is also used for the production of benzene (Fruscella 2002). Small quantities of benzene are also produced from destructive distillation of coal used for coke manufacture. Benzene is derived from the light oil fraction produced during the coking process (Fruscella 2002).

The nationally aggregated production of benzene has held steady between $10x10^{10}$ and $20x10^{10}$ pounds between 2016 and 2019 (EPA 2022a). The companies summarized in Table 5-2 reported benzene manufacturing to the Chemical Data Reporting (CDR) Rule in 2019 (EPA 2022a). This is not an exhaustive list; companies must meet a threshold to trigger reporting to the CDR, and other manufacturers may therefore be unreported.

Table 5-2. U.S. Manufacturers of Benzene Reported to the CDR in 2019

Company	City	State[a]	Production volume (pounds)
BASF Corp.	Port Arthur	TX	NR
Chalmette Refining, LLC	Chalmette	LA	NR
Chevron	Pascagoula	MS	1,400,000,000
Citgo Holding, Inc.	Corpus Christi	TX	350,000,000
	Sulphur	LA	508,404,699
	Lemont	IL	78,000,000
Delek Us Holdings, Inc.	Big Spring	TX	NR
Deltech Corporation	Baton Rouge	LA	NR
Dynachem Inc.	Georgetown	IL	452,360
Equilon Enterprises LLC DBA Shell Oil Products US	Norco	LA	NR
Exxon Mobil Corporation	Beaumont	TX	NR
	Baton Rouge	LA	NR
	Baytown	TX	NR
Husky Energy, Inc.	Lima	OH	134,703,677
Ineos Americas, LLC	Pasadena	TX	132,800

Table 5-2. U.S. Manufacturers of Benzene Reported to the CDR in 2019

Company	City	State[a]	Production volume (pounds)
JX Nippon Chemical Texas, Inc.	Pasadena	TX	1,666,296
Koch Industries, Inc.	Corpus Christi	TX	NR
	Corpus Christi	TX	NR
Lyondell Chemical Company	Houston	TX	NR
	Channelview	TX	NR
Marathon Petroleum Company LP	Robinson	IL	251,150,313
	Texas City	TX	1,234,696,983
	Catlettsburg	KY	236,869,394
New Erie	Kalamazoo	MI	35,531
PBF Energy Inc.	Delaware City	DE	287,298,018
PBF Holding Company LLC	Oregon	OH	148,123,522
PES Ultimate Holdings, LLC	Philadelphia	PA	136,000,000
Phillips 66 Co	Belle Chasse	LA	542,863,463
	Old Ocean	TX	260,000,000
Shell Chemical LP	Norco	LA	170,437,239
	Deer Park	TX	4,330,664
SI Group USA (USAA), LLC	Bay Minette	AL	NR
The Dow Chemical Co	Plaquemine	LA	NR
Total Petrochemicals & Refining USA, Inc.	Port Arthur	TX	181,717,910
WRB Refining LP Wood River Refinery	Roxana	IL	313,717,525

[a]Post office state abbreviations used.

CDR = Chemical Data Reporting; NR = not reported

Source: EPA 2022a (data are for 2019)

5.2.2 Import/Export

Benzene is imported and exported to the United States as both the pure chemical and as a mixture of mineral fuels. Imports and exports are reported in million liters (million L) by the U.S. International Trade Commission (USITC). The import of pure benzene into the United States is dependent on domestic production and demand. Imports of benzene for consumption (from mineral fuels and pure benzene) in the United States were approximately 2,320 million L (4,480 billion pounds) in 2023, 2,054 million L (3,967 billion pounds) in 2022 1,886 million L (3,643 billion pounds) in 2021, and 2,441 million L (4,715 billion pounds) in 2020 (USITC 2024). The largest exporters of benzene to the United States in 2023 were South Korea, Canada, the Netherlands, Japan, and Brazil (USITC 2024).

As in the case of import, the export of benzene from the United States to other countries is dependent on domestic and world production and demand. Domestic exports of benzene (both pure benzene and benzene derived from mineral fuels) to other countries were approximately 125 million L (241 billion pounds) in 2023, 185 million L (357 billion pounds) in 2022, 351 million L (678 billion pounds) in 2021, and 182 million L (352 billion pounds) in 2020 (USITC 2024). These numbers are up from 23 million L (45 billion pounds) in 2001 and 4.7 million L (9.0 billion pounds) in 1993 (USITC 2024). The largest importers of benzene from the United States in 2023 were Canada, the Netherlands, Brazil, South Korea, and Mexico (USITC 2024).

5.2.3 Use

Benzene has been used extensively as a solvent in the chemical and drug industries, as a starting material and intermediate in the synthesis of numerous chemicals, and as a gasoline additive (NTP 1994).

Benzene recovered from petroleum and coal sources is used primarily as an intermediate in the manufacture of other chemicals and end products. The major uses of benzene are in the production of ethylbenzene, cumene, and cyclohexane. Ethylbenzene (52% of benzene production volume in 2008) is an intermediate in the synthesis of styrene, which is used to make plastics and elastomers. Cumene (22%) is used to produce phenol and acetone. Phenols are used in the manufacture of phenolic resins and nylon intermediates; acetone is used as a solvent and in the manufacture of pharmaceuticals. Cyclohexane (15%) is used to make nylon resins. Other industrial chemicals manufactured from benzene include nitrobenzene (7%), which is used in the production of aniline and other products, urethanes, linear alkylbenzenes used for detergents (2%), chlorobenzenes for engineering polymers (1%), and miscellaneous other uses (1%) (Eveleth 1990; Greek 1990; IARC 2018; NLM 2023).

Benzene is also a component of gasoline since it occurs naturally in crude oil and since it is a byproduct of oil refining processes (Brief et al. 1980; Holmberg and Lundberg 1985). Benzene is especially important for unleaded gasoline because of its anti-knock characteristics. Historically, the percentage by volume of benzene in unleaded gasoline previously was approximately 1–2% (NESCAUM 1989); however, beginning in 2011, the annual average benzene content of gasoline is regulated to 0.62% volume (maximum average: 1.3% volume) under the Mobile Source Air Toxics rules (EPA 2023a). Benzene is present at insignificant levels in diesel fuel (EPA 1993).

The widespread use of benzene as a solvent has decreased. This is likely the result of EPA listing benzene as a hazardous air pollutant (HAP), hazardous waste (EPA 1977, 1981), and human carcinogen (IRIS 2003). Many products that used benzene as solvents in the past have replaced it with other organic solvents; however, benzene may still occur as a trace impurity in these products (Kim et al. 2022). Less than 2% of the amount produced is used as a solvent in products such as trade and industrial paints, rubber cements, adhesives, paint removers, artificial leather, and rubber goods.

In the past, benzene has been used in the shoe manufacturing and rotogravure printing industries in the United States (EPA 1978; OSHA 1977) and continues to be used in the printing industry outside of the United States (Shi et al. 2022). Furthermore, small amounts of benzene were present in certain consumer products (such as some paint strippers, carburetor cleaners, denatured alcohol, and rubber cement used in tire patch kits and arts and crafts supplies) contained small amounts of benzene (Young et al. 1978). Other consumer products that contained benzene were certain types of carpet glue, textured carpet liquid detergent, and furniture wax (Wallace et al. 1987).

The Consumer Products Safety Commission (CPSC) withdrew an earlier proposal to ban consumer products, except gasoline and laboratory reagents, that contained benzene as an intentional ingredient or as a contaminant at >0.1% by volume. The withdrawal of the rulemaking was based on CPSC findings that benzene was no longer used as an intentional ingredient and that the contaminant levels remaining in certain consumer products were unlikely to result in significant exposures (NTP 1994). Products containing >5% benzene, and paint solvents and thinners containing <10% of petroleum distillates such as benzene, are required to meet established labeling requirements. In a guidance document targeting school science laboratories, the CPSC recommended that benzene not be used or stored in schools. The document identified benzene as a carcinogen and ascertained that the hazards posed by its use in high school laboratories may be greater than its potential usefulness.

The U.S. Food and Drug Administration (FDA) regulates benzene as an indirect food additive under the Food, Drug, and Cosmetics Act (FDCA). Under the FDCA, benzene is restricted to use only as a component of adhesives used on articles intended for packaging, transport, or holding foods (FDA 1977a). The FDA has set a limit of <1 ppm on residual benzene in modified hop extract (where benzene is a solvent used in sequential extraction during production of this flavoring agent) used as an additive in beer (FDA 1977b). In December 2023, the FDA requested that the U.S. Pharmacopeia-National Formulary avoid use of carbomers manufactured with benzene, which are used as thickening agents in drug and cosmetic products, due to residual benzene levels in end-use products above the maximum

permitted level of 2 ppm (FDA 2023a). The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) classified benzene as a "solvent to be avoided," indicating that it should only be used if absolutely necessary during the manufacture of a drug with a "significant therapeutic advance" (ICH 2021). For these cases, ICH set a maximum permitted level of 2 ppm.

5.2.4 Disposal

Benzene-containing wastes, such as commercial chemical products, manufacturing chemical intermediates, and spent solvents, are subject to federal and/or state hazardous waste regulations. Waste byproducts from benzene production processes include acid and alkali sludges, liquid-solid slurries, and solids (EPA 1982; Saxton and Narkus-Kramer 1975). In the past, landfilling and lagooning have been the major methods of disposal of benzene-containing industrial wastes (EPA 1982). Biodegradation is the primary fate of industrial wastes; however, a portion of the benzene is expected to be lost due to volatilization. Unfortunately, benzene, along with other hazardous contaminants, also leaches into groundwater from the lagooned wastes.

The recommended method of disposal is to incinerate solvent mixtures and sludges at a temperature that ensures complete combustion. The recommended methods for combustion are liquid injection incineration at a temperature range of 650–1,600°C and a residence time of 0.1–2 seconds; rotary kiln incineration at a temperature range of 820–1,600°C and residence times of seconds for liquids and gases, and hours for solids; and fluidized bed incineration at a temperature range of 450–980°C and residence times of seconds for liquids and gases and longer for solids (IRPTC 1985). Since benzene burns with a very smoky flame, dilution with alcohol or acetone is suggested to minimize smoke.

Several methods exist for the treatment of wastewater that contains benzene: biological treatment (aeration or activated sludge process), solvent extraction, air and/or steam stripping, and activated carbon process (EPA 1994a; IRPTC 1985). A combination of steam stripping and air stripping, and a vapor extraction system that removes the separated benzene vapor may be suitable for the treatment of contaminated groundwater and soil (Naft 1992). An *in situ* bioremediation process has been used to decontaminate a site by delivering a controlled amount of nitrate (to accelerate biodegradation of benzene) to the site under hydraulic control (Kennedy and Hutchins 1992).

5.3 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2022b). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ ≥10 full-time employees; if their facility's North American Industry Classification System (NAICS) codes is covered under EPCRA Section 313 or is a federal facility; and if their facility manufactures (defined to include importing) or processes any TRI chemical in excess of 25,000 pounds, or otherwise uses any TRI chemical in excess of 10,000 pounds, in a calendar year (EPA 2022b).

5.3.1 Air

Estimated releases of 3,198,877 pounds (~1,450 metric tons) of benzene to the atmosphere from 1,125 domestic manufacturing and processing facilities in 2022, accounted for about 36% of the estimated total environmental releases from facilities required to report to the TRI (TRI22 2023). These releases are summarized in Table 5-3.

Table 5-3. Releases to the Environment from Facilities that Produce, Process, or Use Benzene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AL	17	96,461	1	0	3,790	19	96,478	3,792	100,270
AK	18	6,281	0	0	225	604	6,285	825	7,110
AZ	17	12,184	0	0	5	510	12,189	510	12,699
AR	8	39,584	1,087	10	74	1,168	40,748	1,175	41,923
CA	47	26,101	4,194	0	50,524	342	72,364	8,796	81,161
CO	26	34,104	3	26,828	908	6,781	34,105	34,519	68,625
CT	3	3,189	2	0	0	0	3,190	0	3,190
DE	1	2,190	9	0	5	0	2,199	5	2,204
FL	24	18,491	0	150	0	1	18,641	1	18,642
GA	7	2,111	1	0	0	0	2,112	0	2,112
HI	10	15,423	0	3	112	0	15,427	112	15,538
ID	3	854	0	0	17	0	854	17	871
IL	34	117,031	112	0	290	96	117,317	212	117,529
IN	33	92,888	16	16,867	1,053	103	109,758	1,169	110,927

Table 5-3. Releases to the Environment from Facilities that Produce, Process, or Use Benzene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
IA	42	64,382	1	0	0	0	64,383	0	64,383
KS	22	35,280	18	172	1,530	960	36,613	1,347	37,960
KY	22	78,976	731	0	201	7,674	79,712	7,871	87,582
LA	75	430,040	543	6,057	803	773	435,923	2,294	438,216
ME	7	1,042	1	0	0	46	1,043	46	1,089
MD	6	960	5	0	0	0	965	0	965
MA	8	2,211	2	0	17	185	2,213	202	2,415
MI	30	128,407	26	0	133	1,366	128,552	1,380	129,932
MN	19	14,884	31	0	14	2	14,915	16	14,931
MS	14	38,867	5	70,056	175	2	38,872	70,233	109,105
MO	14	31,700	0	0	0	169	31,700	169	31,869
MT	9	20,264	6	0	66	11	20,318	30	20,348
NE	21	4,920	0	0	75	1,370	4,920	1,445	6,365
NV	3	396	0	0	0	3	396	3	399
NH	5	0	0	0	0	0	0	0	0
NJ	10	11,816	310	0	6	17	12,126	23	12,149
NM	24	65,825	0	2,706,450	1,078	5,130	2,754,512	23,971	2,778,483
NY	17	5,158	1	0	7	1	5,162	4	5,167
NC	10	1,983	0	0	4	195	1,987	195	2,182
ND	29	28,432	2	17,856	11	0	28,435	17,866	46,302
OH	36	59,324	7	6,564	72	664	59,347	7,284	66,631
OK	31	67,899	28	2,012	14,590	21	67,926	16,623	84,549
OR	4	1,101	2	0	3	0	1,102	4	1,106
PA	21	90,869	143	16	118	1,123	91,011	1,258	92,269
RI	3	853	1	0	0	0	854	0	854
SC	5	60,063	0	0	0	0	60,063	0	60,064
SD	16	6,343	0	0	0	0	6,343	0	6,343
TN	17	17,936	155	0	263	0	17,937	418	18,355
TX	260	1,082,656	2,465	2,528,552	11,719	4,596	1,679,417	1,950,570	3,629,987
UT	15	132,709	1,027	14,564	163	128	132,721	15,870	148,591
VA	6	7,333	1,426	0	0	108	8,713	153	8,867
WA	16	33,109	8	0	222	59	33,168	230	33,398
WV	11	13,850	1	8,115	371	4	14,205	8,136	22,341
WI	18	139,633	0	0	764	3	139,633	767	140,399
WY	17	21,992	0	16,797	92,649	0	22,508	108,930	131,438
GU	2	1,397	0	0	0	0	1,397	0	1,397

Table 5-3. Releases to the Environment from Facilities that Produce, Process, or Use Benzene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
MP	2	208	0	0	0	0	208	0	208
PR	7	6,198	0	0	0	0	6,198	0	6,198
VI	3	22,970	0	0	0	0	22,970	0	22,970
Total	1,125	3,198,877	12,369	5,421,070	182,059	34,235	6,560,134	2,288,475	8,848,609

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, wastewater treatment (metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown.
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI22 2023 (Data are from 2022)

Benzene is released into the atmosphere from both natural and industrial sources. Natural sources include, but are not limited to, crude oil seeps, forest fires, volcanoes, and plant volatiles (Brief et al. 1980; Dickinson et al. 2022; Graedel 1978; NCI 2022). Major anthropogenic sources of benzene include industrial emissions, automobile exhaust, automobile refueling operations, and environmental tobacco smoke.

Industrial and automotive sources of benzene are well characterized (Tables 5-2 and 5-3). Oil and gas production is expected to be the largest industrial contributor to benzene emissions: around 20% of total emissions (EPA 2020a). Benzene composes 3–5% of passenger car tailpipe emissions, depending on the control technology and fuel, and is estimated to be about 1% of evaporative emissions from vehicles (EPA 1993). Mobile sources (including on-road vehicles) were estimated to contribute to 33% of all benzene emissions, and on-road vehicles were estimated to contribute around 12% of all benzene emissions (EPA 2020a).

Emissions from car exhaust began decreasing in localities where federally reformulated gasoline is sold as part of a mandate under the 1990 Clean Air Act (EPA 1995b). This program has had great success, reducing benzene emissions by 43% between 1995 to 1999 in the District of Columbia and the 17 participating states (EPA 1999). Gasoline-related benzene emissions have further been reduced under the mobile source air toxics rule (EPA 2007). These new standards are expected to reduce benzene emissions by 20,000 per year by 2030 (EPA 2017).

Benzene is also released by off-gassing from particle board (Glass et al. 1986), vaporization from oil spills, and emissions from landfills (Bennett 1987; Wood and Porter 1987). An analysis of gas from 20 Class II (municipal) landfills revealed a maximum concentration of 32 ppm for benzene (Wood and Porter 1987). While all of these sources release more benzene into the environment, a large percentage of the benzene inhaled by humans comes from tobacco cigarette smoke. Exhaled breath of smokers contains benzene (Wallace 1989a, 1989b; Wallace and Pellizzari 1986; Wester et al. 1986).

Fires are the natural sources most monitored for benzene releases to the air (Austin et al. 2001; Lowry et al. 1985). Austin et al. (2001) monitored volatile organic compounds (VOCs) released from nine municipal fires in Canada and found a mean concentration of 3.45 ppm of benzene. This study also reported very high relative concentration of other VOCs.

EPA's National Emission Inventory (NEI) database contains information regarding sources that emit criteria air pollutants (CAPs) and their precursors, and HAPs for the 50 United States, Washington DC, Puerto Rico, and the U.S. Virgin Islands. Emissions are estimated from multiple sources, including state and local environmental agencies; the TRI database; computer models for on- and off-road emissions; and databases related to EPA's Maximum Achievable Control Technology (MACT) programs to reduce emissions of HAPs. Benzene emissions estimated from the 2020 inventory are summarized in Table 5-4.

Table 5-4. National Emission Inventory (NEI) Total National Emissions for Benzene Estimated by Sector 2020

Sector	Emissions (pounds)
Fires; wildfires	80,674,309
Industrial processes; oil and gas production	65,225,185
Mobile; non-road equipment; gasoline	49,281,476
Fires; prescribed fires	39,151,086
Mobile; on-road non-diesel light duty vehicles	36,787,086

Table 5-4. National Emission Inventory (NEI) Total National Emissions for Benzene Estimated by Sector 2020

Sector	Emissions (pounds)
Fuel combustion; residential; wood	27,232,070
Waste disposal	9,722,888
Mobile; non-road equipment; diesel	4,142,396
Fires; agricultural field burning	3,063,972
Gas stations	2,435,276
Industrial processes; storage and transfer	2,018,389
Mobile; aircraft	1,361,523
Mobile; on-road non-diesel heavy duty vehicles	1,286,764
Fuel combustion; industrial boilers, internal combustion engines; biomass	1,048,989
Industrial processes; chemical manufacturing	990,792
Mobile; on-road diesel heavy duty vehicles	943,279
Agriculture; livestock waste	905,710
Mobile; locomotives	895,965
Fuel combustion; industrial boilers, internal combustion engines; natural gas	842,202
Bulk gasoline terminals	818,626
Miscellaneous non-industrial not elsewhere classified	800,091
Industrial processes; petroleum refineries	619,480
Solvent; consumer and commercial solvent use	593,762
Industrial processes; not elsewhere classified	504,294
Industrial processes; cement manufacturing	447,863
Commercial cooking	384,291
Mobile; on-road diesel light duty vehicles	379,016
Industrial processes; ferrous metals	337,533
Fuel combustion; electric generation; coal	178,053
Fuel combustion; electric generation; biomass	172,574
Solvent; industrial surface coating and solvent use	169,271
Industrial processes; pulp and paper	158,119
Fuel combustion; electric generation; natural gas	105,881
Mobile; commercial marine vessels	89,440
Fuel combustion; electric generation; oil	89,325
Fuel combustion; industrial boilers, internal combustion engines; other	84,235
Fuel combustion; commercial/institutional; biomass	84,118
Mobile; non-road equipment; other	60,133
Fuel combustion; industrial boilers, ices; oil	53,089
Industrial processes; non-ferrous metals	29,347
Fuel combustion; commercial/institutional; natural gas	28,728
Fuel combustion; commercial/institutional; oil	28,534
Fuel combustion; industrial boilers, internal combustion engines; coal	12,801
Fuel combustion; electric generation; other	11,345

Table 5-4. National Emission Inventory (NEI) Total National Emissions for Benzene Estimated by Sector 2020

Sector	Emissions (pounds)
Fuel combustion; residential; natural gas	10,061
Fuel combustion	3,839
Industrial processes; mining	2,960
Fuel combustion; residential; other	854
Solvent; graphic arts	634
Fuel combustion; residential; oil	597
Solvent; degreasing	477
Fuel combustion; comm/institutional; coal	175
Solvent; dry cleaning	2

Source: EPA 2020a

5.3.2 Water

Estimated releases of 12,369 pounds (~5.61 metric tons) of benzene to surface water from 1,125 domestic manufacturing and processing facilities in 2022, accounted for about 0.14% of the estimated total environmental releases from facilities required to report to the TRI (TRI22 2023). This estimate includes releases to wastewater treatment and publicly owned treatment works (POTWs) (TRI22 2023). These releases are summarized in Table 5-3.

Benzene is released to water from the discharges of both treated and untreated industrial wastewater, gasoline leaks from underground storage tanks, accidental spills during transportation of chemical products or drilling, and leachate from landfills and other contaminated soils (CDC 1994; Crawford et al. 1995; EPA 1979; NESCAUM 1989; Staples et al. 1985; Reddy et al. 2012). A fire in a tire dump site in western Frederick County, Virginia, produced a free-flowing oily tar containing benzene among other chemicals. The seepage from this site contaminated nearby surface water (EPA 1992). Accidental spills released 3,000 gallons of benzene into Newark Bay and its major tributaries in 1991 (Crawford et al. 1995). Following a tanker truck accident that released 3,200 gallons of gasoline in 2020, benzene was detected in the Yellowstone River and monitoring wells (EPA 2020b). Oil spills are an important source of emissions to the marine environment. An estimated 1,600 metric tons of benzene were released from the 2010 Deepwater Horizon oil spill in the Gulf of Mexico (Reddy et al. 2012). In 2020, a tanker truck accident resulted in approximately 3,200 gallons of gasoline spilled at Yellowstone National Park; soil

monitoring was not reported, but increased benzene was detected in the nearby river and monitoring wells (EPA 2020b).

5.3.3 Soil

Estimated releases of 182,059 pounds (~82.6 metric tons) of benzene to soil from 1,125 domestic manufacturing and processing facilities in 2022, accounted for about 2.1% of the estimated total environmental releases from facilities required to report to the TRI (TRI22 2023). An additional 5,421,070 pounds (~2,460 metric tons), constituting about 61% of the total environmental emissions, were released via underground injection (TRI22 2023). These releases are summarized in Table 5-3.

Benzene is released to soils through industrial discharges, land disposal of benzene-containing wastes, and gasoline leaks from underground storage tanks. Limited data on terrestrial emissions are available. This may be due to benzene's expected partitioning to air or migration to groundwater. Benzene was detected in sediment at an NPL site of a former truck terminal, which disposed of wastewater in an unlined lagoon and in underground tanks (ATSDR 2023d), and in the soil of the marine terminal area of a former army air and ground base (ATSDR 2019a). In northern Virginia, approximately 200,000 gallons of liquid hydrocarbons were released from a fuel-storage terminal into the underlying soil (Mushrush et al. 1994).

5.4 ENVIRONMENTAL FATE

5.4.1 Transport and Partitioning

Air. The high volatility of benzene is the controlling physical property in the environmental transport and partitioning of this chemical. Benzene is considered to be highly volatile with a vapor pressure of 94.8 mm Hg at 25°C. Benzene is soluble in water, with a solubility of 1,790 mg/L at 25°C, and the Henry's law constant for benzene (5.5×10^{-3} atm-m^3/mole at 25°C) indicates that benzene partitions readily to the atmosphere from surface water (Mackay and Leinonen 1975; NLM 2023). Since benzene is soluble in water, removal from the atmosphere via wet deposition may occur. A substantial portion of any benzene in rainwater that is deposited to soil or water will be returned to the atmosphere via volatilization.

Water. Benzene is soluble in water and (1,790 mg/L at 25°C) and has low tendency to partition to the organic phase based on the octanol/water partition coefficient (log K_{ow}) of 2.13 (NLM 2023). Due to this and its volatility, benzene will preferentially partition to the atmosphere rather than be removed from the water column through sorption to particulate matter or sediments.

Sediment and Soil. Benzene released to soil surfaces partitions to the atmosphere through volatilization, to surface water through runoff, and to groundwater as a result of leaching. The soil organic carbon sorption coefficient (K_{oc}) for benzene has been measured with a range of 60–85 (Karickhoff 1981; Kenaga 1980; NLM 2023), indicating that benzene is highly mobile in soil and readily leaches into groundwater. Other parameters that influence leaching potential include the soil type (e.g., sand versus clay), amount of rainfall, depth of the groundwater, and extent of degradation. In a study of the sorptive characteristics of benzene to groundwater aquifer solids, benzene showed a tendency to adsorb to aquifer solids. Greater soil adsorption was observed within an aquifer with more organic matter content (4.4%), compared to an aquifer with less organic matter content (2.2%) (Uchrin and Mangels 1987). An investigation of the mechanisms governing the rates of adsorption and desorption of benzene by dry soil grains revealed that periods of hours are required to achieve equilibrium and that adsorption is much faster than desorption (Lin et al. 1994). The rate of volatilization and leaching are the principal factors that determine overall persistence of benzene in sandy soils (Tucker et al. 1986).

Other Media. Studies suggest that benzene does not bioaccumulate in marine organisms. The bioconcentration/ bioaccumulation potential of benzene in aquatic organisms of the open coastal ocean was investigated by sampling final effluent from the Los Angeles County wastewater treatment plant quarterly from November 1980 to August 1981 (Gossett et al. 1983). Benzene has a relatively low log K_{ow} value of 2.13 (Gossett et al. 1983; NLM 2023). In the alga, *Chlorella,* a bioaccumulation factor of 30 was determined experimentally (Geyer et al. 1984). An experimental bioconcentration factor (BCF) of 4.27 (reported as log BCF of 0.63) was measured in goldfish reared in water containing 1 ppm of benzene (Ogata et al. 1984). Based on these measured values, bioconcentration/bioaccumulation of benzene in the aquatic food chains does not appear to be an important fate pathway. These results are consistent with the fact that benzene has a relatively low octanol/water partition coefficient (Gossett et al. 1983; NLM 2023), suggesting relatively low bioaccumulation. There is no evidence in the literature of biomagnification of benzene in aquatic food chains.

Evidence exists for the uptake of benzene by cress and barley plants from soil (Scheunert et al. 1985; Topp et al. 1989). BCFs for barley plants after 12, 33, and 125 days were 17, 2.3, and 4.6, respectively.

BCFs for cress plants after 12, 33, and 79 days were 10, 2.3, and 1.9, respectively. The relative decrease in the BCFs with time was attributed to growth dilution (Topp et al. 1989). Since benzene exists primarily in the vapor phase, air-to-leaf transfer is considered to be the major pathway of vegetative contamination (Hattemer-Frey et al. 1990). Based on an equation to estimate vegetative contamination, the total concentration of benzene on exposed food crops consumed by humans and used as forage by animals was estimated to be 587 ng/kg, 81% of which was from air-to-leaf transfer and 19% was from root uptake (Hattemer-Frey et al. 1990).

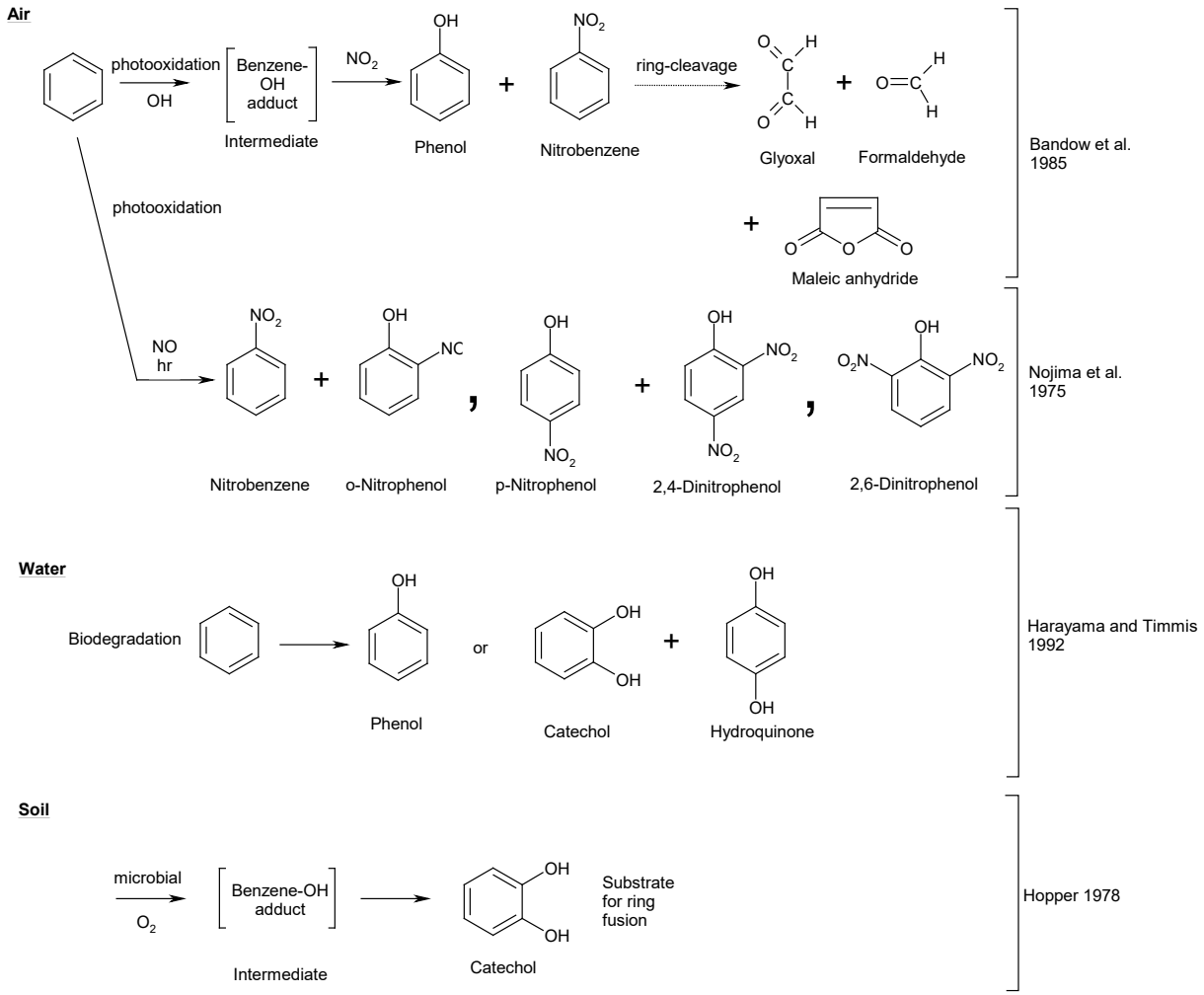
Benzene also accumulates in the leaves and fruits of plants. After 40 days, plants grown in benzene-rich environments showed bioaccumulation in the leaves and fruit that were greater than the air portioning coefficient of benzene in the atmosphere. Blackberries exposed to 0.313 ppm and apples exposed to 2.75 ppm contained about 1,000 and 36 ng/g of benzene, respectively (Collins et al. 2000).

5.4.2 Transformation and Degradation

Benzene undergoes a number of different transformation and degradation reactions in the environment as discussed in the following sections. The resulting environmental transformation products within different media are shown in Figure 5-2.

Air. Benzene in the atmosphere exists predominantly in the vapor phase (Eisenreich et al. 1981). The most important degradation process for benzene is its reaction with atmospheric hydroxyl radicals. The rate constant for the vapor phase reaction of benzene with photochemically produced hydroxyl radicals has been determined to be 1.3×10^{-12} cm^3/molecule-second (Gaffney and Levine 1979), which corresponds to a calculated residence time of 8 days at an atmospheric hydroxyl radical concentration of 1.1×10^6 molecules/cm^3, based on the equation reported by Lyman (1982). Using a hydroxyl radical concentration of 1×10^8 molecules/cm^3, corresponding to a polluted atmosphere (Lyman 1982), the calculated residence time is shortened to 2.1 hours. Benzene may also react with other oxidants in the atmosphere such as nitrate radicals and ozone; however, the rate of degradation is considered insignificant compared to the rate of reaction with hydroxyl radicals. Residence times of 472 years for rural atmospheres and 152 years for urban atmospheres were calculated for the reaction of benzene with ozone (O_3) using a rate constant of 7×10^{-23} cm^3/molecule-second (Pate et al. 1976) and atmospheric concentrations of 9.6×10^{11} O_3 molecules/cm^3 (rural) and 3×10^{12} O_3 molecules/cm^3 (urban) (Lyman 1982).

Figure 5-2. Environmental Transformation Products of Benzene in Various Media



Sources: Bandow et al. 1985; Harayama and Timmis 1992; Hopper 1978; Nojima et al. 1975

The reaction of benzene and nitric oxide in a smog chamber was investigated to determine the role of benzene in photochemical smog formation (Levy 1973). The results showed that benzene exhibited low photochemical smog reactivity in the four categories tested: rate of photooxidation of nitric oxide, maximum oxidant produced, eye-irritation response time, and formaldehyde formation. The study authors concluded that benzene probably does not play a significant role in photochemical smog formation (Levy 1973). In the presence of active species such as nitrogen oxides and sulfur dioxide, the rate of photodegradation of benzene in the gas phase was greater than that in air alone. Its half-life in the presence of such active species (100 ppm benzene in the presence of 10–110 ppm NO_x or 10–100 ppm SO_2) was 4–6 hours, with 50% mineralization to carbon dioxide in approximately 2 days (Korte and Klein 1982). The primary products of the reaction of benzene with nitrogen monoxide gas include

nitrobenzene, *o*- and *p*-nitrophenol, and 2,4- and 2,6-dinitrophenol (Nojima et al. 1975). Photooxidation of benzene in a nitrogen monoxide/nitrogen dioxide-air system formed formaldehyde, formic acid, maleic anhydride, phenol, nitrobenzene, and glyoxal (ethane-1,2-dione) (Bandow et al. 1985).

Direct photolysis of benzene in the atmosphere is not likely because the upper atmosphere effectively filters out wavelengths of light <290 nm, and benzene does not absorb wavelengths of light >260 nm (Bryce-Smith and Gilbert 1976).

Water. Benzene is subject to indirect photolysis in sunlit surface water but does not undergo direct photolysis. For direct photolysis to occur, a substance must absorb photons of light >290 nm. During indirect photolysis, light energy is absorbed by other constituents (photosensitizers) of the media (water, soil) and the excited species can then transfer energy to benzene (indirectly promoting it to an excited electronic state), or lead to the formation of reactive species, such as singlet oxygen or hydroxy radicals, which react with benzene. Humic and fulvic acids are well-known photosensitizing agents and are practically ubiquitous in natural waters. A half-life of 16.9 days was reported for photolysis of benzene dissolved in oxygen-saturated deionized water and exposed to sunlight (Hustert et al. 1981).

Benzene is readily degraded in water under aerobic conditions. Results of a biochemical oxygen demand (BOD) test determined that benzene was completely biodegradable after the second week of static incubation at 25°C at benzene concentrations of 5 and 10 mg/L using domestic wastewater as the microbial inoculum (Tabak et al. 1981). A study of the degradation of benzene by the microbial population of industrial wastewater at 23°C using a shaker flask system showed that after 6 hours, only 8% (4 mg/L) of the initial 50 mg/L dose of benzene remained (Davis et al. 1981). Water from a petroleum production site was successfully biotreated for complete removal of benzene using a flocculated culture of *Thiobacillus denitrificans* strain F and mixed heterotrophs (Rajganesh et al. 1995).

Microbial degradation of benzene in aquatic environments is influenced by many factors including microbial population, dissolved oxygen, nutrients, other sources of carbon, inhibitors, temperature, pH, and initial concentration of benzene. Vaishnav and Babeu (1987) reported biodegradation half-lives for benzene in surface water (river water) and groundwater of 16 and 28 days, respectively. Benzene was found to be resistant to biodegradation in surface water taken from a harbor and supplemented with either nutrients (nitrogen and phosphorus) or acclimated microbes; however, biodegradation did occur, with a half-life of 8 days, in surface water enriched with both nutrients and microbes (Vaishnav and Babeu 1987). At very high levels, as may be the case of a petroleum spill, benzene (and other compounds

contained in petroleum) is toxic to microorganisms and the rate of degradation is slow compared to low initial starting concentrations. In another study, Davis et al. (1994) observed rapid aerobic biodegradation of benzene in aquifer groundwater samples and measured times for 50% disappearance ranging from 4 days for an initial benzene concentration of 1 mg/kg to 14 days for an initial benzene concentration of 10 mg/kg. Under acidic conditions (pH 5.3, 20°C), benzene was completely microbially degraded in 16 days in groundwater taken from a shallow well (Delfino and Miles 1985).

The aerobic biodegradation of benzene is also influenced by the presence of other aromatic hydrocarbons. A bacterial culture grown with aromatic hydrocarbons plus nitrogen-, sulfur-, and oxygen-containing aromatic compounds was much less efficient in degrading benzene than the culture grown with aromatic hydrocarbons alone. Pyrrole strongly inhibited benzene degradation. Benzene degradation was high when either toluene or xylene were present (Arvin et al. 1989).

Laboratory studies on microbial degradation of benzene with mixed cultures of microorganisms in gasoline-contaminated groundwater revealed that both oxygen and nitrogen concentrations are major controlling factors in the biodegradation of benzene. Nitrogen enhanced the biodegradation rate of benzene 4.5-fold, over inoculum-enriched water alone. More than 95% of the benzene in groundwater was removed through microbial action within 73.5 hours (Karlson and Frankenberger 1989).

Benzene biodegradation under anaerobic conditions does not readily occur. When dissolved oxygen is depleted, an alternative electron acceptor such as nitrate, carbonate, or iron (III) must be available, and microbes capable of using the alternative electron acceptor to degrade the benzene must be present (McAllister and Chiang 1994). Using aquifer material obtained from a landfill from Norman, Oklahoma, no significant benzene biodegradation was reported during the first 20 weeks of incubation under anaerobic conditions at 17°C; however, after 40 weeks of incubation, benzene concentrations were reduced by 72 and >99% of the benzene was degraded after 120 weeks (Wilson et al. 1986). No degradation of benzene was observed in 96 days under anaerobic conditions (20°C) using raw water intake from a water treatment plant (Delfino and Miles 1985).

Use of water as an oxygen source in the anaerobic degradation of benzene has been demonstrated. Experiments indicated that incorporation of ^{18}O from ^{18}O-labeled water is the initial step in the anaerobic oxidation of benzene by acclimated methanogenic cultures. Phenol was the first major product (Vogel and Grbić-Galić 1986).

Sediment and Soil. Benzene is biodegraded in soil under aerobic conditions. This process has been well-described, as reviewed by Gibson (1980, 1977) and Hopper (1978). Microbial metabolism of benzene proceeds through the formation of *cis*-dihydrodiols and, with further metabolism, to catechols, which are the substrates for ring fission. *Pseudomonas putida* oxidized benzene through *cis*-1,2-dihydroxy-1,2-dihydrobenzene (Gibson 1977; Hopper 1978). A strain of *Rhodococcus* isolated from contaminated river sediment mineralized 71% of benzene at an initial concentration of 0.7 mg/L in 14 days (Malachowsky et al. 1994). The soil bacterium, *Nitrosomonas europaea*, catabolized benzene to phenol and hydroquinone (Keener and Arp 1994). Another mixotrophic bacteria, a strain of *Pseudomonas sp.* isolated from contaminated soil, grew under both anaerobic and aerobic conditions and used benzene for its growth (Morikawa and Imanaka 1993). Furthermore, *Norcardia sp*. and *Pseudomonas sp.* effectively degraded benzene to carbon dioxide within 7 days (45–90%) (Haider et al. 1981). The biodegradation of 2 mg of radiolabeled benzene in 100 g of soil with a mixed microbial population transformed 47% of the added radioactivity to carbon dioxide after 10 weeks (Haider et al. 1981). The study authors concluded that specific organisms that degrade benzene were present in the soil in only small numbers.

Limited benzene degradation was predicted in shallow sandy soil contaminated with gasoline from an leaking underground storage tank using ULTRA, a fate and transport model used to predict the environmental fate (Tucker et al. 1986). The ULTRA model predicted that only about 1% of the benzene in the gasoline would be degraded over a 17-month period, and 3% would remain in the soil (Tucker et al. 1986). Most of the benzene present in the soil from leaked gasoline was predicted to either volatilize (67%) or move into groundwater (29%).

Salanitro (1993) summarized the aerobic degradation rates for BTEX in laboratory subsoil-groundwater slurries and aquifers. Decay rates for benzene in laboratory microcosms were highest (19–52% per day) for benzene concentrations <1 ppm when initial dissolved oxygen levels were about 8 ppm. Rates were significantly reduced (0–1.1% per day) when benzene levels were 1–2 ppm, and no degradation was observed when benzene levels were >2 ppm. This is particularly relevant in the case of petroleum spills as high concentrations of petroleum compounds are toxic to organisms and decrease the rate of biodegradation.

Benzene has been shown to be anaerobically transformed by mixed methanogenic cultures derived from ferulic acid-degrading sewage sludge enrichments. In most of the experiments, benzene was the only semi-continuously supplied energy sources in the defined mineral medium (Grbić-Galić and Vogel 1987).

After an initial acclimation time of 11 days, at least 50% of the substrate was converted to CO_2 and methane. The intermediates were consistent with benzene degradation via initial oxidation by ring hydroxylation.

Edwards and Grbić-Galić (1992) discussed that where mixtures of benzene, toluene, xylenes, and ethylbenzene are present in an anaerobic environment, there is a sequential utilization of the substrate hydrocarbons, with toluene usually being the first to be degraded, followed by xylene isomers, then finally ethylbenzene and benzene. They proposed that benzene may not be degraded at all. However, in microcosm experiments, Edwards and Grbić-Galić (1992) demonstrated that benzene at initial concentrations of 40–200 μM degraded at rates ranging from 0.36 to 3.7 μM/day, depending upon substrate concentration and the presence of other carbon sources.

Other Media. Twenty-day-old spinach leaves placed in a hermetic chamber containing vapors of ^{14}C-labeled benzene were shown to assimilate benzene. The benzene was subsequently metabolized to various nonvolatile organic acids (Ugrekhelidze et al. 1997).

Bacterium strain, *Mycobacterium cosmeticum* byf-4, has been reported to aerobically biodegrade benzene, toluene, ethylbenzene, and *o*-xylene, simultaneously or individually, via mineralization and incorporation into cell materials (Zhang et al. 2013). Benzene alone or in a mixture of benzene, toluene, ethylbenzene, and *o*-xylene, at an initial concentration of 100 mg/L, was completely degraded within 36–42 hours.

5.5 LEVELS IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to benzene depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of benzene in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on benzene levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

Table 5-5 shows the lowest limit of detections that are achieved by analytical analysis in environmental media. An overview summary of the range of concentrations detected in environmental media is presented in Table 5-6.

Table 5-5. Lowest Limit of Detection Based on Standards[a]

Media	Detection limit	Reference
Air (ppbv)	0.002	EPA 2023b
Drinking water (ppb)	0.028	EPA 2013
Surface water and groundwater (ppb)	0.0067	USGS 2003
Soil (ppb)	0.009	EPA 1996
Sediment (ppb)	0.009	EPA 1996
Whole blood (ppb)	0.024	CDC 2022a

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

Table 5-6. Summary of Environmental Levels of Benzene

Media	Low	High[a]	For more information
Outdoor air (ppbv)	0.082	46.6	Section 5.5.1
Indoor air (ppbv)	<0.02	140	Section 5.5.1
Surface water (ppb)	0.038[b]	7.8	Section 5.5.2
Groundwater (ppb)	0.01	27,000	Section 5.5.2
Drinking water (ppb)	0.02	923	Section 5.5.2
Soil (ppb) (2023 data)	ND	0.026	section 5.5.3
Food (ppb)	ND	190	section 5.5.4

[a]High levels may be representative of monitoring data at localized contaminated sites and may not be reflective of background environmental levels.
[b]Surface water data were limited; this value represents the average level detected in 2023. The lowest level detected was not reported.

ND = not detected (and detection limit not specified)

Detections of benzene in air, water, and soil at NPL sites are summarized in Table 5-7.

Table 5-7. Benzene Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
Water (ppb)	34	82.5	1.25	2	432
Soil (ppb)	1,890	1,440	75.3	261	183

Table 5-7. Benzene Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
Air (ppbv)	3.72	9.11	25.1	224	139

[a]Concentrations found in ATSDR site documents from 1981 to 2022 for 1,868 NPL sites (ATSDR 2022a). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

5.5.1 Air

Benzene is ubiquitous in the atmosphere. It has been identified in outdoor air samples of both rural and urban environments and in indoor air, and concentrations vary seasonally (Kinney et al. 2002). However, ambient air concentrations, particularly in urban environments, have decreased in the past few decades.

In California, motor vehicle exhaust accounted for over 70% of the nonsmoking population's exposure to ambient benzene (Cal EPA 1987). The 1984 population-weighted average benzene concentration in California was estimated to be 3.3 ppbv (Cal EPA 1987). Benzene emissions in a Los Angeles roadway tunnel were measured at a concentration of 382 mg/L (118,420 ppmv) (Fraser et al. 1998). New regulations on benzene content in gasoline have dramatically reduced these levels (EPA 1995b, 2007, 2023a). The California EPA estimated that gasoline-attributed benzene decreased 70–80% between 1996 and 2014, and an updated statewide population-weighted average benzene concentration of approximately 0.25 ppbv was estimated (Cal EPA 2018).

Nationally, a decline in ambient air benzene of 66% has been reported between 1996 and 2009 (EPA 2014). Table 5-8 provides a comparison of benzene levels in outdoor air in various cities in the United States from data collected prior to implementation of national regulations reducing benzene content in gasoline, which were identified as primarily influenced by mobile sources (EPA 1987), to more recent data. This is a broad historical comparison for these urban areas; specific monitoring site locations and site types between the studies were not compared.

Table 5-8. Historical Comparison of Benzene Levels in Urban Air

Location	Year	Concentration (ppbv)	Reference
San Francisco, California	1984	0.8–5.2 (range); 2.6±1.3[a]	Wester et al. 1986
	2020–2023[b]	NS–1.39 (range); 0.110[d]	EPA 2023b[c]
St. Louis, Missouri	Summer 1985	0.63–12.1 (range); 1.85[d]	EPA 1987[c]
	2020–2023[b]	NS–0.52 (range); 0.17[d]	EPA 2023b[c]
Philadelphia, Pennsylvania	Summer 1985	0.32–3.0 (range); 1[d]	EPA 1987[c]
	2020–2023[b]	NS–9.43 (range); 0.18[d]	EPA 2023b[c]
New York (Manhattan), New York	Summer 1986	0.88–5.3 (range); 1.75[d]	EPA 1987[c]
	2020–2023[b]	NS–0.62 (range); 0.20[d]	EPA 2023b[c]
Boston, Massachusetts	1990–1991	0.69–3.1; 1.06[d]	Kelly et al. 1993
	2020–2023[b]	NS–0.37; 0.14[d]	EPA 2023b[c]
New York (Staten Island), New York	Spring 1984	0.1–34 (range); 4.4±6.6[a]	Singh et al. 1985
	2020–2023[b]	NS–0.93 (range); 0.20[d]	EPA 2023b[c]

[a]Average±standard deviation.
[b]As of October 26, 2023.
[c]Values were originally reported in parts per billion carbon (ppbC) and have been converted to ppbv benzene. ppbv = $ppbC_{benzene}$/six carbons.
[d]Median.

NS = not stated

Benzene is a pollutant monitored for in the national Air Quality System (AQS) database, which contains ambient air pollution data collected by EPA, state, local, and tribal air pollution control agencies from monitors throughout the country. Table 5-9 shows the yearly mean 24-hour percentile distributions of benzene at monitoring stations across the United States from 2019 to 2023. The maximum observed concentration for this time period (46.6 ppbv) was recorded from an industrial monitor in Detroit, Michigan in 2020. This value appears to be an outlier, as it was twice as high as the next highest monitoring concentration recorded in 2020, and the maximum concentration observed for the Detroit site in 2021 was of similar magnitude to other sites across the United States (data not shown).

Table 5-9. Summary of Annual Concentrations of Benzene (ppbv) Measured in Ambient Air at Locations Across the United States[a,b]

Year	Number of monitoring locations	Number of samples	Average	10th Percentile	50th Percentile	75th Percentile	95th Percentile	Maximum
2019	136	7,610	0.197	0.083	0.156	0.223	0.494	11.4
2020	172	9,237	0.217	0.082	0.166	0.254	0.485	46.6
2021	178	12,534	0.181	0.083	0.161	0.228	0.386	3.83

Table 5-9. Summary of Annual Concentrations of Benzene (ppbv) Measured in Ambient Air at Locations Across the United States[a,b]

Year	Number of monitoring locations	Number of samples	Average	10th Percentile	50th Percentile	75th Percentile	95th Percentile	Maximum
2022	131	3,291	0.177	0.087	0.155	0.221	0.394	1.68
2023[c]	117	2,613	0.176	0.099	0.158	0.211	0.363	3.01

[a]Values were originally reported in parts per billion carbon (ppbC) and have been converted to ppbv benzene.
[b]24-hour sampling period.
[c]As of October 26, 2023.

Source: EPA 2023b

Dickinson et al. (2022) reported benzene air concentrations from wildfires within a 0–61-mile radius of the fire's origin. In 2019, benzene was detected in the air at mean concentrations of 3.968±6.287 ppbv (range: 0.042–25.000 ppbv) for the Idaho Nethkar wildfire and 0.446±0.167 ppbv (range: 0.165–0.668 ppbv) for the Washington state Williams Flats wildfire. In 2020, benzene air levels were measured at mean concentrations of 0.283±0.181 ppbv (range: 0.024–0.596 ppbv) for the Washington state Chief Timonthy wildfire and 1.826±1.781 ppbv (range: 0.104–4.000 ppbv) for the Idaho Whitetail Loop wildfire.

Benzene has been detected in indoor air. EPA's compilation of 14 studies of background indoor air concentrations found a 31–100% detection rate for benzene in 2,615 U.S. resident samples between 1990 and 2005 (EPA 2011). The background medians ranged from below the reporting level (0.05–1.6 μg/m^3; 0.02–0.50 ppbv) to 4.7 μg/m^3 (1.5 ppbv), with 95th percentiles of 9.9 to 29 μg/m^3 (3.1–9.2 ppbv), and maximum values between 21 and 460 μg/m^3 (6.6–140 ppbv). During the Detroit Exposure and Aerosol Research Study, which measured daily average ambient air concentrations of benzene from 2004 to 2007 in Detroit, Michigan, the mean daily ambient air concentrations were 1.3–4.1 μg/m^3 (0.4–1.3 ppbv; n=1,483) and the mean daily indoor air concentrations were 2.3–6.0 μg/m^3 (0.7–1.8 ppbv; n=934) (George et al. 2011).

Indoor air monitoring conducted in Detroit in November and December 2006, reported an average of 3±5.7 μg/m^3 benzene (0.9±1.8 ppbv) (Johnson et al. 2010). The ratio of indoor to outdoor air concentrations was 1.2, indicating that indoor air sources had a greater influence on the concentrations, likely a seasonal effect due to use of gas appliances and heaters and reduced ventilation. Smoking in homes was considered via questionnaire, but results differentiated by smoking versus nonsmoking homes

were not reported. These levels were comparable to benzene levels of 3 μg/m^3 (0.9 ppbv) measured in the indoor air of a newly built home (Sasahara et al. 2007).

Between December 2003 and April 2006, benzene was detected at an average of 4.07±5.94 μg/m^3 (1.27±1.86 ppbv; 76% detection rate; range: <0.64–42 μg/m^3 or 0.20–13 ppbv) in New Jersey suburban and rural homes (Weisel et al. 2008). Few smokers were included in this study; the likely source of benzene was evaporative emissions from gasoline in cars housed in attached garages. Benzene was detected in the living rooms of two homes in Alaska between 1 and 25 ppbv, likely due to gasoline stored in garages outside of homes (Isbell et al. 2005). In a highly industrialized area of southeast Chicago, benzene was detected at an average of 4.1 μg/m^3 (1.3 ppbv) in homes (Van Winkle and Scheff 2001).

In the winter of 1999, mean benzene levels in New York City were 0.80±0.44 ppbv for outdoor (home) air, 1.8±2.2 ppbv for indoor (home) air, and 1.4±1.0 ppbv for personal air (Kinney et al. 2002). During the summer of 1999, mean benzene levels in New York City were 0.41±0.32 ppbv for outdoor (home) air, 0.53±0.27 ppbv for indoor (home) air, and 1.0±0.7 ppbv for personal air (Kinney et al. 2002). A review reported the benzene results of residential outdoor, indoor, and personal air monitoring from several studies conducted from the summer of 1999 to the spring of 2001 (Weisel 2010). Mean benzene values of 0.46±0.52, 0.84±1.3, and 0.93±1.4 ppbv for outdoor, indoor, and personal air, respectively, were reported for nonsmoking residences in Elizabeth, New Jersey. In Houston, Texas, the mean benzene values for nonsmoking households were 0.86±1.78, 1.6±2.0, and 1.6±1.6 ppbv for outdoor, indoor, and personal air, respectively. Additionally, nonsmoking residences in Los Angeles, California had mean benzene values of 0.84±0.78, 1.0±1.7, and 1.0±2.2 ppbv for outdoor, indoor, and personal air, respectively. The review did not discuss potential sources of indoor benzene pollution in-depth but noted that personal exposures were higher than indoor air exposures.

Residential heating oil is expected to be a source of benzene indoor air pollution. According to the New York Department of Health, the air from about 50% of oil fuel heated homes between the years 1997 and 2003 contained benzene concentrations ≥2.2 μg/m^3 (0.69 ppbv) inside the homes and 1.5 μg/m^3 (0.47 ppbv) in the area outside the homes (NYSDOH 2005). Concentrations in indoor air from 27 homes in Maine that heat with #2 fuel oil with basement tanks or with K1 with outside tanks were 0.39–13.3 μg/m^3 (0.12–4.16 ppbv) (Maine DEP 2014).

A significant source of indoor air benzene pollution is from residences with individuals who smoke tobacco inside the home. One study reported that a median level of benzene in 185 homes without

smokers was 2.2 ppbv and a median level of benzene in 343 homes with one or more smokers was 3.3 ppbv (Wallace 1989a). This finding points to the possible significance of passive smoking as a source of benzene exposure. A study conducted by R.J. Reynolds Tobacco Company in smoking and nonsmoking homes revealed that benzene levels were elevated in smoking homes. In 24 nonsmoking homes, the mean benzene concentration was 1.21 ppbv, with a maximum of 5.93 ppbv. In 25 smoking homes, the mean benzene concentration was 1.73 ppbv, with a maximum of 8.44 ppbv. However, benzene was not significantly correlated or associated with 3-ethenylpyridine, a proposed vapor-phase environmental tobacco smoke marker (Heavner et al. 1995).

Limited monitoring values for commercial indoor air are available. A study conducted from the summer of 2003 to the winter of 2005 reported geometric means of 0.55 ppbv benzene in air of U.S. stores and 0.96 ppbv benzene in air of U.S. restaurants (Loh et al. 2006). Concentrations in restaurants were influenced by the presence of smokers and would therefore be expected to be lower today due to restrictions on smoking in public places.

Benzene may be present in indoor air by vapor intrusion from contaminated groundwater into buildings. The EPA (2016c) includes benzene in its Vapor Intrusion Screening Levels (VISL) Calculator, indicating that it is sufficiently volatile and sufficiently toxic to be considered a concern for vapor intrusion from soil water and groundwater. Accordingly, ATSDR (2016) recommends that health assessors should evaluate potential health implications of vapor intrusion for benzene during site risk assessments. However, there are many other background sources of benzene to indoor air pollution, and several site assessments did not find vapor intrusion to be the main source of benzene.

At a former naval air station and hazardous waste disposal site in California, the highest indoor air level of benzene was 1.18 μg/m^3 (0.369 ppbv) in an aircraft engine facility; vapor intrusion was supported as a source of benzene based on detections in groundwater and soil gas, but these concentrations were not provided in this report (ATSDR 2022c).

In Indiana, possible vapor intrusion from a plume of contaminated groundwater into commercial buildings was investigated. Benzene concentrations were <0.13–3.6 μg/m^3 (<0.04–1.1 ppbv) in the sub-slab gas and 1.7–9.8 μg/m^3 (0.53–3.1 ppbv) in soil below a recycling center, gymnastics center, and ambulance company and electrical contractor (ATSDR 2023a). Adjusted indoor air concentrations (to reflect exposure for 5 days/week, 10 hours/day) were <0.13–1.0 μg/m^3 (<0.04–0.31 ppbv). The ratio of

underground benzene to indoor air benzene was 13 and not suggestive of an underground source at this site, since ratios >33 indicate underground source contribution.

Benzene concentrations in soil gas of a former cleaning products manufacturing site and current Superfund site in Texas ranged from below the detection limit to 96 μg/m^3 (30 ppbv), 0.58–7.7 μg/m^3 (0.18–2.4 ppbv) in indoor air, and 0.3 μg/m^3 (0.09 ppbv) in outdoor air onsite; concentrations in off-site soil gas were 0.1–29 μg/m^3 (0.03–9.1 ppbv) and concentrations in indoor air were 0.3–0.42 μg/m^3 (0.09–0.13 ppbv) (ATSDR 2023b). Vapor intrusion was not likely the source of benzene to indoor air at this site. Near another Superfund site that was contaminated with dry cleaner solvents in Tennessee, a restaurant adjacent to the site had benzene in soil gas up to 9.9 μg/m^3 (3.1 ppbv) and was not detected (limit of detection [LOD]: 0.48 μg/m^3; 0.15 ppbv) in the indoor air of the restaurant (ATSDR 2021).

Townhomes in Minnesota were investigated from possible vapor intrusion due to underlying groundwater contamination from a nearby NPL site (ATSDR 2023c). Benzene air levels measured in these townhomes between May and July of 2015 were 1.2–5.3 μg/m^3 (0.36–1.7 ppbv) in indoor air, 1.3–4.5 μg/m^3 (0.41–1.4 ppbv) in crawl space air, and 2.3–4.4 μg/m^3 (0.71–1.4 ppbv) in sub-slab gas. Measured benzene air levels in outdoor air near townhomes over the same time period were 0.35–0.76 μg/m^3 (0.12–0.24 ppbv). Pollution was determined likely to be from indoor sources rather than vapor intrusion (ATSDR 2023c).

Ambient air is impacted by nearby hazardous waste sites, oil spills, or other accidental releases of hazardous materials. Benzene was measured in the vicinity of the Bridgeton landfill, a solid waste landfill in Missouri, at a maximum concentration of 32.5 ppbv (ATSDR 2022b). In the 5 months following the 2010 Deepwater Horizon oil spill, mean benzene concentrations were 4.83 μg/m^3 (1.51 ppbv; range: 0.12–81.89 μg/m^3; 0.04–25.63 ppbv) in regional areas and 2.96 μg/m^3 (0.927 ppbv; range: 0.14–290 μg/m^3; 0.04-–90.8 ppbv) in coastal areas of Louisiana (IARC 2018). On February 3, 2023, a freight train carrying hazardous materials derailed in East Palestine, Ohio. Some of the cars caught fire, while others spilled their loads into an adjacent stream. In air samples collected at the train derailment site between February and August 2023, benzene was detected at a median of 0.175 ppbv (range: 0.072–3.76 ppbv) (EPA 2023c; Oladeji et al. 2023).

Ambient air is also impacted by nearby oil and gas development. Outdoor monitoring of residential areas in the Dallas/Fort Worth metro area was conducted varying distances from urban drilling and unconventional shale gas exploration and production operations (Rich and Orimoloye 2016). Five of the

six counties monitored were classified as non-attainment areas during the time of sampling. The mean benzene concentration was 18.53±83.75 ppbv (range: 0.6–592 ppbv). Sampling sites were selected to avoid influence of gas station and roadway emissions, and the drilling operations were believed to be the primary source of the benzene (Rich and Orimoloye 2016).

Outdoor air samples were collected between May and December 2019 at an elementary school 1,400 feet north of a wellsite in Colorado (CDPHE 2020). Benzene in air was detected in 93% of all samples, at maximum concentrations of 2.28 ppbv for the baseline measurements (after wells had been drilled but not completed), 2.91 ppbv during hydraulic fracturing, 2.57 ppbv during milling, and 4.52 and 14.72 ppbv during two periods of hybrid-flowback production.

As part of a 2009 air toxics monitoring initiative, the outdoor air of selected schools with a mix of pollution sources were monitored for 60-day periods (EPA 2016a). In the case of Birmingham, Alabama, additional nearby communities near industry, major highways, or urban areas were also monitored. A summary of the monitoring results is presented in Table 5-10. EPA used a screening level of 9.3 ppbv (30 μg/m^3) to identify potential concern for risk of health problems from short- term exposures; the majority of the samples were below this value.

Table 5-10. Summary of Benzene in Outdoor Air (ppbv) Near Selected Schools Across the United States[a,b]

Location	Sampling period	Number of samples	Median	Range	Percent detected[c]
Birmingham, Alabama					
Lewis Elementary School	July 2009–November 2009	22	0.349	0.088–7.01	64%
North Birmingham Elementary School	July 2009–November 2009	24	0.444	0.081 –9.42	71%
Riggins School	July 2009–December 2009	27	1.610	0.131–9.55	37%
Additional community monitoring					
Collegeville	June 2011–August 2012	72	0.427	0.113 –6.86	83%
Fairmont	June 2011–August 2012	75	0.905	0.097–17.1	87%
Harriman Park	June 2011–August 2012	71	0.789	0.163–7.12	85%
North Birmingham	June 2011–August 2012	73	0.319	0.117–6.39	84%
Tarrant City, Alabama					
Tarrant Elementary School	July 2009–November 2009	22	0.363	0.078–7.26	95%

Table 5-10. Summary of Benzene in Outdoor Air (ppbv) Near Selected Schools Across the United States[a,b]

Location	Sampling period	Number of samples	Median	Range	Percent detected[c]
Los Angeles, California					
Felton Elementary School	August 2009–Mar 2010	23	0.398	0.163–1.12	87%
Soto Street Elementary School	August 2009–Mar 2010	37	0.520	0.211–0.986	41%
East Chicago, Indiana					
Abraham Lincoln Elementary School	August 2009–December 2009	13	0.149	0.094–0.319	77%
Gary, Indiana					
Jefferson Elementary School	August 2009–December 2009	16	0.186	0.101–0.304	63%
Ashland, Kentucky					
Charles Russell Elementary School	July 2009–April 2010	25	0.260	0.149–2.35	52%
Crabbe School	July 2009–April 2010	25	0.329	0.176–4.23	48%
Hatcher School	July 2009–April 2010	25	0.274	0.150–2.36	48%
Paulsboro, New Jersey					
Paulsboro High School	August 2009–February 2010	27	0.341	0.183–0.592	44%
Elizabeth, New Jersey					
Mabel Holmes Middle School	August 2009–January 2010	25	0.229	0.145–0.601	48%
New York City, New York					
Intermediate School 143	July 2009–June 2010	25	0.360	0.252–0.642	44%
Wauseon, Ohio					
Elm Street Elementary School	September 2009–November 2009	12	0.180	0.072–0.360	83%
Ironton, Ohio					
Whitwell Elementary School	July 2009–April 2010	25	0.239	0.150–0.770	40%
Warren, Ohio					
Life Skills of Trumbull County and Academy of Arts and Humanities	August 2009–February 2011	36	0.346	0.158–0.917	56%
Portland, Oregon					
Harriet Tubman Middle School	August 2009–November 2009	13	0.242	0.135–0.704	92%
Clairton, Pennsylvania					
Clairton Educational Center	September 2009–December 2009	18	0.526	0.097–2.95	56%
McKeesport, Pennsylvania					
South Allegheny Middle/High School	August 2009–April 2012	59	0.905	0.094–18.9	80%

Table 5-10. Summary of Benzene in Outdoor Air (ppbv) Near Selected Schools Across the United States[a,b]

Location	Sampling period	Number of samples	Median	Range	Percent detected[c]
McKees Rock, Pennsylvania					
Sto-Rox Elementary School and Sto-Rox Middle School	August 2009–January 2010	22	0.181	0.088–0.488	45%
North Charleston, South Carolina					
Chicora Elementary School	July 2009–March 2010	40	0.314	0.146–0.679	25%
Dallas, Texas					
NW Harllee Elementary School	September 2009–December 2009	15	0.194	0.124–0.711	67%
Deer Park, Texas					
San Jacinto Elementary School and Deer Park Junior High School	September 2009–May 2012	56	0.534	0.135–3.79	79%
Houston, Texas					
Young Scholars Academy	September 2009–December 2009	16	0.267	0.226–0.457	63%
Seattle, Washington					
Concord Elementary School	August 2009–November 2009	19	0.284	0.154–0.545	68%
Follansbee, West Virginia					
Follansbee Middle School and Jefferson Primary School	August 2009–August 2012	66	0.826	0.129–6.32	73%
Durango, Colorado (tribal area)					
Sunnyside Elementary School	August 2009–November 2009	15	0.129	0.072–0.256	93%
Lapwai, Idaho (tribal area)					
Lapwai High School	September 2009–December 2009	16	0.152	0.078–0.326	81%

[a]Values were originally reported in micrograms per cubic meter ($\mu g/m^3$) and converted to ppbv.
[b]24-hour sampling period.
[c]Minimum limit of detection: 0.0006 ppbv.

Source: EPA 2016a

5.5.2 Water

The EPA maintains a Water Quality Portal (WQP) database which aggregates air monitoring data from the National Water Information System (NWIS) and STORage and RETrieval (STORET) system. A summary of the data for ambient surface and groundwater from recent years are reported in Table 5-11

(WQP 2023). Benzene was detected at higher concentrations and at greater frequency in groundwater than in surface water.

Table 5-11. Summary of Benzene Concentrations (ppb) in Surface and Groundwater Across the United States

Year	Average	Maximum	Number of samples	Percent detected
Surface water				
2019	0.420	7.8	1,279	9.1%
2020	0.473	1.23	824	7.4%
2021	0.012	0.014	554	0.7%
2022	0.184	0.5	448	0.7%
2023[a]	0.038	0.04	86	2.3%
Groundwater				
2019	5.00	300	1,812	4.1%
2020	159	4,580	1,313	4.9%
2021	1,407	27,000	1,729	15.7%
2022	1,003	24,500	2,774	16.1%
2023[a]	765	16,800	1,126	15.6%

[a]As of December 7, 2023.

Source: WQP 2023

The U.S. Geological Survey (USGS) conducted a national assessment of 55 VOCs in well water samples collected from 2,401 domestic wells around the country during 1985–2002. Benzene was detected in 37 of 1,208 well samples, or 3.1% of the samples, at concentrations mostly <1 μg/L (Rowe et al. 2007). During another assessment of principal aquifers in the United States conducted from 1991 to 2010, benzene was detected in 2.05% of areas sampled that are used for drinking water, 1.47% of shallow groundwater beneath agricultural land, and 5.80% of shallow groundwater beneath urban land (USGS 2014). The median detected concentration was 0.035 ppb (range: 0.01–290 ppb).

In a USGS-conducted groundwater quality monitoring campaign, benzene was detected in 5 of the 336 samples collected between 2013 and 2016 from wells across the United States: 0.014 ppb in one well in South Carolina, 0.021 ppb in one well in Kansas, 0.052 in one well in Arkansas, 0.244 ppb in one well in Oklahoma, and 1.35 ppb in one well in Tennessee (USGS 2020).

Benzene is not a common contaminant of municipal water. EPA conducted their third 6-year compliance monitoring assessment of public water supplies between 2006 and 2011 (EPA 2016b). Benzene was detected in 0.31% of the 372,470 public water supply samples analyzed, with a median value of 0.8 ppb (range: 0.02–230 ppb).

Drinking water can be impacted indirectly by forest fires. Following the 2017 Tubbs Fire in California, benzene was detected at an average of 11.0 ppb in municipal water of Santa Rosa city (maximum: 40,000 ppb 6 months after the fire) during sampling 1–20 months after the fire (Proctor et al. 2020a, 2020b). Eight months following the 2018 Camp Fire in California, benzene was detected at a maximum of 923 ppb in municipal waters. The study authors suggested that thermal degradation of plastic pipes may have released benzene into the water system. Another study following the 2018 Camp Fire reported a mean of 18.97±75.26 ppb benzene in municipal water sampled in Paradise, California, from December 2018 to May 2020 (Solomon et al. 2021).

Proximity to hazardous waste sites, oil spills, or other accidental releases of hazardous materials may result in increased benzene in surface water, groundwater, and drinking water derived from these local sources, depending on the site uses. During a site assessment of a former army air and ground base in Port Heiden, Alaska, benzene was reported in groundwater near a wastewater drainage pond and near a former fuel pipeline, at a maximum concentration of 9.4 ppb (ATSDR 2019a). The groundwater near the pond site was not used for drinking water but some wells near the fuel pipe previously were. Other samples collected near the fuel pipeline corridor ranged from not detected to <0.15 ppb (reporting limit) benzene in groundwater and a maximum of 0.98 ppb benzene in surface water (ATSDR 2019a). Benzene was not detected in public drinking water on St. Lawrence Island, Alaska, after investigation based on concerns of fuel contaminants resulting from the former military surveillance and communications station (ATSDR 2020).

Benzene was detected at a maximum of 7.65 ppb in drinking water sourced from groundwater wells and springs impacted by natural gas drilling in Pennsylvania after unauthorized release of chemicals in order to recover a lost drill bit (ATSDR 2019b). At a former truck terminal and tank-trailer cleaning facility NPL site in New Jersey, which disposed of wastewater in an unlined lagoon and underground tanks onsite, benzene was detected at 92 ppb in monitoring wells near the former lagoon area and in one surface water sample (n=17) at 1.80 ppb at off-site locations along the Grand Sprute Run tributary (ATSDR 2023d).

Benzene was detected at 0.5 ppb in only one groundwater sample used for drinking water near the Dorado NPL site in Puerto Rico (ATSDR 2023e). It was not detected in the aqueduct used for drinking water distribution. Benzene was not detected in groundwater samples (n=82) collected between 2018 and 2020 at the Palermo Wellfield Superfund site (WQP 2023), nor was it detected in surface water samples (reporting limits: 0.27–100 ppb) collected in February 2023 at the East Palestine, Ohio train derailment site (EPA 2023c).

Historically, benzene has been detected in water in the vicinity of industrial facilities using or producing benzene from <1 ppb to a high of 179 ppb (found in plant effluent). In general, benzene in plant effluents quickly dispersed in rivers or streams to levels of 1–≤2 ppb (EPA 1979). Benzene concentrations monitoring wells within aquifers near fuel spills at gasoline service stations were 1,200–19,000 ppb (Salanitro 1993). A monitoring well in the vicinity of a bulk storage facility had a maximum benzene level of 45,000 ppb (Salanitro 1993). In northern Virginia, approximately 200,000 gallons of liquid hydrocarbons were released from a fuel-storage terminal into the underlying soil. A dichloromethane extract of groundwater from a monitoring well in the same area gave a benzene concentration of 52.1 ppm (Mushrush et al. 1994). Benzene has been detected at concentrations of 16–110 ppb in landfill leachate from a landfill that accepted both municipal and industrial wastes (Cline and Viste 1985).

Marine water contains high benzene concentrations when an oceanic oil spill occurs. In water column samples obtained from the Deepwater Horizon oil spill plume, benzene concentrations were 0.4–21.7 ppb at ~1,100 m depths; however, benzene was not detected at depths less than 1,000 m (Reddy et al. 2012).

5.5.3 Sediment and Soil

Limited ambient soil and sediment monitoring data are available. Benzene was not detected in sediment (n=52) and soil (n=14) samples reported between 2019 and 2023 in the EPA Water Quality Portal database (WQP 2023).

Some recent data are available regarding the presence of benzene at hazardous waste sites. Benzene was reported in soil near the marine terminal area of a former army air and ground base in Port Heiden, Alaska, at a maximum of 0.026 ppb (ATSDR 2019a). At an NPL site of a former truck terminal with tank-trailer cleaning in New Jersey, which disposed of wastewater in an unlined lagoon and underground tanks onsite, benzene concentrations were 0.03–39 ppm in sediment samples at off-site locations along the Grand Sprute Run tributary (ATSDR 2023d). Benzene was not detected in sediment samples (n=5)

collected in 2021 at the Palermo Wellfield Superfund Site (WQP 2023). Benzene was not included in soil analysis at the East Palestine, Ohio, train derailment site (EPA 2023c).

Historically, benzene levels of <2–191 ppb in soil were recorded in the vicinity of industrial facilities using or producing benzene (EPA 1979). In northern Virginia, following the release of approximately 200,000 gallons of liquid hydrocarbons from a fuel-storage terminal into the underlying soil, benzene was detected in soil at a concentration of 1,500 ppm at a depth of 10 feet, about 1,000 feet from the storage terminal (Mushrush et al. 1994).

5.5.4 Other Media

Benzene has been detected in a variety of food and beverages. Benzene may form in food and beverages from benzoate salts (e.g., sodium benzoate and potassium benzoate), which are added as a preservative or are naturally present such as in some fruits, and ascorbic acid (Vitamin C), which may also be added or be naturally present (Meadows 2006; Medeiros Vinci et al. 2012). Conditions such as low sugar, low acidity, and storage conditions under strong light and higher temperatures promote benzene formation; changing the product formulation and storage conditions successfully reduces the likelihood of benzene contamination (Salviano Dos Santos et al. 2015). Foods may also absorb benzene during processes such as smoking (Medeiros Vinci et al. 2012) or from VOCs released during cooking with oil (Medeiros Vinci et al. 2012; Pellizzari et al. 1995).

A summary of the available data for benzene in food and beverages is reported in Table 5-12. The most recent large-scale study in the United States was an FDA-sponsored 5-year study of table-ready foods from 1996 to 2000; foods tested included both foods that were purchased uncooked (and cooked prior to testing) as well as ready-to-eat food purchases (Fleming-Jones and Smith 2003). Benzene was detected in all sampled foods except for American cheese and vanilla ice cream. More recent data were available from a sampling of Belgian supermarkets in 2010 (Medeiros Vinci et al. 2012). Selected products not otherwise covered by Fleming-Jones and Smith (2003), and products with high instances of detection are reported in Table 5-12; the products with the highest detected benzene were smoked, canned, and raw fatty fish, while non-fatty fish and raw meat had lower levels.

Table 5-12. Summary of Benzene in Food and Beverages

Location (sampling period)	Product	Mean (ppb)	Range (ppb)	Notes	Source
United States, 1996–2000	Cheddar cheese		20–47	Detected in 2 samples	Fleming-Jones and Smith 2003[a]
	American cheese		–	Not detected in an unknown number of samples	
	Mixed nuts		1–6	Detected in 3 samples	
	Ground beef		9–190	Detected in 12 samples	
	Pork bacon		2–17	Detected in 6 samples	
	Banana, raw		11–132	Detected in 13 samples	
	Frankfurters, beef		2–11	Detected in 4 samples	
	Cream cheese		1–17	Detected in 3 samples	
	Chocolate cake icing		2–23	Detected in 2 samples	
	Tuna canned in oil		4–13	Detected in 7 samples	
	Fruit flavored cereal		2–21	Detected in 5 samples	
	Eggs scrambled		2–40	Detected in 4 samples	
	Peanut butter		2–25	Detected in 5 samples	
	Avocado, raw		3–30	Detected in 10 samples	
	Popcorn, popped in oil		4–22	Detected in 3 samples	
	Blueberry muffin		3–8	Detected in 3 samples	
	Strawberries, raw		1	Detected in 1 sample	
	Cola carbonated		1–138	Detected in 3 samples	
	Orange, raw		11–15	Detected in 2 samples	
	Coleslaw with dressing		11–102	Detected in 14 samples	
	Sweet roll Danish		3	Detected in 1 sample	
	Potato chips		2–7	Detected in 2 samples	
	Fruit flavored sherbet		3–61	Detected in 3 samples	
	Quarter pound hamburger cooked		4–47	Detected in 11 samples	
	Margarine		7	Detected in 1 sample	
	Sandwich cookies		1–39	Detected in 3 samples	
	Butter		4-22	Detected in 6 samples	
	Chocolate chip cookies		1–8	Detected in 2 samples	
	Sour cream		3–15	Detected in 2 samples	
	Apple pie fresh/frozen		2–11	Detected in 4 samples	
	Chicken nuggets fast food		2–5	Detected in 4 samples	
	Graham crackers		1–9	Detected in 2 samples	
	French fries, fast food		2–56	Detected in 3 samples	
	Cheeseburger quarter pound		5–44	Detected in 8 samples	
	Cheese pizza		1–2	Detected in 2 samples	
	Bologna		2–44	Detected in 4 samples	

Table 5-12. Summary of Benzene in Food and Beverages

Location (sampling period)	Product	Mean (ppb)	Range (ppb)	Notes	Source
	Cheese and pepperoni pizza		8–30	Detected in 4 samples	
	Olive/safflower oil		1–46	Detected in 6 samples	
	Sugar cookies		8–30	Detected in 3 samples	
	Cake doughnuts with icing		3	Detected in 2 samples	
	Vanilla ice cream		–	Not detected in an unknown number of samples	
	Popsicle		1–10	Detected in 4 samples	
Belgium, 2010	Ready for consumption beverages	1.30	NS–11.52	Detected in 14/28 samples	Medeiros Vinci et al. 2012
	Smoothies and juices	–	0.48	Detected in 1/9 samples	
	Coffee, tea, and infusions	0.21	NS–1.33	Detected in 2/12 samples	
	Water (carbonated)	–	–	Not detected in 3 samples	
	Sauces	1.18	NS-–6.35	Detected in 11/14 samples	
	Dips and tapenades	1.44	NS–6.48	Detected in 15/17 samples	
	Non-refined oils	2.19	NS–5.04	Detected in 11/12 samples	
	Refined oils	0.74	NS–2.55	Detected in 11/11 samples	
	Fresh fruit	–	–	Not detected in 10 samples	
	Fresh vegetables	–	–	Not detected in 10 samples	
	Canned fruits and vegetables	–	–	Not detected in 11 samples	
	Smoked fish	18.90	NS–76.21	Detected in 10/10 samples	
	Canned fish	7.40	NS–20.81	Detected in 15/16 samples	
	Raw fatty fish	3.15	NS–9.44	Detected in 4/4 samples	
	Raw non-fatty fish	0.52	NS–2.00	Detected in 2/6 samples	
	Processed (dried/cured/fermented) meat	1.77	NS-–6.95	Detected in 6/7 samples	
	Raw meat	–	1.25	Detected in 1/4 samples	
	Fresh eggs	–	–	Not detected in 9 samples	
	Chilled ready to eat meals	1.11	NS-3.68	Detected in 11/14 samples	
	Ready to eat salads	2.79	NS-25.46	Detected in 62/68 samples	

Table 5-12. Summary of Benzene in Food and Beverages

Location (sampling period)	Product	Mean (ppb)	Range (ppb)	Notes	Source
Maryland, Virginia, and Michigan, November 2005–May 2007	Beverages containing benzoate only	1.75	1.1–3.5	Quantifiable in 4/61 samples; detected (>0.2 ppb) but not quantifiable (<1 ppb) in 26/61 samples	FDA 2015
	Beverages containing benzoate and ascorbic acid or erythorbic acid	12.3	1.1–87.9	Quantifiable in 55/102 samples; detected (>0.2 ppb) but not quantifiable (<1 ppb) in 28/102 samples	
	Beverages containing ascorbic acid or erythorbic acid	2.70	1.3–4.8	Quantifiable in 7/24 samples; detected (>0.2 ppb) but not quantifiable (<1 ppb) in 9/24 samples	
	Other beverages (e.g., juice cocktails, may contain natural benzoate)	4.96	1.6-10.7	Quantifiable in 6/12 samples; detected (>0.2 ppb) but not quantifiable (<1 ppb) in 4/12 samples	

[a]Only foods containing over 100 ppb at least one VOC were reported by study authors.

NS = not stated; VOC = volatile organic compound

The FDA analyzed several soft drink and juice beverages between 2005 to 2007 due to concerns of benzene formation from preservatives and additives (FDA 2015; Meadows 2006). Twenty-three samples contained benzene at concentrations >5 ppb (EPA's standard for drinking water); FDA stated that they would follow up with manufacturers about product reformulation (FDA 2015).

Between 2021 and 2023, 17 products were recalled by the FDA for the presence of benzene (FDA 2023b). These products included hand sanitizers and spray products such as an antifungal spray powder, anesthetics, antiperspirants, dry shampoo, and sunscreen. Benzene may be present in these consumer products as a residual contaminant from petroleum-based feedstocks sometimes used during product manufacturing (Kumar Pal et al. 2022). For spray consumer products, contamination is likely due to isobutane spray propellants used in these products (FDA 2023c). Contamination in hand sanitizers became of greater concern during the Covid-19 epidemic, when use and demand increased dramatically. Commercially available hand sanitizers purchased online or available in public places (e.g., restaurants and hospitals) in New York State from April 2021 to July 2021 had a median of 0.081 ppb benzene (range: 0.081–22,300 ppb) detected (Kumar Pal et al. 2022).

Benzene is an incomplete combustion product of organic material, which may be a significant source of pollution to indoor environments. In one study analyzing emission rates from burning wood in a fireplace, pine emitted 383 mg benzene per kg of wood burned (Schauer et al. 2001). Cooking stoves are another significant emitter of benzene. Median emission rates ranged from 0.04 μg benzene emitted per minute for induction heat stovetops on high to 3.89 μg benzene emitted per minute for propane ovens set to 350°F (Kashtan et al. 2023). Furthermore, there were benzene emissions from electric coils and ovens and radiant stoves due to burning of residual organic material at high temperatures. In 9 of the 33 cases, a single gas burner on high or gas oven set to 350°F raised kitchen air benzene concentrations above those expected from second-hand smoke (>0.78 ppbv).

Cigarette smoke remains an important source of human exposure to benzene. The amount of benzene measured in mainstream smoke was 5.9–73 μg/cigarette (Brunnemann et al. 1990). Larger amounts of benzene (345–653 μg/cigarette) were found in sidestream smoke (Brunnemann et al. 1990). Benzene has been found in vapor from cigarette smoke at concentrations of 3.2–61.2 μg/cigarette depending on the brand of cigarette. The amount of tar in the cigarette was not directly related to the benzene concentration (Darrall et al. 1998). Benzene has been detected in the emissions of electronic cigarettes at 0.5–2.6 ppb (one puff per minute) and 0.7–6.6 ppb (two puffs per minute) (Lee et al. 2017).

Benzene monitoring data in biota were not located. Based on experimental results, benzene is not expected to accumulate in animals or plants to a significant degree (see Section 5.4.1).

5.6 GENERAL POPULATION EXPOSURE

The general population is environmentally exposed to benzene primarily through ambient air, and in rarer instances, through water and drinking water. Benzene has been detected at low levels in food as a result of cooking or formation from added preservatives. The most significant general population exposures are in indoor air from sources such as evaporative emissions from cars in attached garages, or from cooking on gas stoves, from smoking, and from automobile related activities such as pumping gasoline.

The CDC conducts continuous monitoring of the non-institutionalized, civilian U.S. population during 2-year study periods under the NHANES program, beginning in 1999. Whole-blood benzene results from the most recent survey period are summarized in Table 5-13. An additional survey comparing the blood benzene levels of smokers versus nonsmokers was conducted, the most recent results are summarized in Table 5-14. In these recent results, median whole-blood levels of benzene in the general population or in

nonsmokers could not be determined because values were below the level of detection (0.024 ng/mL). The median whole-blood benzene level in smokers was 0.178 ng/mL.

Table 5-13. Benzene in Whole-Blood Samples (ng/mL) of the U.S. Population (2017–2018)

Population group	Geometric mean	50th percentile (95% CI)	75th percentile (95% CI)	90th percentile (95% CI)	95th percentile (95% CI)	Sample size
Total population	NA	<LOD	0.034 (0.025–0.046)	0.138 (0.112–0.175)	0.263 (0.207–0.319)	2,840
Age 12–19 years	NA	<LOD	<LOD	0.038 (0.027–0.046)	0.048 (0.040–0.063)	472
Age 20+ years	NA	<LOD	0.038 (0.029–0.052)	0.155 (0.121–0.199)	0.282 (0.221–0.366)	2,368
Males	NA	<LOD	0.039 (0.028–0.059)	0.161 (0.118–0.204)	0.279 (0.210–0.335)	1,380
Females	NA	<LOD	0.029 (<LOD–0.042)	0.117 (0.079–0.150)	0.245 (0.162–0.335)	1,460
Mexican American	NA	<LOD	0.027 (<LOD–0.044)	0.054 (0.042–0.069)	0.079 (0.061–0.143)	412
Non-Hispanic Black	NA	<LOD	0.075 (0.054–0.102)	0.212 (0.179–0.263)	0.334 (0.273–0.399)	636
Non-Hispanic White	NA	<LOD	0.033 (<LOD–0.052)	0.137 (0.086–0.190)	0.279 (0.180–0.366)	972
All Hispanic	NA	<LOD	0.026 (<LOD–0.038)	0.058 (0.044–0.079)	0.111 (0.069–0.146)	672
Non-Hispanic Asian	NA	<LOD	<LOD	0.032 (0.025–0.051)	0.059 (0.041–0.083)	396

CI = confidence interval; LOD = limit of detection (0.024 ng/mL); NA = not available (proportion of results below limit of detection was too high to provide a valid result)

Source: CDC 2022a

Table 5-14. Benzene in Whole Blood Samples (ng/mL) of the U.S. Smoking and Nonsmoking Populations (2015–2016)

Population group	Geometric mean (95% CI)	50th percentile (95% CI)	75th percentile (95% CI)	90th percentile (95% CI)	95th percentile (95% CI)	Sample size
Total smoking population	0.153 (0.135–0.174)	0.178 (0.163–0.198)	0.308 (0.280–0.338)	0.455 (0.395–0.535)	0.642 (0.510–0.826)	824
Age 18–49 years	0.121 (0.096–0.153)	0.144 (0.111–0.178)	0.261 (0.213–0.336)	0.407 (0.347–0.503)	0.602 (0.425–0.826)	456
Age 50+ years	0.224 (0.196–0.256)	0.224 (0.198–0.261)	0.372 (0.303–0.395)	0.541 (0.395–0.796)	0.796 (0.503–1.37)	368

Table 5-14. Benzene in Whole Blood Samples (ng/mL) of the U.S. Smoking and Nonsmoking Populations (2015–2016)

Population group	Geometric mean (95% CI)	50th percentile (95% CI)	75th percentile (95% CI)	90th percentile (95% CI)	95th percentile (95% CI)	Sample size
Males	0.142 (0.123–0.164)	0.169 (0.145–0.185)	0.287 (0.243–0.346)	0.477 (0.395–0.625)	0.741 (0.602–1.01)	494
Females	0.168 (0.145–0.194)	0.188 (0.169–0.213)	0.330 (0.280–0.354)	0.453 (0.379–0.523)	0.541 (0.415–0.796)	330
Total nonsmoking population	NA	<LOD	0.028 (<LOD–0.036)	0.050 (0.042–0.058)	0.067 (0.058–0.080)	2,045
Age 18-49 years	NA	<LOD	0.029 (<LOD–0.039)	0.053 (0.043–0.067)	0.078 (0.057–0.130)	1,031
Age 50+ years	NA	<LOD	0.027 (<LOD–0.036)	0.047 (0.039–0.054)	0.062 (0.051–-0.071)	1,014
Males	NA	<LOD	0.029 (<LOD–0.038)	0.051 (0.041–0.057)	0.070 (0.057–0.086)	924
Females	NA	<LOD	0.026 (<LOD–0.035)	0.050 (0.039–0.062)	0.065 (0.054–0.089)	1,121

CI = confidence interval; LOD = Limit of detection (0.024 ng/mL); NA = not available (proportion of results below limit of detection was too high to provide a valid result)

Source: CDC 2022a

Historical median whole-blood benzene levels (NHANES III, 1988–1994) were 0.061 ng/mL in the general population and 0.047 ng/mL in nonsmokers only (Ashley et al. 1994; Lemire et al. 2004). For previous NHANES data, significantly higher median blood concentrations were seen in individuals who had pumped gasoline into a car or other motor vehicle, and for those who inhaled diesel exhaust, in the 3 days leading up to sampling compared to those who hadn't (IARC 2018).

Because blood benzene is sensitive to recent exposures, *trans,trans*-muconic acid and PhMA, urinary metabolites of benzene, are used as biomarkers of environmental and occupational exposure (IARC 2018). The CDC conducts urinary monitoring as part of the NHANES program, and the results of the most recent survey years are reported in Table 5-15.

Table 5-15. Urinary Metabolites of Benzene (ng/mL) in the U.S. Population (2017–2018)

Population group (sex, age)	Geometric mean	95% CI	Samples below the MDL	Sample size
trans,trans-Muconic acid				
Total population	60.8	58.8–62.8	177	4,734
Age 3–5 years	66.4	58.9–74.8	16	543
Age 6–11 years	89.6	80.2–100	18	568
Age 12–19 years	57.4	51.9–63.5	19	641
Age 20+ years	56.2	53.9–58.7	57	2,982
Male	71.2	67.8–74.8	53	2,350
Female	52.0	49.4–54.7	124	2,384
Mexican American	60.3	54.7–66.5	30	665
Non-Hispanic Black	66.4	61.8–71.3	24	1,058
Non-Hispanic White	71.2	67.0–75.7	45	1,667
Non-Hispanic Asian	33.0	29.9-36.4	50	554
PhMA				
Total population	0.194	0.189–0.200	1,451	4,734
Age 3–5 years	0.148	0.141–0.156	105	543
Age 6–11 years	0.152	0.145–0.159	116	568
Age 12–19 years	0.151	0.144–0.159	117	641
Age 20+ years	0.226	0.217–0.235	522	2,982
Male	0.212	0.204–0.221	642	2,350
Female	0.178	0.172–0.185	809	2,384
Mexican American	0.159	0.151–0.167	238	665
Non-Hispanic Black	0.233	0.219–0.249	255	1,058
Non-Hispanic White	0.206	0.196–0.217	498	1,667
Non-Hispanic Asian	0.141	0.134–0.148	222	554

CI = confidence interval; MDL = method detection limit (9.81 ng/mL for *trans,trans*-muconic acid and 0.150 ng/mL for PhMA); PhMA = *S*-phenylmercapturic acid

Source: CDC 2022b

Between 1999 and 2000, as part of the NHANES, the CDC conducted personal air monitoring via passive exposure monitors (or badges) for 48–72-hour periods, followed by an exposure factors questionnaire (CDC 2005). A summary of the results is presented in Table 5-16. Significantly higher benzene exposures were observed for participants who had homes with attached garages versus participants who did not have an attached garage; participants who had no windows open in the home versus participants who had any open windows; and participants who were smokers versus nonsmoking participants (Symanski et al. 2009). More recent general population personal air monitoring data have been reported from the Detroit Exposure and Aerosol Research Study, which measured ambient breathing zone

concentrations of benzene for from 2004 to 2007 in Detroit, Michigan (George et al. 2011). The mean average daily personal benzene air concentrations were 2.7–7.7 μg/m^3 (858 samples).

Table 5-16. Benzene in Personal Air Monitoring (μg/m^3) of the U.S. Adult Population (1999–2000)

Population group (sex, age)	Geometric mean	95% CI	Samples below the MDL	Sample size
Total population	3.25	3.03–3.49	222	669
Age 20–29 years	3.10	2.70–3.57	75	185
Age 30–39 years	3.35	2.93–3.83	58	172
Age 40–49 years	3.44	2.98–3.96	42	158
Age 50+ years	3.14	2.65–3.79	47	132
Male	3.74	3.35–4.17	84	295
Female	2.90	2.64–3.18	138	352
Mexican American	4.30	3.77–4.91	34	185
Other Hispanic	2.88	2.48–3.35	55	126
Non-Hispanic Black	2.84	2.55–3.18	108	276
Non-Hispanic White	3.03	2.34–3.91	21	45

CI = confidence interval; MDL = method detection limit (not reported)

Source: CDC 2005

Nationally, a decline in ambient air benzene of 66% has been reported between 1996 and 2009 (EPA 2014). Average intake from outdoor air is estimated to be 0.16 μg/kg body weight/day based on an average concentration of 0.562 μg/m^3 (0.176 ppbv) (EPA 2023b) and an inhalation rate of 20 m^3/day for a 70-kg person.

Benzene is not a common pollutant of municipal water, and drinking water is not expected to be an important route of exposure for benzene (Wallace 1989a). A median value of 0.8 μg/L has been reported based on benzene detections in 0.31% of the 372,470 public water supply samples analyzed (EPA 2016b). Based on this value, a water intake of 0.02 μg/kg body weight/day is estimated for these populations based on a water consumption rate of 2 L/day for a 70-kg person.

ATSDR's three-compartment Shower and Household-Use Exposure (SHOWER) model predicts air concentrations in the shower stall, bathroom, and main house throughout the day by estimating the contribution from showering or bathing and the contribution from other water sources in the house, such as the dishwasher, clothes washer, and faucets. This information along with human activity patterns are

used to calculate a daily time-weighted average exposure concentration via inhalation exposure and from dermal uptake from skin contact. ATSDR's SHOWER model is available by sending a request to showermodel@cdc.gov. While benzene is not commonly detected in municipal water, it is expected to be readily volatile from water and exposure during bathing may occur from trace amounts of benzene present for some populations. Using a median treated water concentration of 0.8 μg/L and a representative outdoor air level of 0.562 μg/m^3 (0.176 ppbv), Reasonable Maximum Exposure (RME) levels were calculated for different exposure groups and are reported in Table 5-17.

Table 5-17. Estimated RME Daily Inhalation Dose and Administered Dermal Dose for the Target Person

Exposure group	Inhalation dose (μg/kg/day)	Inhalation dose (μg/m^3)	Dermal dose (μg/kg/day)
Birth–<1 year	1.2	1.2	0.0050
1–<2 years	1.2	1.2	0.0047
2–<6 years	0.85	1.2	0.0040
6–<11 years	0.50	1.2	0.0032
11–<16 years	0.34	1.2	0.0026
16–<21 years	0.27	1.2	0.0024
Adult	0.24	1.2	0.0024
Pregnant and breastfeeding women	0.35	1.2	0.0024

Source: ATSDR 2023f

Benzene may be present at trace amounts in some food and dietary intake is not expected to be a significant route of exposure for the general population (Wallace 1989a). A relatively recent study detected benzene in 58% of the 455 food samples collected from the Belgian market. The highest concentrations of benzene were found in processed foodstuffs, including fish (smoked or canned) with a reported maximum concentration of 76.21 μg/kg; raw meat, fish, eggs, and other unprocessed foods had no or lower concentrations of benzene (Medeiros Vinci et al. 2012). Using this study, an estimated mean benzene intake for all foods in the Belgian market was reported as 0.020 μg/kg body weight/day. Another dietary estimate of 0.003–0.050 μg/kg body weight/day has been reported (Salviano Dos Santos et al. 2015). The total concentration of benzene on exposed food crops consumed by humans was estimated to be 0.587 μg/kg (Hattemer-Frey et al. 1990).

Inhalation of indoor air is a major route of exposure to benzene. Indoor air benzene concentrations are typically greater than outdoor air (George et al. 2011; Kinney et al. 2002; Weisel et al. 2008). Sources

include evaporative emissions of gasoline in cars, particularly in attached garages, heating sources, and cooking. Depending on airflow from garage to living areas, mean indoor benzene concentrations in houses with a garage were 2–5 times higher than outdoor levels in most homes (Thomas et al. 1993). Benzene levels in four garages during different times in a day were 0.94–61.3 ppbv (3.0–196 μg/m^3). The higher concentrations of benzene in these garages were not only from vehicular activity, but also in varying proportions from stored gasoline, paints, and benzene-containing consumer products (Thomas et al. 1993). Inhalation exposure to off-gassing from benzene-containing products and evaporative emissions from automobiles in attached garages has been estimated to be 150 μg/day (Wallace 1989a).

Benzene has been detected in residences where fuel oil was used for heating and in residences with active wood burning fireplaces (Maine DEP 2014; NYSDOH 2005; Schauer et al. 2001). Benzene has been found to be a major component of the emissions from wood burning, especially from efficient flame combustion, and constituted roughly 10–20% by weight of total non-methane hydrocarbons (Barrefors and Petersson 1995). Emissions of 383 mg benzene per kg of wood burned in a fireplace have been reported (Schauer et al. 2001). It should be noted, however, that chimney emissions result in much lower human exposure than equally large emissions at the ground level. Emissions rates of benzene during cooking on stoves or with ovens have been reported at medians of 0.04 μg benzene emitted per minute for induction hobs on high to 3.89 μg benzene emitted per minute for propane ovens set to 350°F (Kashtan et al. 2023). Even electric appliances can emit benzene from burning off of residual organic material. In some cases, a single gas burner on high or gas oven set to 350°F raised kitchen air benzene concentrations above those expected from second-hand smoke (>0.78 ppbv; >2.5 μg/m^3).

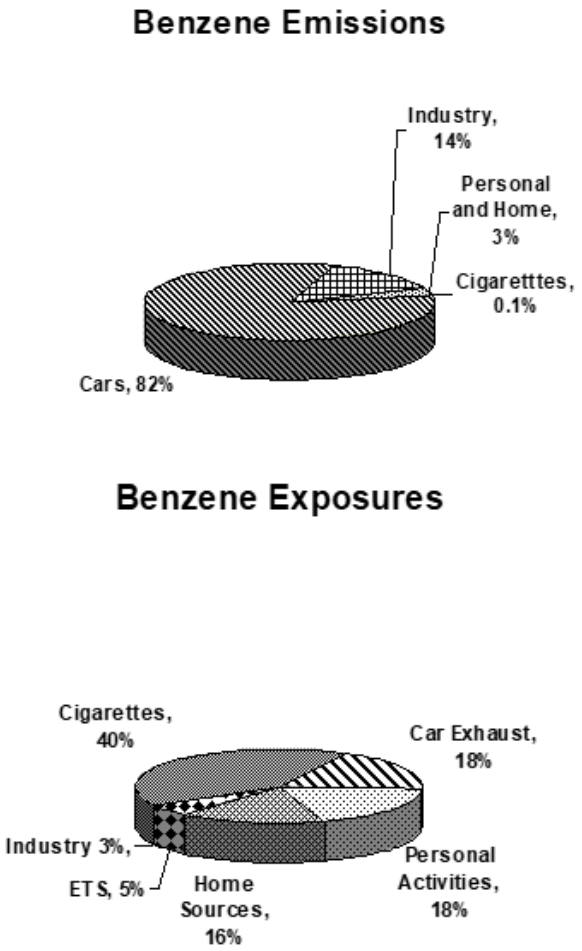
Benzene rapidly volatilizes and there is potential for vapor intrusion as a source of indoor air pollution. However, the majority of recent ATSDR site assessments did not find vapor intrusion as the source of benzene to indoor air (ATSDR 2021, 2023b, 2023c). ATSDR extracted environmental data from 135 ATSDR reports evaluating the vapor intrusion pathway at 121 sites published between 1994 and 2009 (Burk and Zarus 2013). Benzene was detected in the indoor air of 28 sites; only 2 of these sites were declared a public health hazard. EPA's compilation of background indoor air concentrations reported background medians ranging from below the reporting level (0.05–1.6 μg/m^3; 0.02–0.50 ppbv) to 4.7 μg/m^3 (1.5 ppbv), 90th percentiles of 5.2–15 μg/m^3 (1.6–4.7 ppbv), and maximum values of 21–460 μg/m^3 (6.6–140 ppbv), based on 2,615 U.S. resident samples collected between 1990 and 2005 (EPA 2011). Benzene levels in indoor air from the recent site assessments were 0.3–7.7 μg/m^3 (0.1–2.4 ppbv), generally within the median background ranges (ATSDR 2023b).

The Total Exposure Assessment Monitoring (TEAM) studies, carried out by the EPA between 1980 and 1990, suggested that for many chemicals, including benzene, the most important sources of pollution are small and close to the person, and that exposures are not clearly correlated with emissions. For example, the TEAM study findings indicated that nearly 85% of atmospheric benzene in outdoor air is produced by cars burning petroleum products and the remaining 15% is produced by industry (these estimates are expected to differ today as gasoline emissions have decreased dramatically in recent years). Despite the fact that petroleum products contributed to the majority of benzene in the atmosphere, the study found that half of the total national personal exposure to benzene came from cigarette smoke (Wallace 1995). In the United States in 2021, approximately 46 million (18.7%) adults used any tobacco product (CDC 2023).

Even passive exposure to cigarette smoke is responsible for more benzene exposure (about 5% of the total) than the emissions from the entire industrial capacity of the United States (about 3% of the total) (Wallace 1995). A breakdown of the emissions and exposure sources for benzene that was derived from the Los Angeles TEAM study data (Wallace et al. 1991) is provided in Figure 5-3. The reason that a relatively small source of emissions can have such a large effect on exposure is the efficiency of delivery. Wallace (1995) reports that one cigarette delivers an average of 55 μg of benzene with nearly 100% efficiency to the smoker. Benzene from industrial sources is dissipated into the atmosphere.

Smokers (n=200) in the TEAM study had a mean breath concentration of 15 $\mu g/m^3$ (4.7 ppbv), almost 10 times the level of 1.5–2 $\mu g/m^3$ (0.47–0.63 ppbv) observed in >300 nonsmokers (Wallace 1989b). Smokers also had about 6–10 times as much benzene in their blood as nonsmokers (Wallace 1995). In another study, benzene concentrations were compared in the breath of smokers and nonsmokers and in ambient air in both an urban area of San Francisco and in a more remote area of Stinson Beach, California (Wester et al. 1986). In the urban area, benzene in smokers' breath (6.8±3.0 ppbv) was greater than in nonsmokers' breath (2.5±0.8 ppbv) and smokers' ambient air (3.3±0.8 ppbv). In the remote area, the same pattern was observed. This suggests that smoking represented an additional source of benzene above that of outdoor ambient air (Wester et al. 1986). In 10 of 11 homes inhabited by tobacco smokers, mean indoor and personal benzene concentrations were 2–5 times higher than outdoor levels (Thomas et al. 1993).

Figure 5-3. Benzene Emissions and Exposures



Note: A comparison of benzene emission sources (home sources include paints and petroleum products; personal activities include driving and use of consumer products that contain benzene).

Source: Wallace et al. 1991

Assuming that the average sales-weighted tar and nicotine cigarette yields 57 μg benzene in mainstream smoke, Wallace (1989a) estimated that the average smoker (32 cigarettes/day) takes in about 1.8 mg benzene/day from smoking. This is nearly 10 times the average daily intake of nonsmokers (Wallace 1989a). On the assumption that intake of benzene from each cigarette is 30 μg, Fishbein (1992) calculated that a smoker who consumes two packs of cigarettes per day will have an additional daily intake of 1,200 μg.

A British study of rural and urban environments suggested that benzene exposure is greatly affected by proximity to smokers (Duarte-Davidson et al. 2001). Air concentrations of benzene at an urban center in South Hampton averaged about 2.5 ppbv (8.0 μg/m^3), while in a rural location in Hartwell, the average

amount of benzene in the air was 0.41 ppbv (1.3 μg/m^3). Air at a smoky pub was found to contain 22 ppbv (70 μg/m^3) of benzene. Comparing the daily doses of rural nonsmokers, urban nonsmokers, urban passive smokers, and urban smokers, very little difference between the rural nonsmokers' 24 ppbv (77 μg/m^3) daily dose and the urban nonsmokers' 30 ppbv (96 μg/m^3) daily dose was found. Passive urban smokers, on average, have a daily benzene exposure dose of 38 ppbv (120 μg/m^3) of benzene while smokers have a daily exposure dose to benzene of 163 ppbv (521 μg/m^3). On average, nonsmokers in urban and rural environments have estimated benzene intakes of 1.15 and 1.5 μg/kg body weight/day.

Women tend to intake more of benzene per kg body weight than men. Passive smokers' estimated daily intake averages are 2.10 and 1.74 μg/kg body weight/day for women and men, respectively. Urban women and men smokers' estimated intakes are estimated at 9.00 and 7.46 μg/kg body weight/day, respectively; this is equivalent to an atmospheric concentration of 8.2 ppb (Duarte-Davidson et al. 2001).

In 1990, a study in Germany analyzed factors that predicted people's exposures to VOCs and found that while smoking was the most significant determinant of benzene exposure, automobile-related activities, such as refueling and driving, were also significant (Hoffmann et al. 2000). Virtually all (99.9%) of the benzene released into the environment finally distributes itself into the air. The general population may be exposed to benzene through inhalation of contaminated air, particularly in areas of heavy motor vehicle traffic and around gas stations. Compared to inhalation, dermal exposure probably constitutes a minor portion of benzene exposure for the general population. Personal sources account for 18% of the total exposure of the general population to benzene. The main personal sources (other than smoking cigarettes) are driving or riding in automobiles and using products that emit benzene (paints, adhesives, marking pens, rubber products, and tapes) (Wallace 1989a).

Beginning in 2011, benzene in gasoline has been limited to 0.62% volume (EPA 2023a). Since benzene is a constituent of auto exhaust and fuel evaporation, people who spend more time in cars or in areas of heavy traffic have increased personal exposure to benzene. No recent U.S. monitoring data were located. Available exposure estimates and biomonitoring data were published prior to national benzene gasoline reductions and are based on historic levels of benzene in gasoline (1-–2%); these data likely overestimate exposures today. Assuming an average benzene concentration of 40 μg/m^3 (12.5 ppbv) for a moving automobile and an exposure duration of 1 hour/day, the calculated intake for driving or riding in an automobile in the late 1980s was 40 μg/day (Wallace 1989a). In an investigation of exposure to methyl *tert*-butyl ether (MTBE) in oxygenated gasoline in Stamford, Connecticut, venous blood samples were collected from 14 commuters and from 30 other persons who worked in the vicinity of traffic or

automobiles. In addition to MTBE, the samples were analyzed for five chemicals, including benzene. Levels of benzene in the blood of 11 nonsmoking men and women commuters were 0.10–0.20 μg/L (median: 0.12 μg/L). Blood benzene levels of 0.29, 0.14, and 0.58 μg/L were measured in one female and two male smoking commuters, respectively (White et al. 1993).

Pumping gasoline can also be a significant source of exposure to benzene; these studies may be based on historic levels of benzene in gas and therefore overestimate exposures today. A study conducted between July 1998 and March 1999 that comprised of 39 customers of gasoline self-service stations from North Carolina, measured the benzene level in the air around the station as well as the levels of benzene in customers' breath prior to and immediately after fueling (Egeghy et al. 2000). Benzene levels in the air around the station were <0.02–11.16 ppmv (<0.06–35.65 mg/m^3), with a mean (±1 SD) of 0.91 (±1.8) ppmv (2.9±5.6 mg/m^3). The range of benzene levels in the breath of customers prior to fueling was <0.001–0.022 ppmv (<0.003–0.070 mg/m^3) with a mean (±1 SD) of 0.0027 (±0.0034) ppmv (0.0090±0.0110 mg/m^3) while the range of benzene levels in the breath of customers after re-fueling was <0.001–0.434 ppmv (<0.003–1.37 mg/m^3) with a mean (±1 SD) of 0.05 (±0.081) ppmv (0.16±0.259 mg/m^3) (Egeghy et al. 2000). Another study reported a benzene concentration of 1 ppm at the breathing-level of a person pumping gas (Bond et al. 1986b). Using this concentration and an estimated 70 minutes/year of time spent pumping gasoline, a benzene intake of 10 μg/day has been calculated (Wallace 1989a). In a group of 26 subjects who were not occupationally exposed to benzene, but were exposed to benzene during refueling in Fairbanks, Alaska, median blood benzene levels prior to and immediately following refueling were 0.19 ppbv (0.61 μg/m^3; range: 0.08–0.65 ppbv; 0.26–2.1 μg/m^3) and 0.54 ppbv (1.7 μg/m^3; range: 0.13–1.70 ppbv; 0.41–5.4 μg/m^3), respectively (Backer et al. 1997). While most human exposure to benzene is believed to be through inhalation, studies show that benzene can permeate skin with a permeability factor of about 0.14–0.18 cm/hour at 25°C. The permeability factor was not affected by moisturizer, baby oil, or insect repellent; however, it was affected by temperature (50°C) and sunscreen, with the permeability factors increasing to 0.26 and 0.24 cm/hour, respectively (Nakai et al. 1997).

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Children can be subject to increased benzene exposure by inhalation of second-hand smoke. In a study of nonsmoking rural families, urban families, and urban smoking families, infant exposure to benzene was estimated at doses of 15.3, 19.7, and 25.9 μg/day, respectively, with daily intakes of 1.68, 2.16, and 2.55 μg/kg body weight/day, respectively. For children of the same classification, benzene exposure was

measured at doses of 29.3, 37.6, and 49.3 μg/day, respectively, with daily intakes of 0.71, 0.91, and 1.20 μg/kg bodyweight/day, respectively. For all infants and children, benzene exposure predominantly comes from the indoors (Duarte-Davidson et al. 2001).

Depending on the children's living environment, they may have higher exposure to benzene than adults. In a study of two lower-income areas of Minneapolis, children were found to have average personal benzene exposures of 0.66 and 0.53 ppb (2.1 and 1.7 μg/m^3) in the winter and spring, respectively. The highest concentration of benzene in their environment came from the home, with winter and spring concentrations of 0.69 and 0.66 ppbv (2.2 and 2.1 μg/m^3), respectively, while the outdoor and school benzene concentrations were 0.41 and 0.19 ppbv (1.3 and 0.60 μg/m^3), respectively (Adgate et al. 2004). In a follow-up study of Minneapolis lower-income children between 2000 and 2002, blood benzene was detected in 71.4% samples at a median of 0.08 ng/mL (range: 0.04–0.26 ng/mL) (Sexton et al. 2006). In Italy, concentrations of the benzene metabolite*, trans,trans*-muconic acid, were measured in the urine of children from both urban areas in Naples and rural areas in Pollica. The mean urinary concentrations of *trans,trans*-muconic acid detected for rural and urban children were 48.4 and 98.7 μg/L, respectively (Amodio-Cocchieri et al. 2001). These studies also found no strong link between passive smoking and *trans,trans*-muconic acid levels. The only factor that affected levels of *trans,trans*-muconic acid in urine samples was how close the family lived to the road. A study in Rouen, France, compared benzene exposure and concentrations in nonsmoking parents and their children. Despite the fact that the children were exposed to slightly less benzene (3.47 ppbv [11.1 μg/m^3]) than their parents (4.51 ppbv [14.4 μg/m^3]), there was no significant correlation between exposure means and urinary metabolite levels (Kouniali et al. 2003).

Lagorio et al. (2013) conducted a study in Italy assessing the exposure of benzene to children by repeated weekly measurements in breathing zone and ambient outdoor air samples along with determination of cotinine, *trans,trans*-muconic acid, and PhMA in urine. In 108 children, all between the ages of 2 and 12 years, the average benzene concentrations in personal and outdoor air samples were reported as 3 and 2.7 μg/m^3 (0.92 and 0.81 ppbv), respectively. The average urinary cotinine, *trans,trans*-muconic acid, and PhMA concentrations were 3.73, 116.65, and 1.28 μg/g creatinine, respectively.

Benzene in breast milk has been a major concern. Benzene was detected in U.S. breast milk samples from nonsmoking homes, at an average of 0.12 ng/mL (Kim et al. 2007b). Breast milk concentrations trended with indoor air concentrations. While this may provide a mechanism by which infants are exposed to benzene, these concentrations are lower than in other foods.

Individuals who live near hazardous waste sites, leaking underground fuel storage tanks, or oil natural gas drilling might be exposed to potentially high concentrations of benzene in their drinking water if they obtain tap water from wells located near these sources. Benzene was detected at a maximum of 7.65 ppb in drinking water sourced from groundwater impacted by natural gas drilling (ATSDR 2019b). In a series of experiments conducted in a single-family residence from June 11 to 13, 1991, exposure to benzene through contaminated residential water was monitored (Lindstrom et al. 1994). The residential water was contaminated with benzene and other hydrocarbons in 1986. Periodic testing conducted from 1986 to 1991 showed benzene concentrations of 33–673 μg/L (ppb). The experiment involved an individual taking a 20-minute shower with the bathroom door closed, followed by 5 minutes for drying and dressing; then the bathroom door was opened and the individual was allowed to leave the house. Integrated 60- and 240-minute whole-air samples were collected from the bathroom, an adjacent bedroom, the living room, and in ambient air. Glass, gas-tight syringe grab samples were simultaneously collected from the shower, bathroom, bedroom, and living room at 0, 10, 18, 20, 25, 25.5, and 30 minutes. Two members of the monitoring team were measured for 6 hours using personal Tenax gas chromatographic (GC) monitors. For the first 30 minutes of each experiment, one member was based in the bathroom and the other was based in the living room. Benzene concentrations in the shower head were 185–367 μg/L (ppb), while drain level samples ranged from below the detectable limit (0.6 μg/L or ppb) to 198 μg/L (ppb). Analysis of the syringe samples suggested a pulse of benzene moving from the shower stall to the rest of the house over approximately 60 minutes. Peak benzene levels were measured in the shower stall at 18–20 minutes (758–1,670 μg/m^3), in the bathroom at 10–25 minutes (366–498 μg/m^3), in the bedroom at 25.5–30 minutes (81–146 μg/m^3), and in the living room at 36–70 minutes (40–62 μg/m^3). The total benzene dose resulting from the shower was estimated to be approximately 281 μg, with 40% via inhalation and 60% via the dermal pathway (Lindstrom et al. 1994).

The major source of exposure to benzene is cigarette smoke. A smoker of 32 cigarettes per day would have a benzene intake of approximately 1.8 mg/day (at least 10 times the average nonsmoker's intake) (Wallace 1989a). Median benzene concentrations in 343 homes with smokers averaged 3.3 ppb (11 μg/m^3) compared to 2.2 ppb (7.0 μg/m^3) in 185 homes without smokers. This represents a 50% increase in benzene exposure for nonsmokers exposed to passive smoke compared to nonsmokers not exposed to passive smoke (Wallace 1989a). In a study in Germany, the mean benzene concentrations for frequent smokers and nonsmokers were 6.1 and 2.4 ppb (19 and 7.7 μg/m^3), respectively (Hoffmann et al. 2000).

In a study measuring the mean benzene exposure by monitoring urinary benzene excretion in 33 petrochemical plant workers, 30 small town residents 2 km from the plant, 26 small town residents 2–4 km from the plant, and 54 urban residents 25 km from the plant, nonsmokers had median urinary benzene concentrations of 236, 48, 63, and 120 ng/L, respectively, while smokers had median concentrations of 692, 470, 421, and 1,090 ng/L, respectively (Fustinoni et al. 2012).

A study compared the urinary benzene metabolite, *trans,trans*-muconic acid, between smokers and nonsmokers among 81 ceramic factory workers exposed to low levels of benzene and 83 general population controls (Ibrahim et al. 2014). Among the factory workers, 26 smokers had an average urinary *trans,trans*-muconic acid concentration of 0.252 mg/g creatinine, while 55 nonsmokers had an average of 0.183 mg/g creatinine. In the nonexposed control group, 25 smokers had an average urinary *trans,trans*-muconic acid concentration of 0.043 mg/g creatinine, while 58 nonsmokers had an average of 0.035 mg/g creatinine.

Individuals employed in industries that use or make benzene or products containing benzene may be exposed to the highest concentrations of benzene. The National Occupational Exposure Survey (NOES), conducted by the National Institute for Occupational Safety and Health (NIOSH) from 1981 to 1983, estimated that approximately 272,300 workers employed in various professions were potentially exposed to benzene in the United States. Approximately half of these workers were employed in general medical and surgical hospitals, and their occupations included nurses and aides, physicians, technicians, technologists, therapists, dieticians, pharmacists, and janitors (NIOSH 1990). The NOES database does not contain information on the frequency, concentration, or duration of exposure; the survey provides only estimates of workers potentially exposed to chemicals in the workplace. The current OSHA permissible exposure limit (PEL) for an 8-hour TWA exposure to benzene is 1 ppm (3 mg/m^3) and the short-term exposure limit (STEL) in any 15-minute period is 5 ppm (16 mg/m^3) (OSHA 2003). NIOSH recommended exposure limit is 0.1 ppm (0.3 mg/m^3) for a 10-hour workday during a 40-hour workweek and the short-term exposure is 1 ppm (3 mg/m^3) (NIOSH 1992). In 1987, OSHA estimated that approximately 238,000 workers were exposed to benzene in seven major industry sectors, including petrochemical plants, petroleum refineries, coke and coal chemicals, tire manufacturers, bulk terminals, bulk plants, and transportation via tank trucks (see Table 5-18) (OSHA 1987). Approximately 10,000 workers were estimated to be exposed to TWA concentrations in excess of the 1 ppm standard. This estimate did not include firms covered by the exclusions, firms under jurisdiction of other agencies, or firms involved in the use of products containing small quantities of benzene. The uptake of benzene by workers in a municipal waste incinerator in Germany was assessed by measuring benzene levels in blood

(Angerer et al. 1991). No significant difference ($p<0.05$) in blood benzene levels between workers and controls were detected (mean 0.22 μg/L for nonsmoking workers versus 0.25 μg/L for nonsmoking controls). OSHA requires the use of engineering controls and/or respiratory protection in situations where compliance with the TWA is not feasible (OSHA 1987).

Table 5-18. Percentage of Employees Exposed to Benzene by Exposure Level and Industry Division (1987)

Industry sector	Percentage of observations in each exposure category according to range of 8-hour TWA benzene concentrations (ppm)						Total number of employees
	0.0–0.1	0.11–0.5	0.51–1.0	1.1–5.0	5.1–10	10+	
Petrochemical plants[a]		74.6		23.0	2.4	0.0	4,300
Petroleum refineries[b,c]	64.6	26.1	4.6	3.8	0.5	0.4	47,547
Coke and coal chemicals[a]	0.0	39.3	27.6	27.5	4.4	1.3	947[d]
Tire manufacturers[b]	53.4	37.5	6.3	2.8	0.0	0.0	65,000
Bulk terminals[b]	57.8	32.8	5.3	3.7	0.3	0.1	27,095
Bulk plants[b]	57.8	32.8	5.3	3.7	0.3	0.1	45,323
Transportation via tank truck[b]	68.4	23.1	5.3	2.9	0.1	0.2	47,600
Total							237,812

[a]Percentages represent the portion of workers whose average exposures are in each category.
[b]Percentages represent the portion of sampling results in each category.
[c]Data do not reflect respirator use and sampling biases.
[d]Excludes workers employed at the coke ovens.

TWA = time-weighted average

Source: OSHA 1987

Certain jobs, such as gasoline station workers, firefighters, and drycleaners, are believed to put people at a higher risk of benzene exposure. In an analysis of literature, it was estimated that workers in the area of crude petroleum and natural gas are exposed to 0.04 ppmv (0.128 mg/m^3) benzene, while workers in petroleum refining, gas stations, and crude petroleum pipelines are exposed to 0.22, 0.12 and 0.25 ppmv benzene (0.70, 0.38, and 0.80 mg/m^3), respectively. This study also showed that firefighters are exposed to an average of 0.38 ppmv (1.2 mg/m^3) benzene (van Wijngaarden and Stewart 2003). Workers from four different drycleaning facilities in Korea had mean benzene air concentrations of 2.7–3.2 ppbv (8.6–10 μg/m^3). Their exposure to benzene was dependent upon the type of solvent used for cleaning (Jo and Kim 2001). Benzene concentrations of 25.46 and 1,331.29 ppbv (81.33 and 4,253.05 μg/m^3) were found near the kiln and at the rotary line, respectively, inside a hazardous waste incinerator in Turkey (Bakoglu et al. 2004).

In 12 nonsmoking male car repair workers working in Stamford, Connecticut, blood benzene levels were 0.11–0.98 μg/L (median: 0.19 μg/L); in 8 smoking male car repair workers, benzene levels were 0.17–0.67 μg/L (median: 0.42 μg/L). Three nonsmoking male gasoline attendants had blood benzene levels of 0.32–0.47 μg/L (median: 0.36 μg/L) (White et al. 1993).

A study comparing workers who were exposed to benzene regularly at work to people who were not exposed to benzene at work showed that while the general population in Italy had average blood benzene concentration of 165 ng/L, the people who were exposed to high benzene levels at work had an average benzene blood concentration of 186 ng/L. Immediately following their shift, the average benzene blood level samples from of benzene-exposed workers was 420 ng/L. The average blood benzene levels for smoking and nonsmoking occupationally exposed workers were 264 and 123 ng/L, respectively (Brugnone et al. 1998).

Bogen and Sheehan (2014) estimated that workers' dermal exposure to benzene in mineral spirits solvents (MSS), used in parts washing and degreasing operations, averaged 33% of their total (dermal and inhalation) benzene uptake. The estimated average benzene doses from parts washing by dermal exposure and total exposure were reported as 0.0093 and 0.054 mg/day, respectively, using 'low-aromatic' MSS formulations from 1995 to 1999. In a study assessing the dermal exposure and absorption of combustion contaminants in firefighters during six different controlled structure burns, the median increase in breath concentrations of benzene post- versus pre-burn were 48.1, 2.81, 39.2, -0.33, 7.39, and 18.8 μg/m^3 (15.1, 0.880, 12.2, -0.10, 2.31, and 5.88 ppbv) (Fent et al. 2014). The benzene metabolite, PhMA, could not be detected in urine samples collected from the firefighters (minimum detectable concentration 8.5 μg/g).

In a study comparing the urinary benzene metabolite *trans,trans*-muconic acid between 81 ceramic factory workers exposed to low levels of benzene and 83 nonexposed controls, the workers and the control group had mean *trans,trans*-muconic acid concentrations of 0.22 and 0.043 mg/g creatinine, respectively (Ibrahim et al. 2014).

One study assessed the exposure of 133 male petrochemical industry operators to benzene by both environmental (personal air) and biological monitoring (metabolites *trans,trans*-muconic acid and PhMA in end-shift urine). The mean reported values of benzene exposure were 0.014 ppm, 101 μg/g creatinine, and 2.8 μg/g creatinine for benzene, *trans,trans*-muconic acid, and PhMA, respectively (Carrieri et al.

2010). Another study assessed the occupational exposure of urban and rural female workers to benzene, toluene, and xylenes by monitoring urban air for traffic policewomen (street) versus police drivers (vehicle); monitoring urban air versus rural air; and biological monitoring of workers in urban areas versus rural areas (Ciarrocca et al. 2012). Mean personal air exposures to benzene over an 8-hour sampling period were similar for urban street (16.7 μg/m^3) and vehicle workers (18.7 μg/m^3), but were reported to be higher when compared to rural workers (less than the LOD of 1.6 μg/m^3). Mean blood and urine levels of benzene, and *trans,trans*-muconic acid and PhMA, respectively, were similar among the street (244.4 ng/L, 62.0 μg/g creatinine, 3.5 μg/g creatinine) and vehicle workers (241.1 ng/L, 61.8 μg/g creatinine, 3.4 μg/g creatinine), but blood levels of benzene were higher in urban workers compared to rural workers (113.1 ng/L, 40.8 μg/g creatinine, 2.8 μg/g creatinine) (Ciarrocca et al. 2012). In a study measuring the mean benzene exposure in 33 petrochemical plant workers, 30 small town residents 2 km from the plant, 26 small town residents 2–4 km from the plant, and 54 urban residents 25 km from the plant, measured median personal air benzene concentrations were 25, 9, 7, and 6 μg/m^3, respectively, while median urinary metabolite concentrations were 236, 48, 63, and 120 ng/L and 692, 470, 421, and 1,090 ng/L, for nonsmokers and smokers, respectively (Fustinoni et al. 2012).

A study determined benzene exposure in 33 petrochemical industry operators (PIOs), 28 service station attendants (SSAs), 21 gasoline pump maintenance workers (GPMWs), and 51 nonexposed controls by measuring personal air concentrations and benzene metabolites, *trans,trans*-muconic acid and PhMA, in end-of-shift urine samples (Fracasso et al. 2010). The levels of benzene (in μg/m^3) in personal air for PIOs, SSAs, GPMWs, and controls were 1.7–593.50 (median 27.8), 8.00–260.00 (median 40.00), 4.60–514.90 (median 24.20), and 1.97–16.3 (median 5.40), respectively. Urinary levels of metabolites (in μg/g creatinine), *trans,trans*-muconic acid and PhMA, in PIOs, SSAs, GPMWs, and controls were 49.00–422.00 (median 128.00) and 0.40–35.60 (median 8.60), 30.00–418.00 (median 117.00) and 1.55–15.00 (median 5.55), 13.40–242.50 (median 92.00) and 0.21–10.53 (median 1.77), and 3.00–460.50 (median 84.00) and 0.30–10.08 (median 1.90), respectively. The results show that in all groups of workers, the level of personal air exposure to benzene was higher than the control groups, while the level of urinary metabolites was higher in the SSA and PIO groups compared to the control. No increase in urinary metabolites was measured in GPMWs, but it was noted that for these workers, benzene exposure was not continuous and only occurred on specific days.

CHAPTER 6. ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of benzene is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the adverse health effects (and techniques for developing methods to determine such health effects) of benzene.

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.1 EXISTING INFORMATION ON HEALTH EFFECTS

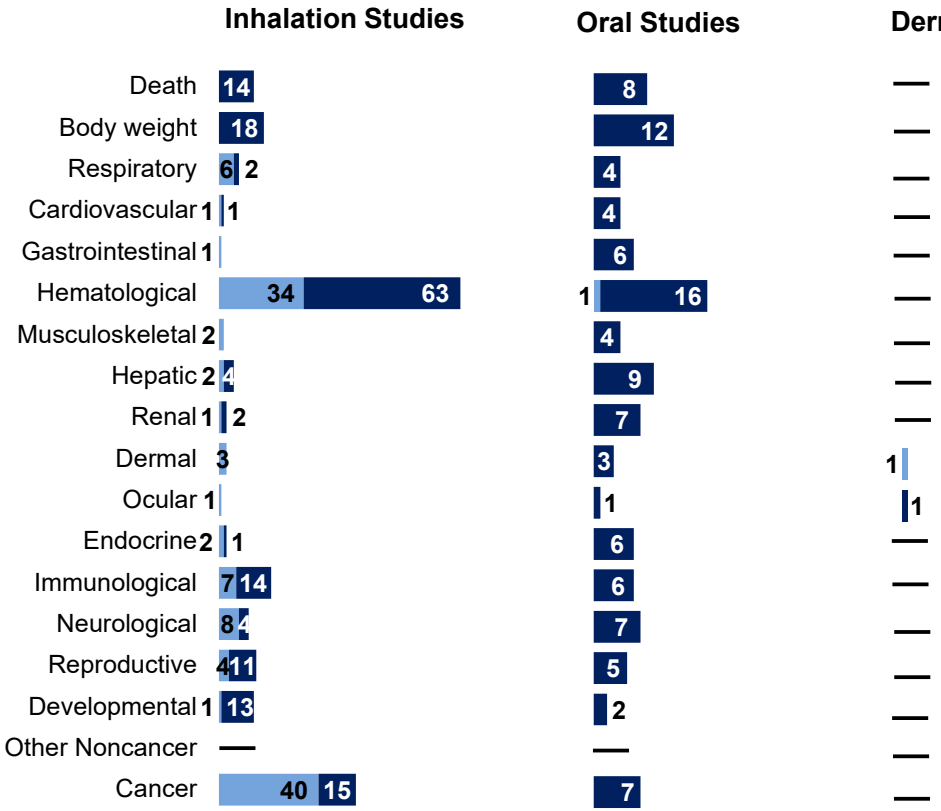
Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to benzene that are discussed in Chapter 2 are summarized in Figure 6-1. The purpose of this figure is to illustrate the information concerning the health effects of benzene. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

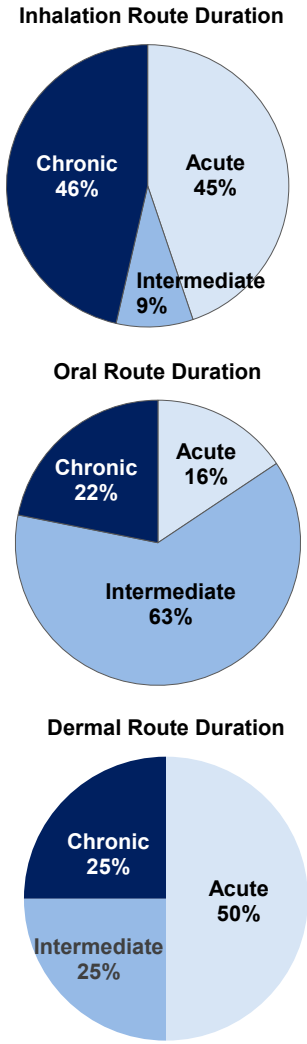
6.2 IDENTIFICATION OF DATA NEEDS

Missing information in Figure 6-1 should not be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 6-1. Summary of Existing Health Effects Studies on Benzene by Route and Endpoint*

Potential hematological, cancer, and body weight effects were the most studied endpoints
The majority of the studies examined inhalation exposure in **animals** (versus **humans**)





*Includes studies discussed in Chapter 2; the number of studies include those finding no effect. If no studies were included in the profile for a specific organ system and route of exposure, this is shown by a dash (–). Note that most studies examined multiple endpoints.

Acute-Duration MRLs. Studies in laboratory animals have identified hematopoietic effects (specifically, decreased peripheral WBCs) as the most sensitive effect of acute-duration inhalation exposure to benzene. A provisional acute-duration inhalation MRL was derived based on a LOAEL of 10.2 ppm for decreased peripheral lymphocytes in mice (Rozen et al. 1984). Dempster and Snyder (1991) also reported similar hematological effects at 10.3 ppm in mice. Additional studies examining hematological effects at lower exposure levels in laboratory animals would provide additional information to define the NOAEL-LOAEL boundary. Acute-duration oral studies did not provide sufficient data to derive an MRL. However, the intermediate-duration oral MRL was adopted for the acute-duration oral MRL. Additional acute-duration inhalation studies examining comprehensive toxicological endpoints, including hematological effects, for extended dose ranges may provide information to derive an acute-duration oral MRL.

Intermediate-Duration MRLs. Provisional intermediate-durations inhalation and oral MRLs have been developed. The intermediate-duration inhalation is based on a LOAEL of 10.2 ppm for immunological effects (delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction) in mice (Rosenthal and Snyder 1987). The LOAEL of 10 ppm is the lowest value for adverse effects in the intermediate-duration inhalation database. However, a NOAEL was not identified in this study. Additional intermediate-duration inhalation studies evaluating effects at lower exposure levels may provide information to define the NOAEL-LOAEL boundary, decreasing uncertainty in the MRL. The intermediate-duration oral MRL is based on the LOAEL of 1 mg/kg/day for hematological effects (decreased number of WBCs, lymphocytes, neutrophils, and monocytes) in mice (Li et al. 2018). This study also identified a NOAEL of 0.1 mg/kg/day. The NOAEL and LOAEL values of 0.1 and 1 mg/kg/day, respectively, are the lowest doses evaluated for intermediate-duration oral studies on benzene. Additional studies at these low doses could provide additional supportive data for the intermediate-duration oral MRL and perhaps identify a lower LOAEL value for adverse health effects. Most intermediate-duration oral studies were conducted at doses much greater than 1 mg/kg/day.

Chronic-Duration MRLs. The primary target for adverse systemic effects of benzene following chronic-duration inhalation exposure is the hematological system. A provisional chronic-duration inhalation MRL was derived based on data from a study in humans that observed hematological effects (decreased B-cell count) (Lan et al. 2004a). Lan et al. (2004a) reported the lowest LOAEL of 0.57 ppm for hematotoxicity in the chronic-duration inhalation database for humans. The MRL is supported by numerous occupational exposure studies and studies in laboratory animals showing that chronic-duration

exposure to benzene is hematotoxic. Additional occupational studies at low exposure levels may identify lower LOAEL values for hematological effects.

No human data are available to evaluate hematological effects following oral exposure. Although chronic-duration oral animal studies are available for hematological effects (Maltoni et al. 1983, 1985; NTP 1986), the most extensive study (NTP 1986) did not conclusively define a NOAEL or a less serious LOAEL for endpoints that could be used to derive an MRL. A provisional chronic-duration oral MRL of 0.0003 mg/kg/day was derived based on a route-to-route extrapolation of the provisional chronic-duration inhalation MRL. The critical effect was decreased number of peripheral lymphocytes (B-cell lymphocytes) in shoe manufacturing workers exposed to benzene (Lan et al. 2004a). A total uncertainty factor of 3 was applied for route-to-route extrapolation. Note that the provisional chronic-duration inhalation included an uncertainty factor of 10 for intraspecies variability. Additional chronic-duration oral animal data at low doses could provide a chronic-duration oral MRL and assist in defining threshold levels for populations living near hazardous waste sites.

Health Effects.

Hematological. The hematological system is a well-established target for benzene toxicity, with numerous studies providing support for adverse effects. Additional occupational and animal studies evaluating low benzene air concentrations may provide additional information to further define the NOAEL-LOAEL boundary. The database of oral exposure studies is much smaller for inhalation. Additional studies evaluating hematological effects at lower oral doses benzene for acute, intermediate, and chronic durations would provide information to more fully define oral dose-response relationships.

Immunological. Immunological effects can be categorized as: (1) indirect effects resulting from decreased WBCs due to hematotoxicity, or (2) direct effects on the function of immune cells. Discussions above consider the indirect effects benzene on the immunological system. However, relatively little information is available on the direct effects on immune function. Additional studies in humans and animals across a wide range of exposures and exposure levels would provide important information. Additional mechanism studies may identify critical effects on the immune system that would broadly inform the immunological effects of benzene.

Neurological. In humans, exposure to high levels of benzene can be neurotoxic and fatal. However, at lower occupational exposures, neurological effects have not been reported. Studies

evaluating neurological function in workers would define the potential for occupational exposure to benzene to produce adverse effects. Few studies in animals have evaluated neurological effects of benzene. Inhalation and oral studies in animals evaluating a full observational battery of effects over a large dose range and all exposure durations would provide information to better determine the neurotoxicity of benzene.

Reproductive. Reproductive effects of benzene exposure were studied in one inhalation study of workers and one study of exposure to contaminated drinking water; no reproductive effects were observed in either study. Additional epidemiological studies evaluating the potential effects of benzene by inhalation and oral exposures would provide important information to determine if reproductive effects are a concern in humans. Studies in animals have identified adverse reproductive effects; however, additional studies specifically designed to evaluate reproductive effects could further define these adverse effects. In addition, no 2-generation reproductive studies were identified; such studies would provide important information in understanding the potential for benzene to produce adverse reproductive effects.

Developmental. No reliable studies evaluating developmental effects in humans were identified. Studies in animals have evaluated developmental effects of benzene. However, additional studies in animals would provide data to further define effects. For example, an inhalation study identified effects on the hematological system in neonates and 6-week-old offspring (Keller and Snyder 1988); however, no other studies have evaluated hematological effects as a developmental endpoint. Studies identifying the potential of benzene to produce hematological or other systemic effects in offspring can provide important information regarding nontraditional developmental endpoints.

Cancer. EPA (IRIS 2003), IARC (2018), and NTP (2021) have concluded that benzene is a human carcinogen based on sufficient data in humans supported by animal evidence. Epidemiological studies and case reports provide clear evidence of associations between occupational exposure to benzene and the occurrence of AML (IARC 2018; IRIS 2003; Yin et al. 1996a, 1996b), as well as suggestive evidence of associations between benzene and NHL and multiple myeloma (Hayes et al. 1997; Rinsky et al. 1987). Strong support for the carcinogenicity of benzene is also provided in numerous animal studies. Additional occupational studies could better characterize exposure level and exposure duration relationships for benzene and leukemia,

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

Epidemiological and Human Dosimetry Studies. For inhalation exposure, several occupational exposure studies provide data that evaluate associations between measured exposure to benzene and hematological effects. Additional studies at low occupational exposure levels would provide more insight into effects of lower measured exposures. In addition, little data are available for associations between urinary benzene and benzene metabolites and hematological effects. Urinary metabolites can confirm that exposure to benzene has occurred; however, due to human variability in benzene metabolism to toxic metabolites, quantitative associations between various benzene metabolites and adverse effects need further investigation. Very little data are available to examine associations between oral exposure to water or food contaminated with benzene and health effects. Additional studies could provide important information to establish these relationships and possibly provide dose-response data.

Biomarkers of Exposure and Effect. Several biomarkers of exposure have been identified to demonstrate exposure to benzene. These include unmetabolized benzene in the expired air and urine and urinary metabolites of benzene, including phenol, *trans,trans*-muconic acid, and PhMA. Urinary metabolites are commonly used as biomarkers of exposure (IARC 2018; Section 3.3.1). Urinary benzene and PhMA are specific biomarkers for benzene exposure; however, urinary *trans,trans*-muconic acid and phenol are not specific for benzene exposure as they also are metabolites of other metabolites (Section 3.3.1). Studies examining the relationships between various urinary metabolites and external benzene exposure would provide additional information for quantifying exposures.

There are no clinical effects that are unique to benzene. However, blood counts of benzene workers are routinely monitored for decreased cell counts. Thus, a combination of blood counts and known exposure to benzene provide information on measures of effect. Additional studies could further define threshold levels of adverse hematological effects based on blood count monitoring. DNA adducts with benzene metabolites, chromosomal aberrations in bone marrow and peripheral blood lymphocytes, and sister chromatid exchanges could be used to monitor for benzene effects; however, other than the formation of DNA adducts with benzene metabolites, these biomarkers are not specific to benzene (IARC 2018; McHale et al. 2012).

Absorption, Distribution, Metabolism, and Excretion. Data from both humans and animals consistently indicate that inhaled benzene is rapidly absorbed through the lungs (Eutermoser et al. 1986;

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

There is no evidence to suggest that the route of administration has any substantial effect on the subsequent metabolism of benzene, either in humans or animals. Benzene is metabolized primarily in the liver; however, production of reactive metabolites in the bone marrow also contributes to toxicity (Section 3.1.3). Benzene is a preferential substrate of CYP2E1, which also metabolizes alcohol. The induction of CYP2E1 by benzene (and some of its metabolites) with subsequent generation of reactive metabolites, oxygen radicals, circulating lipid peroxides, and hydroxyl radicals could be associated with hematopoietic toxicity and carcinogenicity of benzene (Irons 2000; Parke 1989; Ross 1996, 2000; Smith 1996a, 1996b; Snyder 2000a, 2000b, 2002; Snyder and Hedli 1996; Snyder and Kalf 1994). CYP2E1 is not confined to the liver: it has also been detected in bone marrow. Andrews et al. (1979) demonstrated that rabbit bone marrow is capable of metabolizing benzene. Schnier et al. (1989) subsequently found that rabbit bone marrow contains CYP2E1. Irons et al. (1980) demonstrated that benzene metabolism by rat bone marrow (*in situ*) was complete and independent of metabolism by the liver, with concentrations of phenol greater than catechol and hydroquinone. Although the total metabolism by bone marrow was limited (total metabolites present were 25% of those in blood), the concentration of metabolites in the bone marrow exceeded that in the blood. Similar studies have been conducted in mice (Ganousis et al. 1992). Benzene metabolism in bone marrow is not well understood; additional data regarding the initial oxidation step and the comparatively low levels of CYP2E1 activity in bone marrow would be useful in identifying the mechanisms of benzene's hematotoxicity. This aspect of metabolism may have implications for long-term exposures, which could be explored in chronic-duration exposure studies. The intermediary metabolites of benzene are responsible for many of the toxic effects observed (Eastmond et al. 1987; Gad-El-Karim et al. 1985). Biotransformation is believed to be essential for benzene-induced bone marrow damage.

Reactive metabolites of benzene formed in liver and bone marrow contribute to hematologic toxicity to benzene (Section 3.1.5). Studies that quantify the relative contributions of metabolism in liver and bone marrow to hematopoietic toxicity would improve modeling of tissue dosimetry and toxicodynamics. Additionally, more information is needed on the pathways of metabolism in humans, the chemical nature of the toxic metabolites, and the mechanism of toxicity. Data comparing urinary metabolite profiles of orally administered benzene and phenol in mice suggest that zonal differences in metabolism in the liver may be responsible for relative differences in the production of hydroquinone, thus explaining the higher toxicity observed after benzene administration compared with phenol administration (Kenyon et al. 1995). Additional work in this area would aid in further understanding the kinetic determinants of benzene toxicity. Ethanol and dietary factors such as food deprivation and carbohydrate restriction enhance the hematotoxic effects of benzene. Therefore, more information regarding differences in metabolic pattern according to sex, age, nutritional status, and species, and correlation to differences in health effects would be useful.

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

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

Children's Susceptibility. No evidence of age-related differences in susceptibility to benzene toxicity was located. Fetal exposure occurs as benzene crosses the placenta. In addition, nursing infants can be exposed to benzene in the breast milk. Children could potentially be at increased risk for benzene toxicity via the inhalation exposure route based on higher activity levels and ventilation rates than adults. Age-related differences in benzene metabolism could potentially affect susceptibility. However, the

susceptibility of children relative to adults is unknown. Well-designed animal studies should be performed to adequately assess the potential for age-related increased susceptibility to benzene, including gestational exposure and exposure in neonates followed through maturation. Specifically, the most sensitive endpoints (hematological and immunological) should be examined.

Production, Import/Export, Use, Release, and Disposal. Benzene is one of the top 20 highest volume chemicals produced in the United States. In 1994, the U.S. production volume of benzene was 14.7 billion pounds (C&EN 1995). The production volume during the 1984–1994 period increased by 4% annually (C&EN 1995). The United States currently reports nationally aggregated production between $10x10^{10}$ and $20x10^{10}$ pounds (EPA 2022a). Imports of benzene into the United States have generally ranged from 3,643 to 4,715 billion pounds from 2020 to 2022 (USITC 2023). Exports were 352–678 billion pounds during the same time period (USITC 2023). The major use of benzene is in the production of other chemicals (primarily ethylbenzene, cumene, and cyclohexane), accounting for approximately 99% of benzene production volume. Benzene is also used as an anti-knock agent in unleaded gasoline (EPA 2023a; NESCAUM 1989; NTP 1994). The widespread use of benzene as a solvent has decreased in recent years due to benzene's listing as a human carcinogen (IRIS 2003). Many products that used benzene as a solvent in the past have replaced it with other organic solvents; however, benzene may still occur as a trace impurity in these products. Less than 2% of the amount of benzene produced is used as a solvent in such products as trade and industrial paints, rubber cements, adhesives, paint removers, artificial leather, and rubber goods. Benzene has also been used in the shoe manufacturing and rotogravure printing industries (EPA 1978; OSHA 1977). In the past, certain consumer products (such as some paint strippers, carburetor cleaners, denatured alcohol, and rubber cement used in tire patch kits and arts and crafts supplies) contained small amounts of benzene (Young et al. 1978). Other consumer products that contained benzene were certain types of carpet glue, textured carpet liquid detergent, and furniture wax (Wallace et al. 1987). Benzene-containing wastes, such as commercial chemical products, manufacturing chemical intermediates, and spent solvents, are subject to federal and/or state hazardous waste regulations. Currently, the recommended method of disposal is to incinerate solvent mixtures and sludges at a temperature that ensures complete combustion. No additional information on the production, import/export, use, release, or disposal of benzene is needed at this time.

Environmental Fate. Benzene released to the environment partitions mainly to the atmosphere (Mackay and Leinonen 1975; NLM 2023). However, the compound can also be found in surface water and groundwater. Benzene is mobile in soil (Karickhoff 1981; Kenaga 1980); however, there is a need for more information on the leachability potential of benzene to groundwater in different soil types.

Benzene is transformed in the atmosphere by photooxidation. Biodegradation, principally aerobic, is the most important fate process of benzene in water (Delfino and Miles 1985; McAllister and Chiang 1994; Salanitro 1993) and soil (Gibson 1980; Hopper 1978; Salanitro 1993). Benzene can persist in groundwater. Other than leachability potential, no additional information on the environmental fate of benzene is needed at this time.

Bioavailability from Environmental Media. Benzene can be absorbed following oral, dermal, and inhalation exposure (see Section 3.1.1). These routes of exposure may be of concern to humans because of the potential for benzene to contaminate the air, drinking water, and soil (see Section 5.2). Information on inhalation exposure and on the absorption of benzene following ingestion of plants grown in contaminated environments near hazardous waste sites would be helpful in determining bioavailability of the compound in these media.

Food Chain Bioaccumulation. Benzene has an estimated low-to-moderate bioconcentration potential in aquatic organisms (Miller et al. 1985; Ogata et al. 1984) and some plants (Geyer et al. 1984). Most of the benzene accumulation on vegetation results from air-to-leaf transfer (Collins et al. 2000). Root uptake is not believed to be important (Hattemer-Frey et al. 1990). Biomagnification in aquatic food chains does not appear to be important (Ogata et al. 1984). No further information appears to be needed.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of benzene in contaminated media at hazardous waste sites are needed so that the information obtained on levels of benzene in the environment can be used in combination with the known body burden of benzene to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Benzene is widely distributed in the environment and has been detected in air (EPA 2023b; Mohamed et al. 2002; Morello-Frosch et al. 2000), water (Rowe et al. 2007; USGS 2014, 2020; WQP 2023), and some foods (Fleming-Jones and Smith 2003; Medeiros Vinci et al. 2012). Limited soil and sediment monitoring data are available; benzene is typically not detected in ambient samples (WQP 2023) but has been detected at hazardous waste sites (ATSDR 2019a, 2023d). The levels of benzene in air and water are well documented, but there is a need for more current information at hazardous waste sites. Benzene is not expected to be a significant contaminant in aquatic foods (Geyer et al. 1984; Gossett et al. 1983; Miller et al. 1985; Ogata et al. 1984); however, some contamination of food crops consumed by humans may occur, primarily from air-to-leaf transfer (Hattemer-Frey et al. 1990). The total concentration of benzene on exposed food crops consumed by humans was estimated to be 587 ng/kg (Hattemer-Frey et al.

1990). An estimated daily dietary intake of 0.020 μg/kg body weight/day was derived based on detections in food available in Belgian markets (Medeiros Vinci et al. 2012). Humans are at risk of exposure to benzene because of its widespread distribution in the environment, and are typically exposed to higher concentrations in indoor air (George et al. 2011; Kinney et al. 2002; Weisel et al. 2008). Releases to the air from gasoline, smoking, and automobile exhaust constitute the major risk of potential exposure for the general population (Wallace 1995). Indoor air may also be a major risk of potential exposure based on measured benzene pollution from cars in attached garages, pollution in residences that used fuel oil for heating or burned wood in fireplaces, and emission rates during cooking (Kashtan et al. 2023; Maine DEP 2014; NYSDOH 2005; Schauer et al. 2001; Thomas et al. 1993; Weisel et al. 2008). Additional data characterizing the concentration of benzene in drinking water, outdoor and indoor air, and soil surrounding hazardous waste sites would be helpful in assessing human exposure for populations living near these waste sites.

Exposure Levels in Humans. Benzene has been detected in human body fluids and tissues such as blood, urine, and breast milk (CDC 2022a, 2022b; Kim et al. 2007b). Most of the monitoring data have come from occupational studies of specific worker populations exposed to benzene (Inoue et al. 1989; Karacic et al. 1987; OSHA 1987; van Wijngaarden and Stewart 2003). Biological monitoring studies exist for the general population (CDC 2022a). There is information for background levels in breath of smokers and nonsmokers (Wallace 1989b), baseline blood levels (CDC 2022a), and levels of urinary metabolites in unexposed people (CDC 2022b). Information on exposure levels for populations living in the vicinity of hazardous waste sites would be helpful in estimating exposure in these groups. More recent information on worker exposure levels would be helpful in estimating current occupational exposure.

Exposures of Children. Benzene levels have been monitored in children and the environments in which they live. This information gives levels found for infants and children in rural and urban areas as well as the levels found for children in homes of parents who smoke (Duarte-Davidson et al. 2001). There have been many studies relating oil and petroleum exposure to childhood leukemia and other diseases; however, the majority of these studies have not recorded benzene levels. More information about the exposures of children, particularly those subject to high exposures such as smoking, busy roads, and gasoline stations, are needed.

6.3 ONGOING STUDIES

Ongoing studies identified in the National Institute of Health (NIH) RePORTER (2024) database, which tracks projects funded by NIH, are provided in Table 6-1.

Table 6-1. Ongoing Studies on Benzene

Investigator	Affiliation	Research description	Sponsor
Cassidy-Bushrow, Andrea	Wayne State University	Nested case-control study on the effects of volatile organic compounds and preterm birth	NIEHS
Mor, Gil G	Wayne State University	Effect of benzene on fetal T-cell development	NICHD
Sadagurski, Marianna	Wayne State University	Investigation on benzene exposure of neuroinflammation and metabolic dysregulation	NIEHS
Zelko, Igor	University of Louisville	Studies on the effects of benzene and the exacerbation of cardiac function	NIEHS

NICHD = National Institute of Child Health and Human Development; NIEHS = National Institute of Environmental Health Sciences

Source: RePORTER (2024)

CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding benzene in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency.

ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the MRLs for benzene.

Table 7-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
	Air		
EPA	RfC, chronic	0.03 mg/m^3 (0.009 ppm)	IRIS 2003
	Provisional peer-reviewed toxicity values		EPA 2009a
	Provisional RfC, subchronic	0.08 mg/m^3 (0.03 ppm)	
WHO	Air quality guidelines		WHO 2010
	Unit risk of leukemia per 1 μg/m^3 air concentration	6x10^{-6} [a]	
	Water & Food		
EPA	Drinking water standards and health advisories		EPA 2018a
	1-Day health advisory (10-kg child)	0.2 mg/L	
	10-Day health advisory (10-kg child)	0.2 mg/L	
	DWEL	0.1 mg/L	
	Lifetime health advisory	0.003 mg/L	
	10^{-4} Cancer risk	1–10 mg/L	
	National primary drinking water regulations		EPA 2009b
	MCLG	Zero mg/L	
	MCL	0.005 mg/L	
	RfD, chronic	0.004 mg/kg/day	IRIS 2003
	Provisional peer-reviewed toxicity values		EPA 2009a
	Provisional RfD, subchronic	0.01 mg/kg/day	
WHO	Drinking water quality guidelines	0.01 mg/L	WHO 2022

Table 7-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
FDA	Substances added to food (formerly EAFUS)	Permitted for use in food-packaging adhesives (21 CFR 175.105); and permitted in the manufacture of modified hop extract, with limitations (21 CFR 172.560)	FDA 2024
	Allowable level in bottled water	0.005 mg/L	FDA 2023d
	Cancer		
HHS	Carcinogenicity classification	Known to be a human carcinogen	NTP 2021
EPA	Carcinogenicity classification	Group A[b]	IRIS 2003
	Oral slope factor	1.5×10^{-2}–5.5×10^{-2} per (mg/kg)/day	
	Inhalation unit risk	2.2×10^{-6}–7.8×10^{-6} per µg/m^3	
IARC	Carcinogenicity classification	Group 1[c]	IARC 2018
	Occupational		
OSHA	PEL (8-hour TWA) for general industry, shipyards, and construction	1 ppm	OSHA 2022b, 2022c, 2022d
	STEL (15-minute TWA) for general industry, shipyards, and construction	5 ppm	
	PEL (8-hour TWA)	10 ppm[d]	OSHA 2022a
	Acceptable ceiling concentration	25 ppm[d]	
	Acceptable maximum peak above the acceptable ceiling concentration for an 8-hour shift (maximum duration is 10 minutes)	50 ppm[d]	
NIOSH	REL (up to 10-hour TWA)	0.1 ppm[e]	NIOSH 2019
	STEL (15-minute TWA)	1.0 ppm[e]	
	Emergency Criteria		
NIOSH	IDLH	500 ppm[e]	NIOSH 2019
EPA	AEGLs-air		EPA 2018b
	AEGL 1[f]		
	10-minute	130 ppm	
	30-minute	73 ppm	
	60-minute	52 ppm	
	4-hour	18 ppm	
	8-hour	9.0 ppm	
	AEGL 2[f]		
	10-minute	2,000 ppm[g]	
	30-minute	1,100 ppm	
	60-minute	800 ppm	

Table 7-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
	4-hour	400 ppm	
	8-hour	200 ppm	
	AEGL 3[f]		
	10-minute	9,700 ppm[h]	
	30-minute	5,600 ppm[g]	
	60-minute	4,000 ppm[g]	
	4-hour	2,000 ppm[g]	
	8-hour	990 ppm	
DOE	PACs-air		DOE 2018a
	PAC-1[i]	52 ppm	
	PAC-2[i]	800 ppm	
	PAC-3[i]	4,000 ppm[j]	

[a]Guideline summary states that benzene is a genotoxic carcinogen in humans and no safe level of exposure can be recommended.
[b]Group A: known human carcinogen.
[c]Group 1: carcinogenic to humans.
[d]This standard applies to the industry segments exempt from the 1 ppm 8-hour TWA and 5 ppm STEL of the benzene standard at 29 CFR 1910.1028 (OSHA 2022b).
[e]NIOSH potential occupational carcinogen.
[f]Definitions of AEGL terminology are available from EPA (2018c).
[g]Value is ≥10% of the lower explosive limit (LEL), 14,000 ppm; safety considerations against the hazard of explosion must be taken into account.
[h]Value is ≥50% of the LEL; extreme safety considerations against the hazard of explosion must be taken into account.
[i]Definitions of PAC terminology are available from DOE (2018b).
[j]Value is greater ≥10% of the LEL, but <50% of the LEL.

AEGL = acute exposure guideline levels; CFR = Code of Federal Regulations; DOE = Department of Energy; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = U.S. Environmental Protection Agency; FDA = Food and Drug Administration; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; LEL = lower explosive limit; MCL = maximum contaminant level; MCLG = maximum contaminant level goal; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = protective action criteria; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; STEL = short-term exposure limit; TWA = time-weighted average; WHO = World Health Organization

CHAPTER 8. REFERENCES

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APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. LOAELs for serious health effects (such as irreparable damage to the liver or kidneys, or serious birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substances than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Office of Innovation and Analytics, Toxicology Section, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Office of Innovation and Analytics, Toxicology Section, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S106-5, Atlanta, Georgia 30329-4027.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Benzene
CAS Numbers: 71-43-2
Date: October 2024
Profile Status: Draft for Public Comment
Route: Inhalation
Duration: Acute
Provisional MRL: 0.009 ppm (0.03 mg/m^3)
Critical Effect: Hematological/Immunological
Reference: Rozen et al. 1984
Point of Departure: LOAEL of 10.2 ppm ($LOAEL_{HEC}$ of 2.55 ppm)
Uncertainty Factor: 300
LSE Graph Key: 34
Species: Mouse

MRL Summary: A provisional acute-duration inhalation MRL of 0.009 ppm was derived based on decreased number of peripheral lymphocytes and impaired function of marrow lymphocytes (decreased mitogen response of B-lymphocytes) in male C57BL/6J mice (Rozen et al. 1984). The MRL is based on a LOAEL (10.2 ppm) in mice exposed to benzene 6 hours/day for 6 consecutive days. The LOAEL was duration adjusted and converted to a LOAEL human equivalent concentration ($LOAEL_{HEC}$) of 2.55 ppm. A total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from rats to humans after dosimetric adjustment, and 10 for human variability) was applied.

Selection of the Critical Effect: Studies of effects associated with acute-duration inhalation exposure of humans to benzene are limited to case studies of accidental or intentional exposures to near-fatal or fatal levels. Therefore, human data are not suitable for derivation of an acute-duration inhalation MRL. Several acute-duration inhalation studies have been conducted in laboratory animals. To identify the critical effect, ATSDR focused on: (1) reported effects associated with clear biological significance; (2) high-quality, acute-duration studies including a minimum of five animals per exposure group; and (3) studies that reported LOAEL values that were within a factor of 10 from the lowest reported LOAEL (10.2 ppm). The most sensitive LOAELs meeting these criteria are summarized in Table A-1.

Table A-1. Select LOAELs for Acute-Duration Inhalation Exposure to Benzene

Species (number)	Duration	NOAEL/LOAEL (ppm) NOAEL	LOAEL	System: Effect	Reference
Mouse (n=5–8)	6 days (6 hours/day)	ND	10.2	***Hematological:*** ~35% decrease in peripheral lymphocytes ***Immunological:*** 30% decrease in mitogen response of spleen B-lymphocytes	Rozen et al. 1984
Mouse (n=5)	5 days (6 hours/day)	ND	10.3	***Hematological:*** 50% decrease marrow erythroid CFU-E ***Immunological:*** 44% decrease in response of marrow CFU-E to erythropoietin	Dempster and Snyder 1991

Table A-1. Select LOAELs for Acute-Duration Inhalation Exposure to Benzene

Species (number)	Duration	NOAEL/LOAEL (ppm) NOAEL	LOAEL	System: Effect	Reference
Mouse (n=5–7)	5 days (6 hours/day)	10	30	***Hematological:*** ~38% decrease in peripheral lymphocytes	Rosenthal and Snyder 1985
		10	30	***Immunological:*** decreased resistance to bacterial infection	
Mouse (n=5–10)	GDs 6–16 (6 hours/day)	10	20	***Developmental:*** Decreased peripheral erythroid precursors and granulocytic precursor cells in neonates and 6-week-old offspring	Keller and Snyder 1988
Mouse (n=5–8)	14 days (6 hours/day)	ND	48	***Hematological:*** 46% decrease in peripheral WBCs ***Immunological:*** 70% decrease splenic lymphocyte antibody production	Aoyama 1986
Mouse (n=20–48)	GDs 7–14 (24 hours/day)	ND	47	***Developmental:*** 5% decrease in fetal body weight	Tatrai et al. 1980b
Rat (n=17–20)	GDs 6–15 (7 hours/day)	10	50 (SLOAEL)	***Developmental:*** 14% decrease in fetal body weight	Kuna and Kapp 1981

Selected study for the acute-duration inhalation MRL derivation.

CFU-E = erythroid colony-forming unit; GD = gestation day; LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level; SLOAEL = serious lowest-observed-adverse-effect level; WBC = white blood cell

Hematological and immunological effects were selected as the co-critical effects following acute-duration inhalation exposure to benzene because they represent the lowest reliable LOAEL (Rozen et al. 1984). A systematic review (Appendix C) resulted in the hazard identification conclusions that hematological and immunological effects are known health effects for humans. There is a preponderance of evidence that hematopoietic tissues (e.g., marrow) and immune system are sensitive targets for benzene. Observed effects in acute-duration animal studies include the following: (1) decreased peripheral lymphocytes (Aoyama 1986; Cronkite et al. 1985; Rozen et al. 1984; Wells and Nerland 1991 [see Table 2-5 for additional studies]); (2) decreased marrow hematopoietic stem cells (Chertkov et al. 1992; Cronkite et al. 1989; Dempster and Snyder 1991; Farris et al. 1997a) [see Table 2-5 for additional studies]); (3) decreased responses of lymphocytes to mitogens and antigens (Dempster and Snyder 1991; Rozen et al. 1984); (4) decreased splenic production of antibodies (Aoyama 1986); and (5) decreased cellular immunity in whole animals (Rosenthal and Snyder 1985). Collectively, these studies show dose- and duration-dependent effects on hematopoiesis and immune responses. An abundance of mechanistic evidence supports a mode of action for hematological and immunological effects of benzene that involves marrow cytotoxicity and genotoxicity of reactive metabolites of benzene (see Section 2.20).

Selection of the Principal Study: The acute-duration inhalation study in male mice reported by Rozen et al. (1984) was selected as the principal study because it identified the lowest LOAEL for the critical effect (hematological, immunological). Rozen et al. (1984) was rated as a First Tier, High Confidence study during systematic review (Appendix C).

Summary of the Principal Study:

Rozen MG, Snyder CA, Albert RE. 1984. Depressions in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. Toxicol Lett 20(3):343-349. https://doi.org/10.1016/0378-4274(84)90170-x.

Male C57BL/6J mice (7–8/group) were exposed to benzene (mean measured concentrations: 0, 10.2, 31, 100, or 301 ppm) in whole-body dynamic inhalation chambers for 6 hours/day for 6 consecutive days. Control mice were exposed to filtered air, only. Exposure to ≥10.2 ppm resulted in a decrease in peripheral lymphocytes. The decrease was approximately 35% in the 10.2 ppm group (based on Figure 1 of Rozen et al. 1984). Exposure to ≥10.2 ppm resulted in a decrease in mitogen response of marrow B-lymphocytes to lipopolysaccharide (based on a CFU assay of marrow from exposed and control mice). This decrease was 30% in the 10.2 ppm exposure group (Figure 2 of Rozen et al. 1984). Exposure to ≥31 ppm resulted in a decrease in mitogen response of splenic T-lymphocytes to phytohemagglutinin. This decrease was approximately 85% in the 31-ppm group (Figure 3 of Rozen et al. 1984). Exposure to ≥100 ppm resulted in a decrease in peripheral erythrocytes. This decrease was approximately 10% in the 100-ppm group (Figure 1 of Rozen et al. 1984).

Selection of the Point of Departure for the MRL: The LOAEL (10.2) ppm from the Rozen et al. (1984) study was selected as the point of departure (POD) for deriving the acute-duration MRL.

Benchmark dose (BMD) modeling of data on peripheral lymphocyte counts and lipopolysaccharide-induced marrow CFUs was attempted but was not successful. The data (digitized from Figures 1 and 2 of Rozen et al. 1984) could not be fit to BMD models because the responses were non-monotonic. Peripheral lymphocyte counts and marrow CFUs were higher in the 31-ppm group compared to the 10.2-ppm group, although both were significantly below the control group.

Calculations

Adjustment for Intermittent Exposure: The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10.2 ppm) by 6/24 to correct for less than a full day of exposure. The resulting adjusted LOAEL ($LOAEL_{ADJ}$) is 2.55 ppm.

$$LOAEL_{ADJ} = LOAEL\ (10.2\ ppm) \times 6\ hours/24\ hours$$
$$LOAEL_{ADJ} = 2.55\ ppm$$

Human Equivalent Concentration: A review of available PBPK models for benzene did not identify any models that could provide validated interspecies dosimetry extrapolation of doses of reactive benzene metabolites to hematopoietic tissues (see Section 3.1.5). Therefore, the EPA (1994b) methodology for calculating a HEC for extrarespiratory effects of a category 3 gas (such as benzene) was applied to the $LOAEL_{ADJ}$:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times ([H_{b/g}]_A/[H_{b/g}]_H)$$

where:

$LOAEL_{HEC}$ = the LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = the LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A/[H_{b/g}]_H$ = the ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the ratio. According to Wiester et al. (2002), benzene blood:gas partition coefficients for mice and humans are 17.44 and 8.12, respectively. Therefore, the default value of 1 is applied, in which case, the $LOAEL_{HEC}$ is equivalent to the $LOAEL_{ADJ}$ = 2.55 ppm.

Uncertainty Factor: 300

- 10 for use of a LOAEL
- 3 for extrapolation from animals to humans using dosimetric conversion
- 10 for human variability

Provisional acute-duration inhalation MRL = $LOAEL_{ADJ}$ ÷ total uncertainty factor
= 2.55 ppm ÷ 300
= 0.0085 ppm ≈ 0.009 ppm (rounded)

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Numerous human epidemiological and animal studies provide strong support for causal associations between inhalation exposure to benzene and impaired function of hematopoietic tissues and altered immune responses (see Sections 2.7 and 2.14). The LOAEL from Rozen et al. (1984) of 10.2 ppm is corroborated by the results of the Dempster and Snyder (1991) study, which found hematologic effects in mice exposed to 10.3 ppm for 6 days. In the Dempster and Snyder (1991) study, the outcomes were decreased marrow erythroid CFU (CFU-E) and decreased response of marrow CFU-E to erythropoietin. Several other studies found hematological and/or immunological effects in mice exposed to benzene at concentrations of 21–47 ppm for acute durations (Aoyama 1986; Cronkite et al. 1985; Rosenthal and Snyder 1985; Toft et al. 1982; Wells and Nerland 1991). At higher exposures, numerous studies evaluating acute-duration exposures ≥100 have also demonstrated hematotoxicity and immunotoxicity. One developmental study also observed hematological effects in offspring of pregnant mice exposed to benzene during gestation (Keller and Snyder 1988). The LOAEL from this study was 20 ppm (for decreased peripheral erythroid and granulocyte progenitor cells), with a NOAEL of 10 ppm. The MRL based on hematological effects in adult animals at a LOAEL of 10.2 ppm is expected to be protective of observed developmental hematological effects reported at higher concentrations. Other developmental effects (e.g., decreased fetal growth) were observed only at ≥47 ppm (Kuna and Kapp 1981; Tatrai et al. 1980b).

Agency Contacts (Chemical Managers): Gaston Casillas, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Benzene
CAS Numbers: 71-43-2
Date: October 2024
Profile Status: Draft for Public Comment
Route: Inhalation
Duration: Intermediate
Provisional MRL: 0.007 ppm (0.02 mg/m^3)
Critical Effect: Immunological
Reference: Rosenthal and Snyder 1987
Point of Departure: LOAEL of 11.1 ppm ($LOAEL_{HEC}$ of 1.98 ppm)
Uncertainty Factor: 300
LSE Graph Key: 81
Species: Mouse

MRL Summary: A provisional intermediate-duration inhalation MRL of 0.007 ppm was derived based on delayed splenic lymphocyte reaction to foreign antigens in male C57BL/6J mice (Rosenthal and Snyder, 1987). The MRL is based on a LOAEL (11.1 ppm) in mice exposed to benzene 5 days/week, 6 hours/day for 20 days. The LOAEL was converted to a HEC resulting in a value of 1.98 ppm and then divided by a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from rats to humans after dosimetric adjustment, and 10 for human variability).

Selection of the Critical Effect: Epidemiological studies that provide reliable estimates of associations between benzene exposure concentrations and health outcomes evaluated cohorts exposed for periods of >1 year (see Sections 2.7 and 2.14). Therefore, human data are not suitable for derivation of an intermediate-duration inhalation MRL. Several intermediate-duration inhalation studies have been conducted in laboratory animals. To identify the critical effect, ATSDR focused on: (1) reported effects associated with clear biological significance; (2) high-quality, intermediate-duration studies including a minimum of five animals per exposure group; and (3) LOAEL values that are less than 10-fold greater than the lowest LOAEL (10.1 ppm). The most sensitive LOAELs meeting these criteria are summarized in Table A-2.

Table A-2. Select LOAELs for Intermediate-Duration Inhalation Exposure to Benzene

Species (number)	Duration	NOAEL/LOAEL (ppm) NOAEL	LOAEL	System: effect	Reference
Mouse (n=5)	24 weeks (5 days/week, 6 hours/day)	ND	10.1	***Hematological:*** ~36% decrease in peripheral lymphocytes; 94% decrease in marrow CFU-E; decreased marrow and splenic cellularity	Baarson et al. 1984
Mouse (n=5-10)	20 days (5 days/week, 6 hours/day)	ND	11.1	***Immunological:*** Delayed splenic lymphocyte reaction to foreign antigens	Rosenthal and Snyder 1987
Mouse (n=24)	6 weeks (5 days/week, 6 hours/day)	ND	50	***Hematological:*** ~25% decrease in marrow hematopoietic progenitor cells	Malovichko et al. 2021

Table A-2. Select LOAELs for Intermediate-Duration Inhalation Exposure to Benzene

Species (number)	Duration	NOAEL/LOAEL (ppm) NOAEL	LOAEL	System: effect	Reference
Mouse (n=6-11)	6 weeks (5 days/week, 6 hours/day)	ND	50	***Cardiovascular:*** Decreased fractional shortening of the left ventricle during diastole	Zelko et al. 2021
Mouse (n=8)	GDs 6–18 (5 hours/day)	ND	50	***Developmental (LOAEL):*** 5% decrease in fetal weight ***Reproductive (SLOAEL):*** Increased resorptions and pregnancy loss	Maxwell et al. 2023
Mouse (n=5-6)	GDs 1–21 (6 days/week)	ND	50	***Developmental:*** Altered responses to glucose and insulin	Koshko et al. 2021
Mouse (n=20)	6 weeks (6 hours/day)	ND	50	***Endocrine:*** Decreased insulin and glucose tolerances; increased glucose and insulin serum concentrations without challenge	Li et al. 2018

Selected study for the intermediate-duration inhalation MRL derivation.

CFU-E = erythroid colony-forming unit; GD = gestational day; LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level; SLOAEL = serious lowest-observed-adverse-effect level;

Immunological effects were selected as the critical effect following intermediate-duration inhalation exposure to benzene based on the lowest reliable LOAEL (Rosenthal and Snyder 1987). A systematic review (Appendix C) resulted in the hazard identification conclusion that hematological effects are known health effects for humans. There is a preponderance of evidence that hematopoietic tissues (e.g., marrow, spleen) and immune responses are sensitive targets for benzene. Observed effects in intermediate-duration studies include the following: (1) decreased cellular immunity in whole animals (Rosenthal and Snyder 1987); (2) decreased antibody response to antigens in whole animals (Stoner et al. 1981); (3) decreased splenic lymphocyte response to foreign antigens and tumor cells (Rosenthal and Snyder 1987); (4) decreased peripheral lymphocytes (Baarson et al. 1984; Dow 1992; Mukhopadhyay and Nath 2014; Snyder et al. 1984; Vacha et al. 1990; Ward et al. 1985); and (5) decreased marrow and spleen hematopoietic stem cells (Malovichko et al. 2021; Seidel et al. 1989; Vacha et al. 1990). Collectively, these studies show concentration- and duration-dependent effects on hematopoiesis and immune responses. An abundance of mechanistic evidence supports a mode of action for hematological and immunological effects of benzene that involves marrow cytotoxicity and genotoxicity of reactive metabolites of benzene (see Section 2.20).

Selection of the Principal Study: The intermediate-duration inhalation study in male mice reported by Rosenthal and Snyder (1987) was selected as the principal study. Rosenthal and Snyder (1987) was rated as a First Tier, High Confidence study during systematic review (Appendix C). Two studies provided very similar LOAELs for hematological and/or immunological effects in mice: 10.2±0.3 (SD) ppm (Baarson et al. 1984) and 11.1±1.5 (SD) ppm (Rosenthal and Snyder 1987). Rosenthal and Snyder (1987) is considered a stronger study than Baarson et al. (1984) because it evaluated a range of exposure concentrations (see description below), whereas Baarson et al. (1984) evaluated a single exposure

concentration. Excluding the Baarson et al. (1984) study from consideration as the basis of the MRL, the Rosenthal and Snyder (1987) study provided the lowest LOAEL for the critical effect (immunological).

Summary of the Principal Study:

Rosenthal GJ, Snyder CA. 1987. Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice. Toxicol Appl Pharmacol. 88(1):35-43. https://doi.org/10.1016/0041-008x(87)90267-5.

Male C57Bl/6 mice (5–10 per dose group) were exposed to 0, 11.1 (±1.5) ppm, 29.5 (±4.4) ppm, or 99.7 (±7.0) ppm benzene (mean measured concentrations) by inhalation 6 hours/day, 5 days/week for 4 weeks. Differential counts of splenic leukocytes and function of splenic lymphocytes cultured from control and exposed mice were evaluated following 4 weeks of exposure. No changes were observed in the splenic leukocyte differential proportions (lymphocytes, Ig+ cells, granulocytes, esterase+ cells) or T-cell subsets (helper, suppressor cells). Therefore, assays of lymphocyte function could be normalized for particular lymphocyte populations by culturing equal numbers of splenic cells from mice from each exposure group. Exposure to 11.1 or 99.7 ppm delayed the immune response (blastogenesis) of splenic lymphocytes to antigens (splenic cells from DBA/2 mice). The delay was greater at 99.7 ppm (the assay was not performed on mice exposed to 29.5 ppm). Co-culturing experiments showed that the delayed response was not due to induction of splenic suppressor cells. Exposure to 99.7 ppm (but not to 11.1 or 29.5 ppm) decreased splenic lymphocyte cytotoxicity to tumor cells (lysis of P815 cells).

Selection of the Point of Departure for the MRL: The LOAEL of 11.1 ppm from the Rosenthal and Snyder (1987) study was selected as the POD for deriving the intermediate-duration MRL. The critical effect measure was delayed immune response (blastogenesis) of splenic lymphocytes to antigens. These data were presented in plots (see Figure 2 of Rosenthal and Snyder 1987), with the delay assessed from the time profiles of the incorporation of radiolabeled thymidine into cultured lymphocytes, which suggest that the time of peak DNA replication was later in cells from mice exposed to 11.1 ppm (or 99.7 ppm) compared to controls. However, because the peak in thymidine incorporation in cells from benzene exposed mice did not occur within the 5-day observation period of the study, these data do not provide quantitative estimates of the time delay. Since quantitative estimates of the time delay could not be made from the data, BMD modeling of the exposure-time delay relationship could not be performed.

Calculations

Adjustment for Intermittent Exposure: The concentration was adjusted for intermittent exposure by multiplying the LOAEL (11.1 ppm) by 6 hours/24 hours to correct for less than a full day of exposure and 5 days/7 days to correct for less than a full week of exposure. The resulting $LOAEL_{ADJ}$ is 1.98 ppm.

$$LOAEL_{ADJ} = LOAEL\ (11.1\ ppm) \times 6\ hours/24\ hours \times 5\ days/7\ days$$
$$LOAEL_{ADJ} = 1.98\ ppm$$

Human Equivalent Concentration: A review of available PBPK models for benzene did not identify any models that could provide validated interspecies dosimetry extrapolation of doses of reactive benzene metabolites to hematopoietic tissues (see Section 3.1.5). Therefore, the EPA (1994b) methodology for calculating a HEC for extrarespiratory effects of a category 3 gas (such as benzene) was applied to the $LOAEL_{ADJ}$:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times ([H_{b/g}]_A/[H_{b/g}]_H)$$

where:
$LOAEL_{HEC}$ = the LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = the LOAEL adjusted from intermittent to continuous exposure
$[H_{b/g}]_A/[H_{b/g}]_H$ = the ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the ratio. According to Wiester et al. (2002), benzene blood:gas partition coefficients for mice and humans are 17.44 and 8.12, respectively. Therefore, the default value of 1 is applied, in which case, the $LOAEL_{HEC}$ is equivalent to the $LOAEL_{ADJ}$ = 1.98 ppm

Uncertainty Factor: 300

- 10 for use of a LOAEL
- 3 for extrapolation from animals to humans using dosimetric conversion
- 10 for human variability

Provisional intermediate-duration inhalation MRL = $LOAEL_{HEC}$ (1.98 ppm) ÷ total UF (300)
= 0.0066 ppm ≈ 0.007 ppm (rounded)

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Numerous human epidemiological and animal studies provide strong support for associations between inhalation exposure to benzene and impaired function of hematopoietic tissues and altered immune responses (see Sections 2.7 and 2.14). The LOAEL from the Rosenthal and Snyder (1987) of 11.1 ppm is supported by the results of the Baarson et al. (1984) study, which found hematological effects in mice exposed to 10.1 for 24 weeks. In the Baarson et al. (1984) study, the outcomes were decreased peripheral lymphocytes, decreased marrow erythroid stem cells and/or progenitor cells (CFU-E), and decreased marrow and splenic cellularity. Several other studies found hematological and/or immunological effects in mice exposed to benzene at concentrations of 50–200 ppm for intermediate durations (Malovichko et al. 2021; Seidel et al. 1989; Stoner et al. 1981). The Malovichko et al. (2021) study observed hematological effects in mice exposed to 50 ppm for 6 weeks. The lowest intermediate-duration LOAELs for other systemic effects were also 50 ppm (cardiovascular, developmental, endocrine). For reproductive effects, 50 ppm is a SLOAEL for increased resorption and pregnancy loss.

Agency Contacts (Chemical Managers): Gaston Casillas, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Benzene
CAS Numbers: 71-43-2
Date: October 2024
Profile Status: Draft for Public Comment
Route: Inhalation
Duration: Chronic
Provisional MRL: 0.002 ppm (0.006 mg/m^3)
Critical Effect: Hematological
Reference: Lan et al. 2004a
Point of Departure: LOAEL of 0.57 ppm ($LOAEL_{ADJ}$ of 0.16 ppm)
Uncertainty Factor: 100
LSE Graph Key: 90
Species: Human

MRL Summary: A provisional chronic-duration inhalation MRL of 0.002 ppm was derived based on decreased number of peripheral lymphocytes (B-cell lymphocytes) in shoe manufacturing workers exposed to benzene (Lan et al. 2004a). The workers had been employed for an average of 6.1 years. The MRL is based on a LOAEL (0.57 ppm, 8 hours/day, 6 days/week), which was adjusted to a continuous exposure (0.16 ppm) and a total uncertainty factor of 100 (10 for use of a minimal LOAEL and 10 for human variability).

Selection of the Critical Effect: Epidemiological studies that provide reliable estimates of associations between chronic-duration benzene exposure concentrations and health outcomes evaluated hematological outcomes. Several chronic-duration inhalation studies have been conducted in mice and rats. To identify the critical effect, ATSDR focused on: (1) reported effects associated with clear biological significance; and (2) high-quality, chronic-duration human studies and animal studies that included a minimum of five animals per exposure group. The most sensitive LOAELs meeting these criteria are summarized in Table A-3.

Table A-3. Select LOAELs for Chronic-Duration Inhalation Exposure to Benzene

Species (number)	Duration	NOAEL/LOAEL (ppm) NOAEL	LOAEL	System: effect	Reference
Human (n=250 exposed workers,140 unexposed)	6.1 years	ND	0.57	***Hematological:*** 15% decrease in peripheral lymphocytes (B-cells)	Lan et al. 2004a
Human (n=928 exposed workers, 73 unexposed)	≥4 years	ND	2.3	***Hematological:*** Trend for decrease in peripheral WBCs	Schnatter et al. 2010
Human (n=131 exposed, 51 controls)	≥3 years	ND	3.2	***Hematological:*** Trend for decrease in peripheral WBCs	Qu et al. 2002
Mouse (n=40–60)	Lifetime (5 days/week, 6 hours/day)	ND	100	***Hematological:*** Decrease in peripheral WBCs and RBCs	Snyder et al. 1978, 1984

Table A-3. Select LOAELs for Chronic-Duration Inhalation Exposure to Benzene

Species (number)	Duration	NOAEL/LOAEL (ppm) NOAEL	LOAEL	System: effect	Reference
Rat (n=27–47)	Lifetime (5 days/week, 6 hours/day)	ND	300	***Hematological:*** Decrease in peripheral WBCs and RBCs	Snyder et al. 1978, 1984

Selected study for the chronic-duration inhalation MRL derivation.

LOAEL = lowest-observed-adverse-effect level; ND = not determined; NOAEL = no-observed-adverse-effect level; RBC = red blood cell; WBCs = white blood cell

Hematological effects were selected as the critical effect following chronic-duration inhalation exposure to benzene because it represents the lowest reliable LOAEL (Lan et al. 2004a). A systematic review (Appendix C) resulted in the hazard identification conclusions that hematological and immunological effects are known health effects for humans. There is a preponderance of evidence that hematopoietic tissues (e.g., marrow) and immune responses are sensitive targets for benzene. Human epidemiological studies provide evidence for hematological effects (e.g., decrease in peripheral WBCs and lymphocytes) in association with exposures ≥0.57 ppm (Lan et al. 2004a; Qu et al. 2002; Rothman et al. 1996a; Schnatter et al. 2010) [see Table 2-4 for additional studies]). Several chronic-duration inhalation studies have been conducted in mice and rats. These studies provide evidence for hematological effects (decrease in peripheral WBCs and lymphocytes) at exposure levels ≥100 ppm (Snyder et al. 1978, 1982, 1984, 1988). An abundance of mechanistic evidence supports a mode of action for hematological and immunological effects of benzene that involves marrow cytotoxicity and genotoxicity of reactive metabolites of benzene (see Section 2.20). An abundance of mechanistic evidence supports a mode of action for hematological and immunological effects of benzene that involves marrow cytotoxicity and genotoxicity of reactive metabolites of benzene (see Section 2.20).

Other effects observed in chronic-duration studies occurred in mice or rats exposed to ≥200 ppm. These effects include body weight loss and decreased lifespan (Snyder et al. 1978, 1982, 1984, 1988).

Selection of the Principal Study: The chronic-duration inhalation study in workers reported by Lan et al. (2004a) was selected as the principal study because it identified the lowest LOAEL for the critical effect (hematological). Lan et al. (2004a) was rated as a First Tier, Moderate Confidence study during systematic review (Appendix C).

Summary of the Principal Study:

Lan Q, Zhang L, Li G, Vermeulen R, et al. 2004. Hematotoxicity in workers exposed to low levels of benzene. Science 306(5702):1774-1776. https://doi.org/10.1126/science.1102443.

A cross-sectional study was performed on 250 workers (approximately two-thirds female) exposed to benzene at two shoe manufacturing facilities in Tianjin, China, and 140 age- and gender-matched controls (workers in clothing manufacturing facilities that did not use benzene). The benzene-exposed workers had been employed for an average of 6.1±2.9 (SD) years. Benzene and toluene exposures were monitored by individual organic vapor monitors (full shift) ≥5 times during 16 months prior to phlebotomy. Post-shift urine samples were collected from every worker. Urinary benzene concentrations correlated with mean individual air levels. Benzene was not found (detection limit 0.04 ppm) in workplace or home air samples of control workers taken at three different time periods. The worker

cohort was stratified into three groups based on mean air benzene concentrations measured twice during the month prior to phlebotomy: <1 ppm (109 workers), 1–<10 ppm (110 workers), and ≥10 ppm (31 workers). Workers in the <1 ppm group worked at the larger of the two facilities included in the study. Exposure concentrations were generally higher at the smaller facility due to a less adequate ventilation system. Complete blood counts and differential counts were performed on each subject. Laboratory coefficients of variation for measurements of cell counts were <10%.

Mean 1-month benzene exposure levels in the four groups (controls, <1 ppm, 1–<10 ppm, and ≥10 ppm) were <0.04, 0.57±0.24, 2.85±2.11, and 28.73±20.74 ppm, respectively (see Table A-4). The mean toluene exposure concentration in the <1-ppm group was 0.67±0.84 ppm. Benzene and toluene exposures were correlated (r=0.44). An evaluation of potential confounding factors showed that age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index were associated with at least one hematological endpoint. All statistical comparisons were adjusted for these co-variables. Trend analyses were adjusted for toluene, in addition to the above co-variables.

Table A-4. Significantly Reduced Blood Values in Workers Exposed to Benzene in Tianjin, China

	Mean exposure level in ppm[a] (number of subjects)			
Endpoint	<0.04 (n=140)	0.57±0.24 (n=109)	2.85±2.11 (n=110)	28.73±20.74 (n=31)
Leukocytes[b]	6,480±1,710	5,540±1,220[d]	5,660±1,500	4,770±892[e]
Granulocytes[b]	4,110±1,410	3,360±948[d]	3,480±1,170	2,790±750[e]
Monocytes[b]	241±92	217±97[d]	224±93	179±74
Lymphocytes[b]	2,130±577	1,960±541[d]	1,960±533	1,800±392[e]
CD4+ T-cells[b]	742±262	635±187[d]	623±177	576±188[e]
CD4+/CD8+ ratio	1.46±0.58	1.26±0.41[d]	1.22±0.45	1.09±0.35[e]
B-cells[b]	218±94	186±95[d]	170±75	140±101[e]
Platelets[c]	230±59.7	214±48.8[d]	200±53.4	172±44.8[e]

[a]Arithmetic mean of an average of two measurements per subject collected during the month prior to phlebotomy.
[b]Mean cell numbers per microliter blood±standard deviation.
[c]Mean number of platelets ($x10^3$).
[d]Covariate-adjusted lower mean compared to controls (p<0.05) based on linear regression using natural logarithm of each endpoint.
[e]Covariate-adjusted trend (p<0.05) using individual benzene air concentrations as a continuous variable.

Source: Lan et al. 2004a

Mean counts for leukocytes and all subtypes of leukocytes, except CD8+ T-cells and natural killer cells, were lower in the <1 ppm (mean 0.57 ppm) exposure group compared to the control group, with the differences ranging from 8 to 15% (B-cells). Numbers of leukocytes decreased further at higher exposures. Mean counts in the highest exposure group were 15–36% (B-cells) lower than the control group.

Tests for a linear trend in cell counts using individual benzene air concentrations as a continuous variable were significant for all leukocyte measures except monocytes and CD8+ T-cells. When the linear trend analysis was restricted to workers exposed to <10 ppm benzene, excluding controls, associations between increasing benzene exposure concentrations and decreasing cell counts persisted for leukocytes, granulocytes, lymphocytes, B-cells, and platelets.

The above associations were based on exposures measured over a period of 1 month. When the cohort was restricted to workers who had been exposed to <1 ppm benzene over the previous year (n=60) and workers who had experienced <40 ppm-years lifetime cumulative benzene exposure (n=50), leukocytes, granulocytes, lymphocytes, and B-cells in both worker groups were lower than the control group. These results suggest that an association between benzene exposure and leukocyte numbers was robust for measures of longer-term exposure.

When the cohort was restricted to workers (n=30; mean 1-month exposure level 0.29±0.15 ppm) who experienced negligible exposure to solvents other than benzene (e.g., toluene, pentane, ethyl benzene, hexane, *m*-xylene, *p*-xylene, 1,1,1-trichloroethane, and heptane), counts of leukocytes, granulocytes, lymphocytes, and B-cells were lower in workers compared to controls. These results provide further support that associations with benzene were not the result of co-exposure to other solvents.

Blood samples from a subset of 29 workers and 24 matched controls were tested for peripheral leukocyte counts and progenitor cells (based on CFU assays). When workers were stratified into <10 or >10 ppm exposure levels, a co-variate adjusted trend was found for increasing benzene exposure and progenitor CFUs for granulocytes, erythroid cells, macrophages, and megakaryocytes, as well as decreasing peripheral counts of leukocytes and granulocytes. The decrease in CFUs was approximately 50–70% in the >10-ppm group compared to the control group. These results provide further evidence for an effect of benzene exposure on leukocyte progenitor cells and peripheral leukocyte numbers in this worker cohort.

Selection of the Point of Departure for the MRL: The LOAEL (0.57 ppm) from the Lan et al. (2004a) study was selected as the POD for deriving the chronic-duration MRL. The critical effect was decreased peripheral B-cell numbers. Decreased B-cell numbers was selected as the critical effect because it showed the greatest change in response to benzene exposure. The decreases in B-cell numbers relative to the control group (218 cells/μL) were 15% (186 cells/μL), 22% (170 cells/μL) and 36% (140 cells/μL) in the low, middle, and high exposure groups, respectively. The mean B-cell count at the LOAEL was 186 cells/μL, which was above clinical threshold for a "low B-cell count" (<170 cells/μL) (Mitchell et al. 2019; Morbach et al. 2010).

To identify the most sensitive POD, BMD modeling of the B-cell data was performed. The data were fit to all available continuous models in EPA's Benchmark Dose Software (BMDS; version 3.3) using a benchmark response (BMR) of: (1) 1 standard deviation and (2) a relative deviation with BMR of 0.225, which corresponds to a B-cell count <170 cells/μL (Mitchell et al. 2019; Morbach et al. 2010). Adequate model fit was judged by the following criteria: goodness-of-fit statistics (p-value >0.1), visual inspection of the dose-response curve, a 95% lower confidence limit on the BMC (BMCL) that is not 10 times lower than the lowest non-zero dose, a BMC that is not greater than the maximum dose, and scaled residual within ±2 units at the data point (except the control) closest to the predefined BMR.

BMD models did not be fit the data without dropping the highest exposure concentration. After excluding the highest exposure concentration, only nonconstant variance models achieved adequate fit to the data. All models that successfully fit the data with a BMR of 1 standard deviation or with a relative difference of 0.225 yielded BMCs that were higher than the maximum exposure level (2.85 ppm). Therefore, BMCLs were not used for the POD and instead, the LOAEL of 0.57 ppm from the Lan et al. (2004a) study was selected as the POD.

Calculations

Adjustment for Intermittent Exposure: Lan et al. (2004a) reported that workers in the largest factory worked 8 hours/day and 6 days/week. Therefore, the LOAEL (0.57 ppm) concentration was adjusted for

intermittent exposure by multiplying by 8 hours/24 hours to correct for less than a full day of exposure and 6 days/7 days to correct for less than a full week of exposure. The resulting $LOAEL_{ADJ}$ is 0.16 ppm.

$$LOAEL_{ADJ} = LOAEL\ (0.57\ ppm) \times 8\ hours/24\ hours \times 6\ days/7\ days$$
$$LOAEL_{ADJ} = 0.16\ ppm$$

Uncertainty Factor: 100

- 10 for use of a LOAEL
- 10 for human variability

$$\text{Provisional chronic-duration inhalation MRL} = LOAEL_{ADJ}\ (0.16\ ppm) \div \text{total UF}\ (100)$$
$$= 0.0016\ ppm \approx 0.002\ ppm\ (\text{rounded})$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Numerous human epidemiological and animal studies provide strong support for associations between inhalation exposure to benzene and impaired function of hematopoietic tissues and altered immune responses (see Sections 2.7 and 2.14). The LOAEL from the Lan et al. (2004a) of 0.57 ppm is supported by more recent analyses of the data from this same cohort, which found associations between increasing benzene exposure concentration and decreasing peripheral leukocytes and lymphocytes when the cohort was stratified in to <1, 1–10, and >10 ppm, and statistically significant differences in peripheral leukocytes (15% decrease) in the <1 ppm exposure group compared to the control group (Bassig et al. 2016). Two other studies found associations between increasing benzene exposure concentration and decreasing peripheral leukocytes and lymphocytes in other worker cohorts in which the median benzene exposure concentrations were 2.3 and 3.2 ppm (Schnatter et al. 2010; Qu et al. 2002). The lowest chronic-duration LOAELs from animal studies were also for hematological effects (decreases in peripheral leukocytes) and were >100-fold higher (100–300 ppm) than the lowest LOAEL for humans (0.57 ppm) (Lan et al. 2004a).

Agency Contacts (Chemical Managers): Gaston Casillas, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Benzene
CAS Numbers: 71-43-2
Date: October 2024
Profile Status: Draft for Public Comment
Route: Oral
Duration: Acute
Provisional MRL: 0.0009 mg/kg/day ($9x10^{-4}$ mg/kg/day)
Critical Effect: Hematological
Reference: Li et al. 2018
Point of Departure: NOAEL of 0.1 mg/kg/day ($NOAEL_{ADJ}$ of 0.09 mg/kg/day)
Uncertainty Factor: 100
LSE Graph Key: 22
Species: Mouse

MRL Summary: The provisional intermediate-duration oral MRL of 0.0009 mg/kg/day ($9x10^{-4}$ mg/kg/day) was adopted for the provisional acute-duration oral MRL for benzene. The provisional intermediate-duration oral MRL is based on decreased peripheral WBC, lymphocyte, monocyte, and neutrophil counts in mice administered 1 mg/kg/day benzene 6 days/week for 4 weeks (Li et al. 2018). The provisional acute-duration MRL is based on an intermediate-duration NOAEL of 0.1 mg/kg/day that was adjusted to continuous exposure (0.09 mg/kg/day) and divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Rationale for Adopting the Intermediate-Duration Oral MRL: A small number of studies have evaluated the toxicity of benzene following acute-duration oral exposure. The observed effects include decreased peripheral WBCs, alopecia, and decreased maternal body weight. There is strong support for identifying hematological effects as the critical effect for benzene toxicity; a systematic review (see Appendix C) categorized it as a known human health effect; decreased body weight gain and hair loss have not been identified as sensitive targets of benzene toxicity because these two effects were not observed in other acute oral studies.

The only study that examined hematological endpoints following acute-duration oral exposure reported decreased peripheral leukocytes, lymphocytes, and basophils in mice administered 200 mg/kg/day for 14 days (Huang et al. 2013); the study tested one benzene dose. It is likely that this LOAEL far exceeds the actual NOAEL/LOAEL boundary for hematological effects resulting from acute-duration oral exposure to benzene. The LOAEL is approximately 2 orders of magnitude higher than the lowest LOAELs identified in three 4-week studies. Decreased peripheral WBCs, lymphocytes, neutrophils, monocytes, and/or RBCs have been observed in mice exposed to 1 mg/kg/day for 4 weeks (Cui et al. 2022; Li et al. 2018) or 8 mg/kg/day for 4 weeks (Hsieh et al. 1988); a NOAEL of 0.1 mg/kg/day was identified in the Li et al. (2018) study. Additionally, the lowest LOAELs identified in acute- and intermediate-duration inhalation studies are similar and very similar PODs are used to derive acute- and intermediate-duration inhalation MRLs.

There is considerable uncertainty that an acute-duration oral MRL based on the LOAEL of 200 mg/kg/day identified in the Huang et al. (2013) study would be health-protective. Thus, the intermediate-duration oral MRL of 0.0009 mg/kg/day was adopted as an acute-duration oral MRL. The intermediate-duration oral MRL is based on a NOAEL of 0.1 mg/kg/day for decreased peripheral WBCs, lymphocytes, neutrophils, and monocytes in mice administered benzene 6 days/week for 4 weeks (Li et al. 2018).

Agency Contacts (Chemical Managers): Gaston Casillas, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Benzene
CAS Numbers: 71-43-2
Date: October 2024
Profile Status: Draft for Public Comment
Route: Oral
Duration: Intermediate
Provisional MRL: 0.0009 ($9x10^{-4}$) mg/kg/day
Critical Effect: Hematological
Reference: Li et al. 2018
Point of Departure: NOAEL of 0.1 mg/kg/day ($NOAEL_{ADJ}$ of 0.09 mg/kg/day)
Uncertainty Factor: 100
LSE Graph Key: 22
Species: Mouse

MRL Summary: A provisional intermediate-duration oral MRL of 0.0009 mg/kg/day ($9x10^{-4}$ mg/kg/day) was derived for benzene based on decreased peripheral WBC, lymphocyte, monocyte, and neutrophil counts in mice administered 1 mg/kg/day benzene 6 days/week for 4 weeks (Li et al. 2018). The MRL is based on a NOAEL of 0.1 mg/kg/day that was adjusted to continuous exposure (0.09 mg/kg/day) and divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). The study identified a $LOAEL_{ADJ}$ of 0.9 mg/kg/day.

Selection of the Critical Effect: A number of studies have evaluated the toxicity of benzene following intermediate-duration oral exposure. As presented in Table A-5, the hematological and immunological systems are the most sensitive targets of benzene toxicity. There are strong data supporting the identification of the hematological and immunological alterations as the most sensitive targets of benzene toxicity. A systematic review of the hematological effects (Appendix C) concluded that hematological and immunological effects are known health effects for humans. A summary of the lowest LOAELs for hematological and immunological endpoints is presented in Table A-6. The lowest LOAEL is 1 mg/kg/day for decreases in peripheral WBCs, lymphocytes, neutrophils, and monocytes in mice administered benzene 6 days/week for 4 weeks (Li et al. 2018); the NOAEL is 0.1 mg/kg/day.

Table A-5. Summary of the Lowest LOAEL Values for Various Endpoints in Animals Following Intermediate-Duration Oral Exposure to Benzene

Target endpoint	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Hematological	Decreased peripheral WBCs, lymphocytes, neutrophils, and monocytes	0.1	1	Li et al. 2018
Immunological	Altered splenic lymphocyte proliferative response to mitogens, altered splenic lymphocyte cytotoxic response to tumor cells		8	Hsieh et al. 1988
Neurological	Impaired short-term memory		41	Banik and Lahiri 2005
Hepatic	Decreased plasma cholesterol	10	100	Cui et al. 2022

Table A-5. Summary of the Lowest LOAEL Values for Various Endpoints in Animals Following Intermediate-Duration Oral Exposure to Benzene

Target endpoint	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Endocrine	Alterations in blood glucose and insulin		200	Bahadar et al. 2015a, 2015b
Death	Increased mortality		250 (SLOAEL)	

LOAEL = lowest-observed adverse-effect level; NOAEL = no-observed-adverse effect level; SLOAEL = serious lowest-observed adverse-effect level; WBC = white blood cell

Table A-6. Summary of the Lowest LOAEL Values for Hematological and Immunological Effects Following Intermediate-Duration Oral Exposure to Benzene

Species, duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
Hematological effects				
Mouse, 6 days/week, 4 weeks	0.1	1	Decreased peripheral WBCs, lymphocytes, neutrophils, and monocytes	Li et al. 2018
Mouse, 6 days/week, 4 weeks		1	Decreased peripheral WBCs	Cui et al. 2022
Mouse, 4 weeks 7 days/week		8	Decreased peripheral lymphocytes and RBCs	Hsieh et al. 1988
Rat, 5 days/week 120 days		25	Decreased peripheral WBCs	NTP 1986
Mouse, 5 days/week, 120 days	25	50	Decreased peripheral WBCs	NTP 1986
Immunological effects				
Mouse, 4 weeks 7 days/week		8	Altered splenic lymphocyte proliferative response to mitogens, altered splenic lymphocyte cytotoxic response to tumor cells	Hsieh et al. 1988
Mouse, 28 days 7 days/week		27	Decreased splenic lymphocyte production of IL-2	Fan 1992
Mouse, 4 weeks 7 days/week		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. 1990

Table A-6. Summary of the Lowest LOAEL Values for Hematological and Immunological Effects Following Intermediate-Duration Oral Exposure to Benzene

Species, duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
Mouse, 4 weeks 7 days/week	8	40	Decreased splenic lymphocyte IL-2 production in response to mitogens	Hsieh et al. 1991

Selected study for the intermediate-duration oral MRL derivation.

IL-2 = interleukin-2; LOAEL = lowest-observed adverse-effect level; NOAEL = no-observed-adverse effect level; RBC = red blood cell; WBC = white blood cell

Selection of the Principal Study: The Li et al. (2018) study was selected as the principal study because it identified the lowest LOAEL for hematological endpoints. Li et al. (2018) was rated as a First Tier, High Confidence study during systematic review (Appendix C).

Summary of the Principal Study:

Li H, Li D, He Z, et al. 2018. The effects of Nrf2 knockout on regulation of benzene-induced mouse hematotoxicity. Toxicol Appl Pharmacol 358:56-67.

Male wild-type $Nrf2^{+}/^{+}$ and $NrF2^{-}/^{-}$ knockout mice (15/group) were exposed to 0, 0.1, 1, 10, and 100 mg/kg/day benzene via gavage in corn oil vehicle for 6 days/week for 4 weeks. At the end of the exposure duration, animals were euthanized, and blood was collected to measure the numbers of peripheral blood leukocytes (WBCs), lymphocytes, neutrophils, monocytes, RBCs and platelets. Frequency of reticulocytes was also determined. Bone marrow and peripheral blood smears were used to examine cellular morphology. Bone marrow from the femur was also harvested for stem cell counts, CFUs, and histopathology and immunohistochemistry staining for cell cycle proliferation via Ki-67.

In wild-type mice, statistically significant and dose-dependent decreases of WBCs (by ~40%), lymphocytes (by ~36%), neutrophils (by ~30%), and monocytes (by ~55%) were observed at ≥1.0 mg/kg/day. A significant decrease in RBCs was observed at 100 mg/kg/day; no differences in platelets were observed at any dose. Reticulocyte frequency was significantly increased at 100 mg/kg/day, but no differences were observed at lower doses. Significantly decreased frequency of proliferative Ki-67+ cells, decreased CFUs, and increased frequency of bone marrow cells displaying abnormal morphology were observed at 100 mg/kg/day. There was no significant difference in frequency of erythrocytes displaying normal pathology.

Decreases in peripheral cell counts were less severe in knockout mice dosed with benzene compared to wild-type mice. Knockout mice dosed with 100 mg/kg/day benzene also exhibited, relative to wild-type mice, increased proliferation and differentiation of marrow of hematopoietic cells and aberrant morphological changes in peripheral erythrocytes and bone marrow cells.

Selection of the Point of Departure for the MRL: The NOAEL of 0.1 mg/kg/day was selected as the POD for the MRL.

A BMD approach was considered for identifying a potential POD for derivation of the intermediate-duration oral MRL for benzene. The WBC, lymphocyte, neutrophil, and monocyte counts (Table A-7)

were fit to all available continuous models in EPA's BMDS (version 3.3) with extra risk. Adequate model fit was judged by four criteria: goodness-of-fit statistics (p-value >0.1), scaled residual at the data point (except the control) closest to the predefined BMR, benchmark dose lower confidence limit (BMDL) that is not 10 times lower than the lowest non-zero dose, and visual inspection of the dose-response curve. A BMR of 1 SD was used.

Table A-7. Hematological Alterations in Mice Administered Benzene 6 Days/Week for 4 Weeks

	Dose[a] (mg/kg/day)				
Endpoint	0	0.1	1	10	100
Peripheral WBC counts	4,105±8.1	4,608±13.8	2,466±7.3[b]	1,417±6.5[b]	884±4.5[b]
Peripheral lymphocyte counts	2,995±6.3	3,592±20.0	1,912±4.8[b]	928±3.6[b]	592±0.9[b]
Peripheral monocyte counts	101±0.5	104±0.5	45±0.3[b]	41±0.3[b]	30±0.2[b]
Peripheral neutrophil counts	847±1.3	967±1.1	576±0.9[b]	461±0.5[b]	346±0.6[b]

[a]Mean±standard deviation; n=15 mice/group.
[b]Significantly different from controls; p<0.05.

WBC = white blood cell

Source: Li et al. 2018

None of the BMD models provided adequate fit to the data for peripheral WBC, lymphocyte, monocyte, or neutrophil counts. Thus, a NOAEL/LOAEL approach was used to select the NOAEL of 0.1 mg/kg/day as the POD.

Calculations

Intermittent Exposure: The NOAEL of 0.1 mg/kg/day was adjusted from intermittent exposure to account for continuous exposure scenario:

$$NOAEL_{ADJ} = \text{NOAEL of 0.1 mg/kg/day x 6 days/7 days} = 0.09 \text{ mg/kg/day}$$

Uncertainty Factors: The $NOAEL_{ADJ}$ is divided by a total uncertainty factor (UF) of 100:

- 10 UF for extrapolation from animals to humans
- 10 UF for human variability

$$\begin{aligned}\text{Provisional intermediate-duration oral MRL} &= NOAEL_{ADJ} \div UFs \\ &= 0.09 \text{ mg/kg/day} \div (10x10) \\ &= 0.0009 \text{ mg/kg/day } (9x10^{-4} \text{ mg/kg/day})\end{aligned}$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: In addition to the oral exposure studies listed in Table A-6, numerous human epidemiological and animal studies provide strong support for associations between inhalation exposure to benzene and impaired function of hematopoietic tissues and decreases in peripheral leukocytes and lymphocytes (see Section 2.7). An abundance of mechanistic evidence supports a mode of action for hematological effects of benzene that involves marrow cytotoxicity and genotoxicity of reactive metabolites of benzene (see Section 2.20).

Agency Contacts (Chemical Managers): Gaston Casillas, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:**	Benzene
CAS Numbers:	71-43-2
Date:	October 2024
Profile Status:	Draft for Public Comment
Route:	Oral
Duration:	Chronic
Provisional MRL:	0.0003 ($3x10^{-4}$) mg/kg/day
Critical Effect:	Hematological
Reference:	Lan et al. 2004a
Point of Departure:	Chronic-duration inhalation MRL (0.002 ppm)
Uncertainty and Modifying Factors:	3
LSE Graph Key:	25
Species:	Human

MRL Summary: A provisional chronic-duration oral MRL of 0.0003 mg/kg/day was derived based on a route-to-route extrapolation of the chronic-duration inhalation MRL (0.002 ppm). The critical effect was decreased number of peripheral lymphocytes (B-cell lymphocytes) in shoe manufacturing workers exposed to benzene (Lan et al. 2004a). The workers had been employed for an average of 6.1 years. A modifying factor of 3 was applied for route-to-route extrapolation.

Rationale for route-to-route extrapolation: Chronic noncancer effects of oral benzene exposure have been studied in two studies, which provide a LOAEL of 25 mg/kg/day (5 days/week) for hematological effects, but do not provide a NOAEL. In the NTP (1986) study, the lowest LOAEL was 25 mg/kg/day for hematologic effects (decreased peripheral leukocytes and lymphocytes) in mice and rats, which was also the lowest dose level in the study. In the Maltoni et al. (1983, 1985) study, the lowest LOAEL was 50 mg/kg/day for hematological effects (decreased leukocytes and erythrocytes) and was also the lowest dose level in the study.

The chronic-duration oral LOAEL (25 mg/kg/day) is 25 times higher than the intermediate-duration oral LOAEL (1 mg/kg/day for hematologic effects in mice; Li et al. 2018), which is also a LOAEL for hematological effects (decreased peripheral leukocytes and lymphocytes) and the basis for the provisional intermediate-duration oral MRL. Given that the chronic-duration oral LOAEL (25 mg/kg/day) is substantially higher than the intermediate-duration oral LOAEL (1 mg/kg/day), the chronic-duration oral LOAEL cannot be used as a basis for the chronic-duration oral MRL. In the absence of chronic-duration studies that have evaluated benzene doses at or below the intermediate-duration oral LOAEL, the chronic-duration inhalation MRL was adopted as the POD for extrapolating from inhalation to oral exposure.

Calculations

The chronic-duration inhalation MRL (0.002 ppm) was converted to mg/m^3 using the molecular weight of 78.11 g/mol for benzene and a benzene gas volume of 24.24 L/mol at 25°C and 760 mm Hg:

$$\text{Chronic-duration inhalation } MRL_{mg/m3} = 0.002 \text{ ppm} \times 78.11 \div 24.45 = 0.0064 \text{ mg/m}^3$$

The chronic-duration inhalation $MRL_{mg/m3}$ was converted to an equivalent oral dose ($MRL_{mg/kg/day}$) using EPA (1988) human reference values for inhalation rate (IR=20 m^3/day) and body weight (BW=70 kg) and a relative bioavailability factor (RBA= 0.5) to adjust for differences in absorption of benzene following inhalation versus oral exposure (50 from inhalation versus 100% from oral) as follows:

$MRL_{mg/kg/day} = MRL_{mg/m3}$ x IR x RBA ÷ BW = 0.00091 mg/kg/day

$MRL_{mg/kg/day}$ = 0.0064 mg/m^3 x 20 m^3/day x 0.5 ÷ 70 kg = 0.00091 mg/kg/day

$MRL_{mg/kg/day}$ = 0.00091 mg/kg/day

Uncertainty Factors: An uncertainty factor for human variability was not applied in deriving the chronic-duration oral MRL because a factor of 10 for human variability was included in deriving the chronic-duration inhalation MRL.

Modifying Factor: The $MRL_{mg/kg/day}$ (0.00091 mg/kg/day) was divided by a modifying factor of 3 for the route-to-route extrapolation, resulting in a chronic-duration oral MRL of 0.0003 mg/kg/day ($3x10^{-4}$ mg/kg/day).

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Numerous human epidemiological and animal studies provide strong support for associations between oral and inhalation exposure to benzene and impaired function of hematopoietic tissues (see Section 2.7). The chronic-duration inhalation LOAEL from the Lan et al. (2004a) study of 0.57 ppm is supported by more recent analyses of the data from this same cohort, which found associations between increasing benzene exposure concentration and decreasing peripheral leukocytes and lymphocytes when the cohort was stratified to <1, 1–10, and >10 ppm groups and statistically significant differences in peripheral leukocytes (15% decrease) in the <1 ppm exposure group compared to the control group (Bassig et al. 2016). Two other studies found associations between increasing benzene exposure concentration and decreasing peripheral leukocytes and lymphocytes in other worker cohorts in which the median benzene exposure concentrations were 2.3 and 3.2 ppm (Qu et al. 2002; Schnatter et al. 2010). The lowest intermediate- and chronic-duration LOAELs from animal studies were also for hematological effects (see Section 2.7). The intermediate-duration oral LOAEL in mice (1 mg/kg/day) is also for hematological effects (decreased peripheral leukocytes and lymphocytes (Li et al. 2018).

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APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR BENZENE

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to benzene.

B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen were conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for benzene. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Foreign language studies are reviewed based on available English-language abstracts and/or tables (or summaries in regulatory assessments, such as International Agency for Research on Cancer [IARC] documents). If the study appears critical for hazard identification or MRL derivation, translation into English is requested. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of benzene have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of benzene are presented in Table B-1.

Table B-1. Inclusion Criteria for the Literature Search and Screen

- Health Effects
  - Species
    - Human
    - Laboratory mammals
  - Route of exposure
    - Inhalation
    - Oral
    - Dermal (or ocular)
    - Parenteral (these studies will be considered supporting data)
  - Health outcome
    - Death
    - Systemic effects
    - Body weight effects
    - Respiratory effects
    - Cardiovascular effects
    - Gastrointestinal effects
    - Hematological effects
    - Musculoskeletal effects
    - Hepatic effects
    - Renal effects
    - Dermal effects
    - Ocular effects
    - Endocrine effects
    - Immunological effects
    - Neurological effects
    - Reproductive effects

Table B-1. Inclusion Criteria for the Literature Search and Screen

Developmental effects
Other noncancer effects
Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models
Biomarkers
Biomarkers of exposure
Biomarkers of effect
Interactions with other chemicals
Potential for human exposure
Releases to the environment
Air
Water
Soil
Environmental fate
Transport and partitioning
Transformation and degradation
Environmental monitoring
Air
Water
Sediment and soil
Other media
Biomonitoring
General populations
Occupation populations

B.1.1. Literature Search

The current literature search was intended to update the 2007 Toxicological Profile for Benzene; thus, the literature search was restricted to studies published between January 2005 and June 2023. The following main databases were searched in June 2023:

- PubMed
- National Technical Reports Library (NTRL)
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, Medical Subject Headings (MeSH) headings, and keywords for benzene. The query strings used for the literature search are presented in Table B-2.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures and Results (NIH RePORTER) databases using the queries presented in Table B-3. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance Priority List (SPL) resource page, and other items as needed. Regulations applicable to benzene were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

Table B-2. Database Query Strings

Database search date	Query string
PubMed	
06/2023	(Benzene[mh] OR 71-43-2[rn]) AND 2005:3000[dp] AND ("Benzene/toxicity"[mh] OR "Benzene/adverse effects"[mh] OR "Benzene/poisoning"[mh] OR "Benzene/pharmacokinetics"[mh] OR "environmental exposure"[mh] OR ci[sh] OR toxicokinetics[mh:noexp] OR "Benzene/blood"[mh] OR "Benzene/cerebrospinal fluid"[mh] OR "Benzene/urine"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR ("computational biology"[mh] OR "medical informatics"[mh] OR genomics[mh] OR genome[mh] OR proteomics[mh] OR proteome[mh] OR metabolomics[mh] OR metabolome[mh] OR genes[mh] OR "gene expression"[mh] OR phenotype[mh] OR genetics[mh] OR genotype[mh] OR transcriptome[mh] OR ("systems biology"[mh] AND ("environmental exposure"[mh] OR "epidemiological monitoring"[mh] OR analysis[sh])) OR "transcription, genetic "[mh] OR "reverse transcription"[mh] OR "transcriptional activation"[mh] OR "transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, messenger"[mh] OR "RNA, transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "reverse transcriptase polymerase chain reaction"[mh] OR "base sequence"[mh] OR "trans-activators"[mh] OR "gene expression profiling"[mh]) OR "Benzene/antagonists and inhibitors"[mh] OR ("Benzene/metabolism"[mh] AND ("humans"[mh] OR "animals"[mh])) OR "Benzene/pharmacology"[majr] OR ("Neoplasms"[mh] OR "Carcinogens"[mh] OR "Lymphoproliferative disorders"[mh] OR "Myeloproliferative disorders"[mh] OR "Toxicity Tests"[mh] OR ((cancer*[tiab] OR carcinogen*[tiab]) AND (risk*[tiab] OR health[tiab]) AND assessment*[tiab]) OR "Mutagens"[mh] OR "Mutagenicity Tests"[mh] OR "Chromosome Aberrations"[mh] OR "DNA Damage"[mh] OR "DNA Repair"[mh] OR "DNA Replication/drug effects"[mh] OR "DNA/drug effects"[mh] OR "DNA/metabolism"[mh] OR "Genomic Instability"[mh] OR "Salmonella typhimurium/drug effects"[mh] OR "Salmonella typhimurium/genetics"[mh] OR "Sister Chromatid Exchange"[mh] OR strand-break*[tiab])) OR ((Benzene[mh] OR 71-43-2[rn]) AND 2005:3000[dp] AND (indexingmethod_automated OR indexingmethod_curated) AND ("RNA"[mh] OR "DNA"[mh] OR "DNA Replication"[mh] OR "Salmonella typhimurium"[mh] OR antagonist*[tw] OR inhibitor*[tw] OR "blood"[tw] OR "serum"[tw] OR "plasma"[tw] OR pharmacokinetic*[tw] OR toxicokinetic*[tw] OR "pbpk"[tw] OR "poisoned"[tw] OR "poisoning"[tw] OR "urine"[tw] OR "urinary"[tw] OR "toxicity"[sh] OR "occupational diseases"[mh] OR "hazardous substances"[mh] OR "epidemiology"[sh] OR "epidemiologic studies"[mh])) OR ((((("(6)Annulene"[tw] OR "[6]Annulene"[tw] OR "Benzene"[tw] OR "Benzin"[tw] OR "Benzine"[tw] OR "Benzol"[tw] OR "Benzole"[tw] OR "Benzolene"[tw] OR "Bicarburet of hydrogen"[tw] OR "Carbon oil"[tw] OR "Coal naphtha"[tw] OR "Cyclohexatriene"[tw] OR "Mineral naphtha"[tw] OR "Phenyl hydride"[tw]

Table B-2. Database Query Strings

Database search date	Query string
	OR "Polystream"[tw] OR "Pyrobenzol"[tw] OR "Pyrobenzole"[tw]) NOT medline[sb])) AND 2005:3000[dp] AND (toxicity[ti] OR death OR lethal OR fatal OR fatality OR necrosis OR LC50* OR LD50* OR "body weight" OR "weight loss" OR "weight gain" OR weight-change* OR overweight OR obesity OR inhal* OR respiratory OR "pulmonary edema" OR "pulmonary effect" OR "pulmonary system" OR "pulmonary function" OR "pulmonary organ" OR "pulmonary toxicity" OR airway OR trachea OR tracheobronchial OR lung OR lungs OR nose OR nasal OR nasopharyngeal OR larynx OR laryngeal OR pharynx OR bronchial OR bronchi OR bronchioles OR bronchitis OR hemothorax OR alveolar OR alveoli OR irritation OR irritant OR sensitization OR sensitizer OR cilia OR mucocilliary OR cvd OR cardio OR vascular OR cardiovascular OR "circulatory system" OR "circulatory function" OR "circulatory effect" OR "circulatory organ" OR "circulatory toxicity" OR "cardiac arrest" OR "cardiac palpitation" OR "cardiac arrhythmia" OR "cardiac edema" OR "heart rate" OR "heart failure" OR "heart attack" OR "heart muscle" OR "heart beat" OR "myocardial-infarction" OR "chest pain" OR artery OR arteries OR veins OR venules OR cardiotox* OR "gastro-intestinal" OR gastrointestinal OR "digestive system" OR "digestive function" OR "digestive effect" OR "digestive organ" OR "Intestinal system" OR "intestinal function" OR "intestinal microbiota" OR "intestinal effect" OR "intestinal organ" OR "gi tract" OR "gi disorder" OR abdominal OR esophagus OR stomach OR intestine OR pancreas OR pancreatic OR diarrhea OR nausea OR vomit OR ulcer OR constipation OR emesis OR "gut microbes" OR "gut flora" OR "gut microflora" OR anorexia OR hematological OR hematology OR hemato OR haemato OR blood OR anemia OR cyanosis OR erythrocytopenia OR leukopenia OR thrombocytopenia OR hemoglobin OR erythrocyte OR hematocrit OR "bone marrow" OR reticulocyte OR methemoglobin OR red-blood-cell OR musculoskeletal OR skeletal OR muscle OR muscular OR arthritis OR "altered bone" OR "joint pain" OR "joint-ache" OR "limb pain" OR "limb ache" OR hepatic OR "liver system" OR "liver function" OR "liver effect" OR "liver organ" OR "Liver enzyme" OR "liver weight" OR "liver congestion" OR "liver changes" OR "liver biochemical changes" OR "liver toxicity" OR hepatocytes OR gallbladder OR cirrhosis OR jaundice OR "hepatocellular degeneration" OR "hepatocellular hypertrophy" OR hepatomegaly OR hepatotox* OR renal OR "kidney system" OR "kidney function" OR "Kidney effect" OR "kidney toxicity" OR "urinary system" OR "urinary function" OR "urinary effect" OR "Urinary toxicity" OR "bladder system" OR "bladder effect" OR "bladder function" OR "bladder toxicity" OR "Urine volume" OR "blood urea nitrogen" OR bun OR nephropathy OR nephrotox* OR dermal OR "skin rash" OR "skin itch" OR "skin irritation" OR "skin redness" OR "skin effect" OR "skin necrosis" OR "skin exposure" OR "skin contact" OR acanthosis OR dermatitis OR psoriasis OR edema OR ulceration OR acne OR ocular OR "eye function" OR "eye effect" OR "eye irritation" OR "eye drainage" OR "eye tearing" OR blindness OR myopia OR cataracts OR endocrine OR "hormone changes" OR "hormone excess" OR "hormone deficiency" OR "hormone gland" OR "hormone secretion" OR "hormone toxicity" OR "sella turcica" OR thyroid OR adrenal OR pituitary OR immunological OR immunologic OR immune OR lymphoreticular OR lymph-node OR spleen OR thymus OR macrophage OR leukocyte* OR white-blood-cell OR immunotox* OR neurological OR neurologic OR neurotoxic OR neurotoxicity OR neurodegenerat* OR "nervous system" OR brain OR neurotoxicant OR neurochemistry OR neurophysiology OR neuropathology OR "motor activity" OR motor change* OR behavior-change* OR behavioral-change* OR sensory-change* OR cognitive OR vertigo OR drowsiness OR headache OR ataxia OR reproductive OR "reproduction system" OR "reproduction function" OR "reproduction effect" OR "reproduction toxicity" OR fertility OR "maternal toxicity" OR developmental OR "in utero" OR terata* OR terato* OR embryo* OR fetus* OR foetus* OR fetal* OR foetal* OR prenatal* OR "pre-natal" OR perinatal* OR "post-natal" OR postnatal* OR neonat* OR

Table B-2. Database Query Strings

Database search date	Query string
	newborn* OR zygote* OR child OR children OR infant* OR offspring OR elderly OR "altered food consumption" OR "altered water consumption" OR "metabolic effect" OR "metabolic toxicity" OR fever OR cancer OR cancerous OR neoplas* OR tumor OR tumors OR tumour* OR malignan* OR carcinoma OR carcinogen OR carcinogen* OR angiosarcoma OR blastoma OR fibrosarcoma OR glioma OR leukemia OR leukaemia OR lymphoma OR melanoma OR meningioma OR mesothelioma OR myeloma OR neuroblastoma OR osteosarcoma OR sarcoma OR mutation OR mutations OR genotoxicity OR genotoxic OR mutagenicity OR mutagenic OR "mechanism of action"[tiab:~0] OR "mechanism of absorption"[tiab:~0] OR "mechanism of distribution"[tiab:~0] OR "mechanism of excretion"[tiab:~0] OR "mechanism of metabolism"[tiab:~0] OR "mechanism of toxic effect"[tiab:~0] OR "mechanism of toxicity" OR "adverse effect" OR "adverse effects" OR "health effects" OR noncancer OR poisoning OR morbidity OR inflammation OR antagonist OR inhibitor OR metabolism OR "environmental exposure" OR toxicokinetics OR pharmacokinetics OR "gene expression" OR "population health" OR epidemiology OR epidemiological OR case-control* OR case-referent OR case-report OR case-series OR cohort* OR correlation-stud* OR cross-sectional-stud* OR ecological-studies OR ecological-study OR follow-up-stud* OR longitudinal-stud* OR metaanalyses OR metaanalysis OR meta-analysis OR prospective-stud* OR record-link* OR retrospective-stud* OR seroepidemiologic-stud* OR occupation* OR worker* OR workmen* OR workplace* OR "human health" OR "oral intake" OR "oral feed" OR "oral ingestion" OR "oral exposure" OR "oral administration" OR ingest* OR gavage* OR "drinking-water" OR NHANES OR "National Health and Nutrition Examination Survey" OR (human AND (risk OR toxic* OR safety)) OR mammal* OR ape OR apes OR baboon* OR balb OR beagle* OR boar OR boars OR bonobo* OR bovine OR C57 OR C57bl OR callithrix OR canine OR canis OR capra OR capuchin* OR cats OR cattle OR cavia OR chicken OR chickens OR chimpanzee* OR chinchilla* OR cow OR cows OR cricetinae OR dog OR dogs OR equus OR feline OR felis OR ferret OR ferrets OR flying-fox OR Fruit-bat OR gerbil* OR gibbon* OR goat OR goats OR guinea-pig* OR guppy OR hamster OR hamsters OR horse OR horses OR jird OR jirds OR lagomorph* OR leontopithecus OR longevans OR macaque* OR marmoset* OR medaka OR merione OR meriones OR mice OR monkey OR monkeys OR mouse OR muridae OR murinae OR murine OR mustela-putorius OR nomascus OR non-human-primate* OR orangutan* OR pan-paniscus OR pan-troglodytes OR pig OR piglet* OR pigs OR polecat* OR pongopygmaeus OR quail OR rabbit OR rabbits OR rat OR rats OR rhesus OR rodent OR rodentia OR rodents OR saguinus OR sheep OR sheeps OR siamang* OR sow OR sows OR Sprague-Dawley OR swine OR swines OR symphalangus OR tamarin* OR vervet* OR wistar OR wood-mouse OR zebra-fish OR zebrafish))
NTRL	
06/2023	Date limit 2005-2023 Search Titles OR Keywords; "(6)Annulene" OR "[6]Annulene" OR "Benzene" OR "Benzin" OR "Benzine" OR "Benzol" OR "Benzole" OR "Benzolene" OR "Bicarburet of hydrogen" OR "Carbon oil" OR "Coal naphtha" OR "Cyclohexatriene" OR "Mineral naphtha" OR "Phenyl hydride" OR "Polystream" OR "Pyrobenzol" OR "Pyrobenzole"
Toxcenter	
06/2023	FILE 'TOXCENTER' ENTERED AT 15:31:13 ON 27 JUN 2023 --------- L1 (63989)SEA 71-43-2 L2 (63556)SEA L1 NOT TSCATS/FS

Table B-2. Database Query Strings

Database search date	Query string
	L3 (55364)SEA L2 NOT PATENT/DT L4 (26587)SEA L3 AND PY>2004 L5 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L6 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L7 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L8 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L9 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L10 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L11 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) L12 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE)) L13 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L14 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L15 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L16 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L17 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?) L18 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?) L19 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?) L20 QUE (ENDOCRIN? AND DISRUPT?) L21 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?) L22 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?) L23 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?) L24 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?) L25 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?) L26 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?) L27 QUE (NEPHROTOX? OR HEPATOTOX?) L28 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?) L29 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?) L30 QUE L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR

Table B-2. Database Query Strings

Database search date	Query string
	L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29
	L31 (11443)SEA L4 AND L30
	L32 (1977)SEA L31 AND MEDLINE/FS
	L33 (2222)SEA L31 AND BIOSIS/FS
	L34 (7218)SEA L31 AND CAPLUS/FS
	L35 (26)SEA L31 NOT (L32 OR L33 OR L34)
	L36 (9174)DUP REM L32 L33 L35 L34 (2269 DUPLICATES REMOVED)
	L37 (1975)SEA L36
	L38 (1474)SEA L36
	L39 (5702)SEA L36
	L40 (23)SEA L36
	L41 7199 SEA (L37 OR L38 OR L39 OR L40) NOT MEDLINE/FS

	L42 1918 SEA L41 AND CAPLUS/FS AND PY>2016
	L43 1474 SEA L41 AND BIOSIS/FS
	L44 23 SEA L41 AND L35
	D SCAN L44
	D SCAN L43
	D SCAN L42

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS via ChemView	
06/2023	Compounds searched: 71-43-2
NTP	
06/2023	Match exact word or phrase With no date limit or content type limit: 71-43-2 With date limit 2000-present or Not Dated, and content types: Reports & Publications, Systematic Review, Report & Publications, or Reports & Publication: Benzene With date limit 2000-present or Not Dated: Benzin Benzine Benzol Benzole Cyclohexatriene Phenyl hydride Pyrobenzol (6)Annulene Benzolene Bicarburet of hydrogen Carbon oil Coal naphtha Mineral naphtha Polystream

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
	Pyrobenzole
Regulations.gov	
06/2023	Posted 01/01/2005 to 06/26/2023 71-43-2 Benzene
NIH RePORTER	
01/2024	Search Criteria Fiscal Year: Active Projects Text Search: "(6)Annulene" OR "[6]Annulene" OR "Benzene" OR "Benzin" OR "Benzine" OR "Benzol" OR "Benzole" OR "Benzolene" OR "Bicarburet of hydrogen" OR "Carbon oil" OR "Coal naphtha" OR "Cyclohexatriene" OR "Mineral naphtha" OR "Phenyl hydride" OR "Polystream" OR "Pyrobenzol" OR "Pyrobenzole" (advanced) Limit to: Project Title, Project Terms, Project Abstracts
Other	Identified throughout the assessment process

The 2023 results were:

- Number of records identified from PubMed, NTRL, and TOXCENTER (after duplicate removal): 6,673
- Number of records identified from other strategies: 171
- Total number of records to undergo literature screening: 6,844

B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on benzene:

- Title and abstract screen
- Full text screen

Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

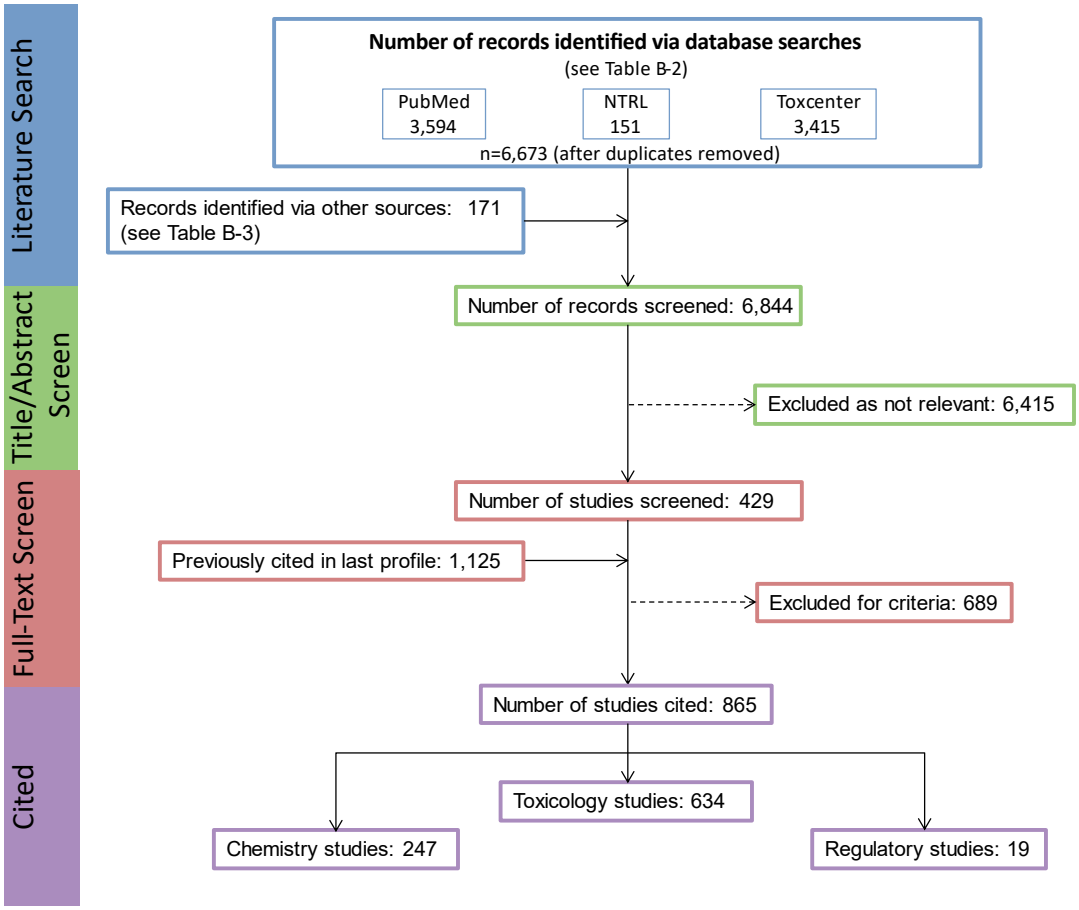
- Number of titles and abstracts screened: 6,844
- Number of studies considered relevant and moved to the next step: 429

Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 429
- Number of studies cited in the pre-public draft of the toxicological profile: 1,125
- Total number of studies cited in the profile: 865

A summary of the results of the literature search and screening is presented in Figure B-1.

Figure B-1. June 2023 Literature Search Results and Screen for Benzene



APPENDIX C. FRAMEWORK FOR ATSDR'S SYSTEMATIC REVIEW OF HEALTH EFFECTS DATA FOR BENZENE

To increase the transparency of ATSDR's process of identifying, evaluating, synthesizing, and interpreting the scientific evidence on the health effects associated with exposure to benzene, ATSDR utilized a slight modification of NTP's Office of Health Assessment and Translation (OHAT) systematic review methodology (NTP 2013, 2015; Rooney et al. 2014). ATSDR's framework is an eight-step process for systematic review with the goal of identifying the potential health hazards of exposure to benzene:

- Step 1. Problem Formulation
- Step 2. Literature Search and Screen for Health Effects Studies
- Step 3. Extract Data from Health Effects Studies
- Step 4. Identify Potential Health Effect Outcomes of Concern
- Step 5. Assess the Risk of Bias for Individual Studies
- Step 6. Rate the Confidence in the Body of Evidence for Each Relevant Outcome
- Step 7. Translate Confidence Rating into Level of Evidence of Health Effects
- Step 8. Integrate Evidence to Develop Hazard Identification Conclusions

C.1 PROBLEM FORMULATION

The objective of the toxicological profile and this systematic review was to identify the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to benzene. The inclusion criteria used to identify relevant studies examining the health effects of benzene are presented in Table C-1.

Data from human and laboratory animal studies were considered relevant for addressing this objective. Human studies were divided into two broad categories: observational epidemiology studies and controlled exposure studies. The observational epidemiology studies were further divided: cohort studies (retrospective and prospective studies), population studies (with individual data or aggregate data), and case-control studies.

Table C-1. Inclusion Criteria for Identifying Health Effects Studies

Species
Human
Laboratory mammals
Route of exposure
Inhalation
Oral
Dermal (or ocular)
Parenteral (these studies will be considered supporting data)
Health outcome
Death
Systemic effects
Body weight effects
Respiratory effects
Cardiovascular effects

Table C-1. Inclusion Criteria for Identifying Health Effects Studies

Gastrointestinal effects
Hematological effects
Musculoskeletal effects
Hepatic effects
Renal effects
Dermal effects
Ocular effects
Endocrine effects
Immunological effects
Neurological effects
Reproductive effects
Developmental effects
Other noncancer effects
Cancer

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 result from reactive metabolites of benzene formed in hematopoietic tissue, as well as in liver and other tissues. The primary enzymes involved in generating reactive metabolites of benzene include CYP2E1, MPO, and NQO1, although other enzymes are also involved. The most sensitive effects of benzene exposure are to the hematological and immunological systems.

Prioritization of Human Data. 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 maintenance) examined and found associations between benzene exposure and health outcomes, primarily hematologic and immunologic. Several studies evaluated associations between measured exposures and effects in these target organs. Studies meeting the following criteria were included in this systematic review: (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. Many studies in workers did not meet these criteria and have limitations that precluded their use in estimating exposure-outcome relationships. These limitations include lack of accurate exposure data, uncontrolled co-exposure to other chemicals, and lack of appropriate control groups. These studies were not considered in the systematic review, although they do provide supportive information for hazard identification and are included in the toxicological profile.

Prioritization of Animal Data. The inhalation database for hematological and immunological endpoints in animals is extensive. Oral exposure studies also examine these endpoints, although the database is much less extensive. Therefore, animal studies evaluating the most sensitive effects of exposure (hematological and immunological) were prioritized for efficient review. Inclusion of hematological and immunological animal inhalation studies in this systematic review was based on the following criteria:

- all single-dose studies with a dose that is ≤2-fold of the LOAEL for the critical study for each exposure-duration category;
- all multi-dose studies where the lowest dose tested is ≤10-fold higher than the LOAEL for the critical study for each exposure-duration category.

Given that the oral exposure database has relatively few studies compared to the inhalation database, all animal studies evaluating effects on the hematological and immunological effects were considered in this systematic review. Note that no human studies on hematological and immunological effects were identified.

C.2 LITERATURE SEARCH AND SCREEN FOR HEALTH EFFECTS STUDIES

A literature search and screen were conducted to identify studies examining the health effects of benzene. The literature search framework for the toxicological profile is discussed in detail in Appendix B.

C.2.1 Literature Search

As noted in Appendix B, the current literature search was intended to update the 2007 Toxicological Profile for Benzene; thus, the literature search was restricted to studies published between January 2005 and June 2023. See Appendix B for the databases searched and the search strategy.

A total of 6,843 records relevant to all sections of the toxicological profile were identified (after duplicate removal).

C.2.2 Literature Screening

As described in Appendix B, a two-step process was used to screen the literature search to identify relevant studies examining the health effects of benzene.

Title and Abstract Screen. In the Title and Abstract Screen step, 6,843 records were reviewed; 57 documents were considered to meet the health effects inclusion criteria in Table C-1 and were moved to the next step in the process.

Full Text Screen. In the second step in the literature screening process for the systematic review, a full text review of 198 health effect documents (documents identified in the update literature search and documents cited in older versions of the profile) was performed. From those 198 documents (240 studies), 49 documents (52 studies) were included in the qualitative review.

C.3 EXTRACT DATA FROM HEALTH EFFECTS STUDIES

Relevant data extracted from the individual studies selected for inclusion in the systematic review were collected in customized data forms. A summary of the type of data extracted from each study is presented in Table C-2. For references that included more than one experiment or species, data extraction records were created for each experiment or species.

Table C-2. Data Extracted From Individual Studies

Citation
Chemical form
Route of exposure (e.g., inhalation, oral, dermal)
Specific route (e.g., gavage in oil, drinking water)
Species
Strain
Exposure duration category (e.g., acute, intermediate, chronic)
Exposure duration
Frequency of exposure (e.g., 6 hours/day, 5 days/week)
Exposure length
Number of animals or subjects per sex per group
Dose/exposure levels
Parameters monitored
Description of the study design and method
Summary of calculations used to estimate doses (if applicable)
Summary of the study results
Reviewer's comments on the study
Outcome summary (one entry for each examined outcome)
No-observed-adverse-effect level (NOAEL) value
Lowest-observed-adverse-effect level (LOAEL) value
Effect observed at the LOAEL value

A summary of the extracted data for each study is presented in the Supplemental Document for Benzene and overviews of the results of the inhalation, oral, and dermal exposure studies are presented in Sections 2.2–2.18 of the profile and in the Levels Significant Exposures tables in Section 2.1 of the profile (Tables 2-1, 2-2, and 2-3, respectively).

C.4 IDENTIFY POTENTIAL HEALTH EFFECT OUTCOMES OF CONCERN

Overviews of the potential health effect outcomes for benzene identified in human and animal studies are presented in Tables C-3 and C-4, respectively. It is well-established that benzene is hematotoxic and immunotoxic based on many years of research in both humans and animals; this is not in dispute. Animal studies evaluating comprehensive toxicological endpoints also demonstrated that the hematological system and the immunological system are the most sensitive effects of benzene exposure. Inclusion of studies to undergo systematic review are discussed in Section C.1 above. There were 52 studies (published in 49 documents) examining these potential outcomes carried through to Steps 4–8 of the systematic review.

Table C-3. Overview of the Health Outcomes for Benzene Evaluated In Human Studies

	Body weight	Respiratory	Cardiovascular	Gastrointestinal	Hematological	Musculoskeletal	Hepatic	Renal	Dermal	Ocular	Endocrine	Immunological	Neurological	Reproductive	Developmental	Other Noncancer	Cancer
Inhalation studies																	
Acute duration		6		1	1			1	3								
		6		1	1			1	3								
Chronic duration	1				33	2	2			1		7	2	3	1		40
	1				31	2				1		7	2	1	1		25
Oral studies																	
Acute duration					1												
					1												
Chronic duration					1												
					1												
Dermal studies																	

Number of studies examining endpoint	0	1	2	3	4	5-9	≥10
Number of studies reporting outcome	0	1	2	3	4	5-9	≥10

Table C-4. Overview of the Health Outcomes for Benzene Evaluated in Experimental Animal Studies

	Body weight	Respiratory	Cardiovascular	Gastrointestinal	Hematological	Musculoskeletal	Hepatic	Renal	Dermal	Ocular	Endocrine	Immunological[a]	Neurological[a]	Reproductive[a]	Developmental	Other Noncancer	Cancer
Inhalation studies																	
Acute-duration	9				28		2					6	4	6	10		
	6				27		1					6	4	1	10		
Intermediate-duration	6		1		28						1	6	1	4	3		9
	1		1		27						1	6	1	3	3		8
Chronic-duration	3	2			5		2	2									6
	3	0			5		0	0									3
Acute-duration	1			1	1		1	1	1				1	1	2		
	1			1	1		1	0	1				1	0	1		
Intermediate-duration	10	2	2	3	13	2	7	4			4	6	4	3			3
	5	0	0	0	13	0	1	0			2	6	3	2			3
Chronic-duration	2	2	2	2	3	2	2	2	2	1	2		2	2			5
	2	1	0	2	3	0	0	0	0	0	1		0	0			5
Dermal studies																	
Acute-duration																	
Intermediate-duration																	
Chronic-duration																	

Number of studies examining endpoint	0	1	2	3	4	5-9	≥10
Number of studies reporting outcome	0	1	2	3	4	5-9	≥10

[a]Number of studies examining endpoint includes study evaluating histopathology, but not evaluating function.

C.5 ASSESS THE RISK OF BIAS FOR INDIVIDUAL STUDIES

C.5.1 Risk of Bias Assessment

The risk of bias of individual studies was assessed using OHAT's Risk of Bias Tool (NTP 2015). The risk of bias questions for observational epidemiology studies, human-controlled exposure studies, and animal experimental studies are presented in Tables C-5, C-6, and C-7, respectively. Each risk of bias question was answered on a four-point scale:

- **Definitely low risk of bias (++)**
- **Probably low risk of bias (+)**
- **Probably high risk of bias (-)**
- **Definitely high risk of bias (– –)**

In general, "definitely low risk of bias" or "definitely high risk of bias" were used if the question could be answered with information explicitly stated in the study report. If the response to the question could be inferred, then "probably low risk of bias" or "probably high risk of bias" responses were typically used.

Table C-5. Risk of Bias Questionnaire for Observational Epidemiology Studies

Selection bias
Were the comparison groups appropriate?
Confounding bias
Did the study design or analysis account for important confounding and modifying variables?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

Table C-6. Risk of Bias Questionnaire for Human-Controlled Exposure Studies
Selection bias
Was administered dose or exposure level adequately randomized?
Was the allocation to study groups adequately concealed?
Performance bias
Were the research personnel and human subjects blinded to the study group during the study?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

Table C-7. Risk of Bias Questionnaire for Experimental Animal Studies
Selection bias
Was administered dose or exposure level adequately randomized?
Was the allocation to study groups adequately concealed?
Performance bias
Were experimental conditions identical across study groups?
Were the research personnel blinded to the study group during the study?
Attrition/exclusion bias
Were outcome data complete without attrition or exclusion from analysis?
Detection bias
Is there confidence in the exposure characterization?
Is there confidence in outcome assessment?
Selective reporting bias
Were all measured outcomes reported?

After the risk of bias questionnaires were completed for the health effects studies, the studies were assigned to one of three risk of bias tiers based on the responses to the key questions listed below and the responses to the remaining questions.

- Is there confidence in the exposure characterization? (only relevant for observational studies)
- Is there confidence in the outcome assessment?
- Does the study design or analysis account for important confounding and modifying variables? (only relevant for observational studies)

First Tier. Studies placed in the first tier received ratings of "definitely low" or "probably low" risk of bias on the key questions **AND** received a rating of "definitely low" or "probably low" risk of bias on the responses to at least 50% of the other applicable questions.

Second Tier. A study was placed in the second tier if it did not meet the criteria for the first or third tiers.

Third Tier. Studies placed in the third tier received ratings of "definitely high" or "probably high" risk of bias for the key questions **AND** received a rating of "definitely high" or "probably high" risk of bias on the response to at least 50% of the other applicable questions.

The results of the risk of bias assessment for the different types of benzene health effects studies (observational epidemiology and animal experimental studies) are presented in Tables C-8 and C-9, respectively.

Table C-8. Summary of Risk of Bias Assessment for Benzene—Observational Epidemiology Studies

Reference	Risk of bias criteria and ratings: Selection bias: Were the comparison groups appropriate?	Confounding bias: Did the study design or analysis account for important confounding and modifying variables?*	Attrition / exclusion bias: Were outcome data complete without attrition or exclusion from analysis?	Detection bias: Is there confidence in the exposure characterization?*	Detection bias: Is there confidence in the outcome assessment?*	Selective reporting bias: Were all measured outcomes reported?	Risk of bias tier
Outcome: Hematological effects							
Cohort studies							
Dosemeci et al. 1996	+	–	++	++	+	++	First
Tsai et al. 2004	– –	–	–	–	+	++	Third
Case-control studies							
Ward et al. 1996	++	–	–	++	+	+	First
Cross-sectional studies							
Collins et al. 1991	–	+	–	–	+	–	Third
Collins et al. 1997	–	+	–	–	–	–	Third
Ibrahim et al. 2014	+	+	+	+	+	+	First
Lan et al. 2004a, 2004b	+	++	++	++	++	++	First
Li et al. 2018	+	+	+	++	+	++	First
Qu et al. 2002	+	++	+	++	+	+	First
Rothman et al. 1996a, 1996b	+	+	++	++	++	++	First
Schnatter et al. 2010	++	++	++	++	+	+	First
Swaen et al. 2010	– –	–	–	–	+	++	Third

Table C-8. Summary of Risk of Bias Assessment for Benzene—Observational Epidemiology Studies

	Risk of bias criteria and ratings						
	Selection bias	Confounding bias	Attrition / exclusion bias	Detection bias		Selective reporting bias	
Reference	Were the comparison groups appropriate?	Did the study design or analysis account for important confounding and modifying variables?*	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?*	Is there confidence in the outcome assessment?*	Were all measured outcomes reported?	Risk of bias tier
Wang et al. 2021a	+	+	+	+	–	++	Second
Wang et al. 2021b	–	++	+	– –	++	++	Second
Zhang et al. 2020	+	+	+	+	–	++	Second

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias; NA = not applicable
*Key question used to assign risk of bias tier

Table C-9. Summary of Risk of Bias Assessment for Benzene—Experimental Animal Studies

Reference	Risk of bias criteria and ratings: Selection bias: Was administered dose or exposure level adequately randomized?	Selection bias: Was the allocation to study groups adequately concealed?	Performance bias: Were experimental conditions identical across study groups?	Performance bias: Were the research personnel blinded to the study group during the study?	Attrition/ exclusion bias: Were outcome data complete without attrition or exclusion from analysis?	Detection bias: Is there confidence in the exposure characterization?	Detection bias: Is there confidence in the outcome assessment?*	Selective reporting bias: Were all measured outcomes reported?	Risk of bias tier
Outcome: Hematological Effects									
Inhalation acute exposure									
Aoyama 1986; 7 days	+	+	+	+	–	–	+	+	First
Aoyama 1986; 14 days	+	+	+	+	–	–	+	+	First
Cronkite 1986	–	+	–	+	–	– –	–	– –	Third
Cronkite et al. 1985	–	+	–	+	–	–	++	–	Second
Dempster and Snyder 1991	+	+	+	+	–	+	+	+	First
Green et al. 1981a	+	+	+	+	–	+	+	+	First
Green et al. 1981b	+	+	+	+	–	+	+	+	First
Li et al. 1986	–	+	–	+	+	–	++	–	Second
Rosenthal and Snyder 1985	+	+	+	+	+	–	+	+	First
Rozen et al. 1984	+	+	+	+	–	–	+	+	First
Toft et al. 1982; 2 weeks	–	+	–	+	+	–	+	+	First
Toft et al. 1982; 1–10 days	–	+	+	+	+	–	+	+	First
Toft et al. 1982; 2 weeks, 0, 14 ppm	–	+	–	+	+	–	+	–	Second
Ward et al. 1985; rat	–	+	+	+	–	+	+	–	First
Ward et al. 1985; mouse	–	+	+	+	–	+	+	–	First

Table C-9. Summary of Risk of Bias Assessment for Benzene—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition/ exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	Is there confidence in the outcome assessment?*	Were all measured outcomes reported?	
Wells and Nerland 1991	+	+	+	+	–	–	+	+	First
Inhalation intermediate exposure									
Baarson et al. 1984	+	+	+	+	+	–	+	+	First
Cronkite et al. 1989	–	+	–	+	–	–	+	–	Second
Dow 1992	–	+	+	+	++	+	+	++	First
Farris et al. 1997a	–	+	+	+	+	+	+	+	First
Farris et al. 1997b	–	+	+	+	+	+	+	+	First
Green et al. 1981a	+	+	+	+	+	–	+	+	First
Green et al. 1981b	+	+	+	+	+	–	+	+	First
Ward et al. 1985; rat	–	+	+	+	–	+	+	–	First
Oral acute exposure									
Huang et al. 2013	–	+	+	+	++	+	++	+	First
Oral intermediate exposure									
Cui et al. 2022	+	+	+	+	++	–	++	+	First
Fan 1992	+	+	+	+	+	+	–	+	Second
Heijne et al. 2005	+	+	+	+	+	++	+	–	First
Hsieh et al. 1988	+	+	+	+	++	+	+	+	First
Hsieh et al. 1990	+	+	+	+	++	+	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Benzene—Experimental Animal Studies

Reference	Risk of bias criteria and ratings								Risk of bias tier
	Selection bias		Performance bias		Attrition/ exclusion bias	Detection bias		Selective reporting bias	
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	Is there confidence in the outcome assessment?*	Were all measured outcomes reported?	
NTP 1986; rat	++	+	+	+	++	++	+	–	First
NTP 1986; mouse	++	+	+	+	++	++	+	–	First
Karaulov et al. 2017	–	+	–	+	–	–	+	+	Second
Li et al. 2018	+	+	+	+	++	–	++	++	First
Maltoni et al. 1983, 1985	+	+	+	+	–	–	+	–	First
Shell 1992	+	+	–	+	+	–	+	+	First
Wolf et al. 1956	–	+	+	+	–	+	–	–	Second
Oral chronic									
NTP 1986; rat	++	+	+	+	+	++	+	–	First
NTP 1986; mouse	++	+	+	+	+	++	+	–	First
Maltoni et al. 1983, 1985	+	+	+	+	–	–	+	+	First
Outcome: Immunological Effects									
Inhalation acute exposure									
Aoyama 1986; 7 days	+	+	+	+	–	–	+	+	First
Aoyama 1986; 14 days	+	+	+	+	–	–	+	+	First
Dempster and Snyder 1991	+	+	+	+	–	+	+	+	First
Li et al. 1986	–	+	–	+	+	–	++	–	Second
Rosenthal and Snyder 1985	+	+	+	+	+	–	+	+	First

Table C-9. Summary of Risk of Bias Assessment for Benzene—Experimental Animal Studies

Reference	Risk of bias criteria and ratings: Selection bias		Performance bias		Attrition/ exclusion bias	Detection bias		Selective reporting bias	Risk of bias tier
	Was administered dose or exposure level adequately randomized?	Was the allocation to study groups adequately concealed?	Were experimental conditions identical across study groups?	Were the research personnel blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Is there confidence in the exposure characterization?	Is there confidence in the outcome assessment?*	Were all measured outcomes reported?	
Rozen et al. 1984	+	+	+	+	–	–	–	+	Second
Ward et al. 1985	–	+	+	+	–	+	+	–	First
Inhalation intermediate exposure									
Rosenthal and Snyder 1987	+	+	+	+	++	–	+	+	First
Stoner et al. 1981	–	+	+	+	++	–	+	+	First
Ward et al. 1985	–	+	+	+	–	+	+	–	First
Oral intermediate exposure									
Fan 1992	+	+	+	+	+	+	+	+	First
Karaulov et al. 2017	–	+	–	+	–	–	+	+	Second
Hsieh et al. 1988	+	+	+	+	++	+	++	+	First
Hsieh et al. 1990	+	+	+	+	++	+	++	+	First
Hsieh et al. 1991	–	+	+	+	+	+	–	+	Second

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias

*Key question used to assign risk of bias tier.

C.6 RATE THE CONFIDENCE IN THE BODY OF EVIDENCE FOR EACH RELEVANT OUTCOME

Confidences in the bodies of human and animal evidence were evaluated independently for each potential outcome. ATSDR did not evaluate the confidence in the body of evidence for carcinogenicity; rather, the Agency defaulted to the cancer weight-of-evidence assessment of other agencies including HHS, EPA, and IARC. The confidence in the body of evidence for an association or no association between exposure to benzene and a particular outcome was based on the strengths and weaknesses of individual studies. Four descriptors were used to describe the confidence in the body of evidence for effects or when no effect was found:

- **High confidence:** the true effect is highly likely to be reflected in the apparent relationship
- **Moderate confidence:** the true effect may be reflected in the apparent relationship
- **Low confidence:** the true effect may be different from the apparent relationship
- **Very low confidence:** the true effect is highly likely to be different from the apparent relationship

Confidence in the body of evidence for a particular outcome was rated for each type of study: case-control, case series, cohort, population, human-controlled exposure, and experimental animal. In the absence of data to the contrary, data for a particular outcome were collapsed across animal species, routes of exposure, and exposure durations. If species (or strain), route, or exposure duration differences were noted, then the data were treated as separate outcomes.

C.6.1 Initial Confidence Rating

In ATSDR's modification to the OHAT approach, the body of evidence for an association (or no association) between exposure to benzene and a particular outcome was given an initial confidence rating based on the key features of the individual studies examining that outcome. The presence of these key features of study design was determined for individual studies using four "yes or no" questions, which were customized for epidemiology, human controlled exposure, or experimental animal study designs. Separate questionnaires were completed for each outcome assessed in a study. The key features for observational epidemiology (cohort, population, and case-control) studies, human controlled exposure, and experimental animal studies are presented in Tables C-10, C-11, and C-12, respectively. The initial confidence in the study was determined based on the number of key features present in the study design:

- **High Initial Confidence:** Studies in which the responses to the four questions were "yes".

- **Moderate Initial Confidence:** Studies in which the responses to only three of the questions were "yes".

- **Low Initial Confidence:** Studies in which the responses to only two of the questions were "yes".

- **Very Low Initial Confidence:** Studies in which the response to one or none of the questions was "yes".

Table C-10. Key Features of Study Design for Observational Epidemiology Studies
Exposure was experimentally controlled
Exposure occurred prior to the outcome
Outcome was assessed on individual level rather than at the population level
A comparison group was used

Table C-11. Key Features of Study Design for Human-Controlled Exposure Studies
A comparison group was used or the subjects served as their own control
A sufficient number of subjects were tested
Appropriate methods were used to measure outcomes (i.e., clinically-confirmed outcome versus self-reported)
Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis

Table C-12. Key Features of Study Design for Experimental Animal Studies
A concurrent control group was used
A sufficient number of animals per group were tested
Appropriate parameters were used to assess a potential adverse effect
Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis

The presence or absence of the key features and the initial confidence levels for studies examining hematological and immunological effects observed in the observational epidemiology and animal experimental studies are presented in Tables C-13 and C-14, respectively.

Table C-13. Presence of Key Features of Study Design for Benzene—Observational Epidemiology Studies

	Key features				
Reference	Controlled Exposure	Exposure prior to outcome	Outcome assess on individual level	Comparison group	Initial study confidence
Outcome: Hematological effects					
Cohort studies					
Dosemeci et al. 1996	No	Yes	Yes	Yes	Moderate
Tsai et al. 2004	No	Yes	Yes	Yes	Moderate

Table C-13. Presence of Key Features of Study Design for Benzene—Observational Epidemiology Studies

Reference	Key features: Controlled Exposure	Exposure prior to outcome	Outcome assess on individual level	Comparison group	Initial study confidence
Case-control studies					
Ward et al. 1996	No	Yes	Yes	Yes	Moderate
Cross-sectional studies					
Collins et al. 1991	No	Yes	Yes	Yes	Moderate
Collins et al. 1997	No	Yes	Yes	Yes	Moderate
Ibrahim et al. 2014	No	Yes	Yes	Yes	Moderate
Lan et al. 2004a, 2004b	No	Yes	Yes	Yes	Moderate
Li et al. 2018	No	Yes	Yes	Yes	Moderate
Qu et al. 2002	No	Yes	Yes	Yes	Moderate
Rothman et al. 1996a, 1996b	No	Yes	Yes	Yes	Moderate
Schnatter et al. 2010	No	Yes	Yes	Yes	Moderate
Swaen et al. 2010	No	Yes	Yes	Yes	Moderate
Wang et al. 2021a	No	Yes	Yes	Yes	Moderate
Wang et al. 2021b	No	Yes	Yes	Yes	Moderate
Zhang et al. 2020	No	Yes	Yes	Yes	Moderate

Table C-14. Presence of Key Features of Study Design for Benzene—Experimental Animal Studies

Reference	Key feature: Concurrent control group	Sufficient number of animals per group	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Outcome: Hematological Effects					
Inhalation acute exposure					
Aoyama 1986; 7 days	Yes	Yes	Yes	Yes	High
Aoyama 1986; 14 days	Yes	Yes	Yes	Yes	High
Cronkite 1986	No	No	Yes	No	Very Low

Table C-14. Presence of Key Features of Study Design for Benzene—Experimental Animal Studies

Reference	Key feature: Concurrent control group	Sufficient number of animals per group	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
Cronkite et al. 1985	Yes	No	Yes	No	Low
Dempster and Snyder 1991	Yes	Yes	Yes	Yes	High
Green et al. 1981a	Yes	Yes	Yes	Yes	High
Green et al. 1981b	Yes	Yes	Yes	Yes	High
Li et al. 1986	Yes	Yes	Yes	Yes	High
Rosenthal and Snyder 1985	Yes	Yes	Yes	Yes	High
Rozen et al. 1984	Yes	Yes	Yes	Yes	High
Toft et al. 1982; 2 weeks	Yes	Yes	Yes	No	Moderate
Toft et al. 1982; 1–10 days	Yes	Yes	Yes	No	Moderate
Toft et al. 1982; 2 weeks, 0, 14 ppm	Yes	Yes	Yes	No	Moderate
Ward et al. 1985; rat	Yes	Yes	Yes	Yes	High
Ward et al. 1985; mouse	Yes	Yes	Yes	Yes	High
Wells and Nerland 1991	Yes	Yes	Yes	Yes	High
Inhalation intermediate exposure					
Baarson et al. 1984	Yes	No	Yes	No	Low
Cronkite et al. 1989	Yes	Yes	Yes	No	Moderate
Dow 1992	Yes	Yes	Yes	Yes	High
Farris et al. 1997a	Yes	Yes	Yes	Yes	High
Farris et al. 1997b	Yes	Yes	Yes	Yes	High
Green et al. 1981a	Yes	Yes	Yes	Yes	High
Green et al. 1981b	Yes	Yes	Yes	Yes	High
Ward et al. 1985; rat	Yes	Yes	Yes	Yes	High
Ward et al. 1985; mouse	Yes	Yes	Yes	Yes	High
Oral acute exposure					
Huang et al. 2013	Yes	Yes	Yes	Yes	High
Oral intermediate exposure					
Cui et al. 2022	Yes	Yes	Yes	Yes	High
Fan 1992	Yes	Yes	No	Yes	Moderate
Heijne et al. 2005	Yes	Yes	Yes	Yes	High
Hsieh et al. 1988	Yes	Yes	Yes	Yes	High
Hsieh et al. 1990	Yes	Yes	Yes	Yes	High

Table C-14. Presence of Key Features of Study Design for Benzene—Experimental Animal Studies

Reference	Key feature: Concurrent control group	Sufficient number of animals per group	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	Initial study confidence
NTP 1986; rat	Yes	Yes	Yes	Yes	High
NTP 1986; mouse	Yes	Yes	Yes	Yes	High
Karaulov et al. 2017	Yes	Yes	Yes	Yes	High
Li et al. 2018	Yes	Yes	Yes	Yes	High
Maltoni et al. 1983, 1985	Yes	Yes	Yes	Yes	High
Shell 1992	Yes	Yes	Yes	Yes	High
Wolf et al. 1956	Yes	Yes	Yes	No	Moderate
Oral chronic exposure					
NTP 1986; rat	Yes	Yes	Yes	Yes	High
NTP 1986; mouse	Yes	Yes	Yes	Yes	High
Maltoni et al. 1983, 1985	Yes	Yes	Yes	Yes	High
Outcome: Immunological Effects					
Inhalation acute exposure					
Aoyama 1986; 7 days	Yes	Yes	Yes	Yes	High
Aoyama 1986; 14 days	Yes	Yes	Yes	Yes	High
Dempster and Snyder 1991	Yes	Yes	Yes	Yes	High
Li et al. 1986	Yes	Yes	Yes	Yes	High
Rosenthal and Snyder 1985	Yes	Yes	Yes	Yes	High
Rozen et al. 1984	Yes	Yes	Yes	Yes	High
Ward et al. 1985	Yes	Yes	Yes	No	Moderate
Inhalation intermediate exposure					
Rosenthal and Snyder 1987	Yes	Yes	Yes	Yes	High
Stoner et al. 1981	Yes	Yes	Yes	No	Moderate
Ward et al. 1985	Yes	Yes	Yes	No	Moderate
Oral intermediate exposure					
Fan 1992	Yes	Yes	No	Yes	Moderate
Karaulov et al. 2017	Yes	Yes	Yes	Yes	High
Hsieh et al. 1988	Yes	Yes	Yes	Yes	High
Hsieh et al. 1990	Yes	Yes	Yes	Yes	High
Hsieh et al. 1991	Yes	Yes	No	Yes	Moderate

A summary of the initial confidence ratings for each outcome is presented in Table C-15. If individual studies for a particular outcome and study type had different study quality ratings, then the highest confidence rating for the group of studies was used to determine the initial confidence rating for the body of evidence; any exceptions were noted in Table C-15.

Table C-15. Initial Confidence Rating for Benzene Health Effects Studies

	Initial study confidence	Initial confidence rating
Outcome: Hematological Effects		
Inhalation acute		
Animal studies		
Aoyama 1986; 7 days	High	High
Aoyama 1986; 14 days	High	
Cronkite 1986	Very Low	
Cronkite et al. 1985	Low	
Dempster and Snyder 1991	High	
Green et al. 1981a	High	
Green et al. 1981b	High	
Li et al. 1986	High	
Rosenthal and Snyder 1985	High	
Rozen et al. 1984	High	
Toft et al. 1982; 2 weeks	Moderate	
Toft et al. 1982; 1-10 days	Moderate	
Toft et al. 1982; 2 weeks, 0, 14 ppm	Moderate	
Ward et al. 1985; Rat	High	
Wells and Nerland 1991	High	
Inhalation intermediate		
Animal studies		
Baarson et al. 1984	Low	High
Cronkite et al. 1989	Moderate	
Dow 1992	High	
Farris et al. 1997a	High	
Farris et al. 1997b	High	
Green et al. 1981a	High	
Green et al. 1981b	High	
Ward et al. 1985; Rat	High	
Ward et al. 1985; Mouse	High	
Inhalation chronic		
Human studies		
Dosemeci et al. 1996	Moderate	Moderate
Tsai et al. 2004	Moderate	
Ward et al. 1996	Moderate	
Collins et al. 1991	Moderate	
Collins et al. 1997	Moderate	

Table C-15. Initial Confidence Rating for Benzene Health Effects Studies

	Initial study confidence	Initial confidence rating
Ibrahim et al. 2014	Moderate	
Lan et al. 2004a, 2004b	Moderate	
Li et al. 2018	Moderate	
Qu et al. 2002	Moderate	
Rothman et al. 1996a, 1996b	Moderate	
Schnatter et al. 2010	Moderate	
Swaen et al. 2010	Moderate	
Wang et al. 2021a	Moderate	
Wang et al. 2021b	Moderate	
Zhang et al. 2020	Moderate	
Oral acute		
Animal studies		
Huang et al. 2013	High	High
Oral intermediate		
Animal studies		
Cui et al. 2022	High	
Fan 1992	Moderate	
Heijne et al. 2005	High	
Hsieh et al. 1988	High	
Hsieh et al. 1990	High	
NTP 1986); rat	High	High
NTP 1986; mouse	High	
Karaulov et al. 2017	High	
Li et al. 2018	High	
Maltoni et al. 1983, 1985	High	
Shell 1992	High	
Wolf et al. 1956	Moderate	
Oral chronic		
Animal studies		
NTP 1986; rat	High	
NTP 1986; mouse	High	High
Maltoni et al. 1983, 1985	High	
Outcome: Immunological Effects		
Inhalation acute information		
Animal studies		
Aoyama 1986; 7 days	High	
Aoyama 1986; 14 days	High	
Dempster and Snyder 1991	High	High
Li et al. 1986	High	
Rosenthal and Snyder 1985	High	

Table C-15. Initial Confidence Rating for Benzene Health Effects Studies

	Initial study confidence	Initial confidence rating
Rozen et al. 1984	High	
Ward et al. 1985	Moderate	
Inhalation intermediate		
Animal studies		
Rosenthal and Snyder 1987	High	
Stoner et al. 1981	Moderate	High
Ward et al. 1985	Moderate	
Oral intermediate		
Animal studies		
Fan 1992	Moderate	
Karaulov et al. 2017	High	
Hsieh et al. 1988	High	High
Hsieh et al. 1990	High	
Hsieh et al. 1991	Moderate	

C.6.2 Adjustment of the Confidence Rating

The initial confidence rating was then downgraded or upgraded depending on whether there were substantial issues that would decrease or increase confidence in the body of evidence. The nine properties of the body of evidence that were considered are listed below. The summaries of the assessment of the confidence in the body of evidence for hematological and immunological effects are presented in Table C-16. If the confidence ratings for a particular outcome were based on more than one type of human study, then the highest confidence rating was used for subsequent analyses. An overview of the confidence in the body of evidence for all health effects associated with benzene exposure is presented in Table C-17.

Table C-16. Adjustments to the Initial Confidence in the Body of Evidence

	Initial confidence	Adjustments to the initial confidence rating	Final confidence
Hematological Effects:			
Human studies	Moderate	+1 Consistency in the body of evidence; +1 Monotonic dose-response gradient	High
Animal studies	High	+1 Consistency in the body of evidence; +1 Monotonic dose-response gradient	High
Immunological Effects:			
Human studies	Not applicable		
Animal studies	High	+1 Consistency in the body of evidence	High

Table C-17. Confidence in the Body of Evidence for Benzene

Outcome	Confidence in body of evidence	
	Human studies	Animal studies
Hematological	High	High
Immunological	Not applicable	High

Five properties of the body of evidence were considered to determine whether the confidence rating should be downgraded:

- **Risk of bias.** Evaluation of whether there is substantial risk of bias across most of the studies examining the outcome. This evaluation used the risk of bias tier groupings for individual studies examining a particular outcome (Tables C-8 and C-9). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for risk of bias:
  - No downgrade if most studies are in the risk of bias first tier
  - Downgrade one confidence level if most studies are in the risk of bias second tier
  - Downgrade two confidence levels if most studies are in the risk of bias third tier

- **Unexplained inconsistency.** Evaluation of whether there is inconsistency or large variability in the magnitude or direction of estimates of effect across studies that cannot be explained. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for unexplained inconsistency:
  - No downgrade if there is little inconsistency across studies or if only one study evaluated the outcome
  - Downgrade one confidence level if there is variability across studies in the magnitude or direction of the effect
  - Downgrade two confidence levels if there is substantial variability across studies in the magnitude or direct of the effect

- **Indirectness.** Evaluation of four factors that can affect the applicability, generalizability, and relevance of the studies:
  - Relevance of the animal model to human health—unless otherwise indicated, studies in rats, mice, and other mammalian species are considered relevant to humans
  - Directness of the endpoints to the primary health outcome—examples of secondary outcomes or nonspecific outcomes include organ weight in the absence of histopathology or clinical chemistry findings in the absence of target tissue effects
  - Nature of the exposure in human studies and route of administration in animal studies—inhalation, oral, and dermal exposure routes are considered relevant unless there are compelling data to the contrary
  - Duration of treatment in animal studies and length of time between exposure and outcome assessment in animal and prospective human studies—this should be considered on an outcome-specific basis

  Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for indirectness:
  - No downgrade if none of the factors are considered indirect
  - Downgrade one confidence level if one of the factors is considered indirect
  - Downgrade two confidence levels if two or more of the factors are considered indirect

- **Imprecision.** Evaluation of the narrowness of the effect size estimates and whether the studies have adequate statistical power. Data are considered imprecise when the ratio of the upper to lower 95% CIs for most studies is ≥10 for tests of ratio measures (e.g., odds ratios) and ≥100 for absolute measures (e.g., percent control response). Adequate statistical power is determined if the study can detect a potentially biologically meaningful difference between groups (20% change from control response for categorical data or risk ratio of 1.5 for continuous data). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for imprecision:
  - No downgrade if there are no serious imprecisions
  - Downgrade one confidence level for serious imprecisions
  - Downgrade two confidence levels for very serious imprecisions

- **Publication bias.** Evaluation of the concern that studies with statistically significant results are more likely to be published than studies without statistically significant results.
  - Downgrade one level of confidence for cases where there is serious concern with publication bias

Four properties of the body of evidence were considered to determine whether the confidence rating should be upgraded:

- **Large magnitude of effect.** Evaluation of whether the magnitude of effect is sufficiently large so that it is unlikely to have occurred as a result of bias from potential confounding factors.
  - Upgrade one confidence level if there is evidence of a large magnitude of effect in a few studies, provided that the studies have an overall low risk of bias and there is no serious unexplained inconsistency among the studies of similar dose or exposure levels; confidence can also be upgraded if there is one study examining the outcome, provided that the study has an overall low risk of bias

- **Dose response.** Evaluation of the dose-response relationships measured within a study and across studies. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
  - Upgrade one confidence level for evidence of a monotonic dose-response gradient
  - Upgrade one confidence level for evidence of a non-monotonic dose-response gradient where there is prior knowledge that supports a non-monotonic dose-response and a non-monotonic dose-response gradient is observed across studies

- **Plausible confounding or other residual biases.** This factor primarily applies to human studies and is an evaluation of unmeasured determinants of an outcome such as residual bias towards the null (e.g., "healthy worker" effect) or residual bias suggesting a spurious effect (e.g., recall bias). Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
  - Upgrade one confidence level for evidence that residual confounding or bias would underestimate an apparent association or treatment effect (i.e., bias toward the null) or suggest a spurious effect when results suggest no effect

- **Consistency in the body of evidence.** Evaluation of consistency across animal models and species, consistency across independent studies of different human populations and exposure scenarios, and consistency across human study types. Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:

- Upgrade one confidence level if there is a high degree of consistency in the database

C.7 TRANSLATE CONFIDENCE RATING INTO LEVEL OF EVIDENCE OF HEALTH EFFECTS

In the seventh step of the systematic review of the health effects data for benzene, the confidence in the body of evidence for specific outcomes was translated to a level of evidence rating. The level of evidence rating reflected the confidence in the body of evidence and the direction of the effect (i.e., toxicity or no toxicity); route-specific differences were noted. The level of evidence for health effects was rated on a five-point scale:

- **High level of evidence:** High confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Moderate level of evidence:** Moderate confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Low level of evidence:** Low confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Evidence of no health effect:** High confidence in the body of evidence that exposure to the substance is not associated with the health outcome
- **Inadequate evidence:** Low or moderate confidence in the body of evidence that exposure to the substance is not associated with the health outcome OR very low confidence in the body of evidence for an association between exposure to the substance and the health outcome

A summary of the level of evidence of health effects for benzene is presented in Table C-18.

Table C-18. Level of Evidence of Health Effects for Benzene

Outcome	Confidence in body of evidence	Direction of health effect	Level of evidence for health effect
Human studies			
Hematological	High	Health effect	High
Immunological	Not applicable		
Animal studies			
Hematological	High	Health effect	High
Immunological	High	Health effect	High

C.8 INTEGRATE EVIDENCE TO DEVELOP HAZARD IDENTIFICATION CONCLUSIONS

The final step involved the integration of the evidence streams for the human studies and animal studies to allow for a determination of hazard identification conclusions. For health effects, there were four hazard identification conclusion categories:

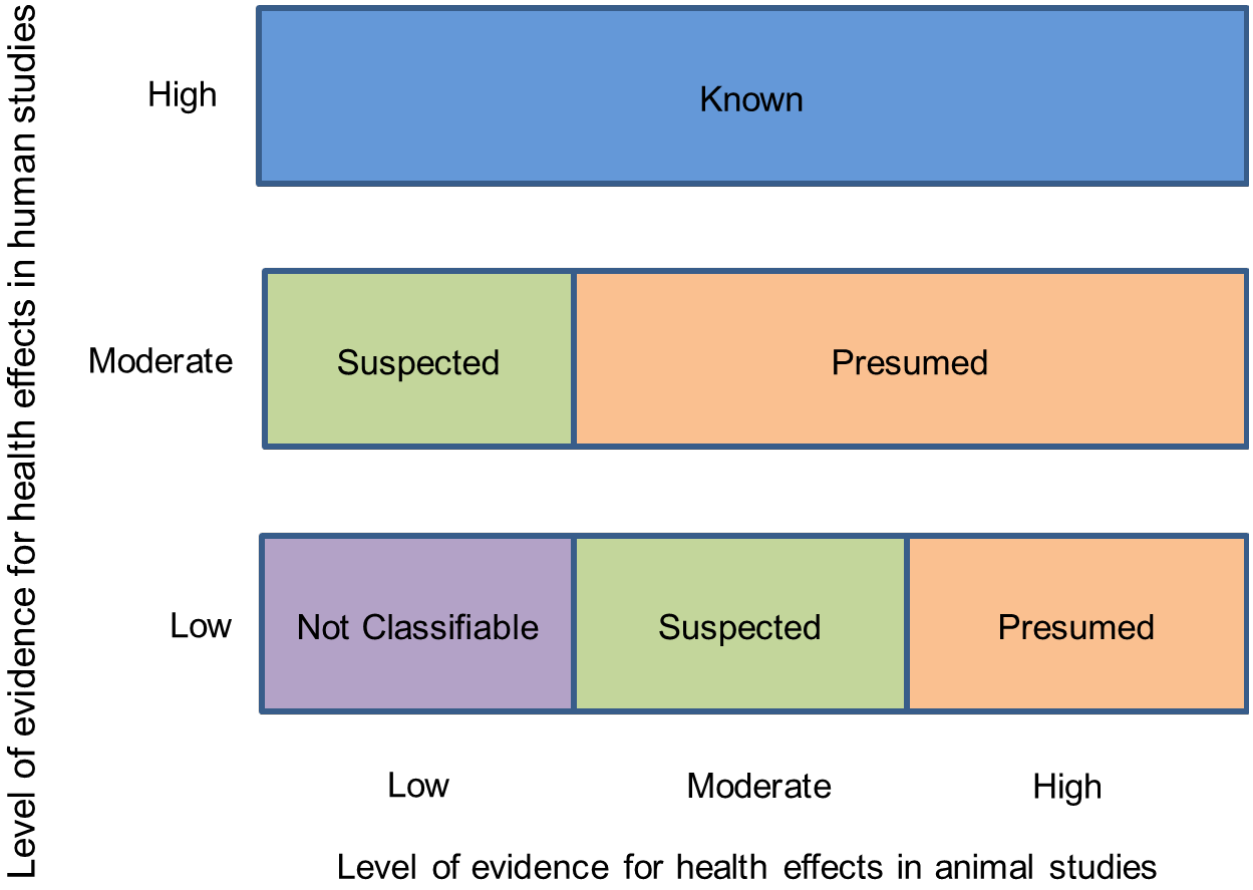
- **Known** to be a hazard to humans
- **Presumed** to be a hazard to humans
- **Suspected** to be a hazard to humans
- **Not classifiable** as to the hazard to humans

The initial hazard identification was based on the highest level of evidence in the human studies and the level of evidence in the animal studies; if there were no data for one evidence stream (human or animal), then the hazard identification was based on the one data stream (equivalent to treating the missing evidence stream as having low level of evidence). The hazard identification scheme is presented in Figure C-1 and described below:

- **Known:** A health effect in this category would have:
  - High level of evidence for health effects in human studies **AND** a high, moderate, or low level of evidence in animal studies.
- **Presumed:** A health effect in this category would have:
  - Moderate level of evidence in human studies **AND** high or moderate level of evidence in animal studies **OR**
  - Low level of evidence in human studies **AND** high level of evidence in animal studies
- **Suspected:** A health effect in this category would have:
  - Moderate level of evidence in human studies **AND** low level of evidence in animal studies **OR**
  - Low level of evidence in human studies **AND** moderate level of evidence in animal studies
- **Not classifiable:** A health effect in this category would have:
  - Low level of evidence in human studies **AND** low level of evidence in animal studies

Other relevant data such as mechanistic or mode-of-action data were considered to raise or lower the level of the hazard identification conclusion by providing information that supported or opposed biological plausibility.

Figure C-1. Hazard Identification Scheme



Two hazard identification conclusion categories were used when the data indicated that there may be no health effect in humans:

- **Not identified** to be a hazard in humans
- **Inadequate** to determine hazard to humans

If the human level of evidence conclusion of no health effect was supported by the animal evidence of no health effect, then the hazard identification conclusion category of "not identified" was used. If the human or animal level of evidence was considered inadequate, then a hazard identification conclusion category of "inadequate" was used. As with the hazard identification for health effects, the impact of other relevant data was also considered for no health effect data.

The hazard identification conclusions for benzene are listed below and summarized in Table C-19.

Known Health Effects

- Hematological
  - High level of evidence for hematology changes (decreased WBCs, lymphocytes, granulocytes, monocytes, neutrophils, platelets) in humans exposed by inhalation in epidemiological studies of occupational populations (Dosemeci et al. 1996; Ibrahim et al.

2014; Lan et al. 2004a, 2004b; Li et al. 2018; Qu et al. 2002; Rothman et al. 1996a, 1996b; Schnatter et al. 2010; Wang et al. 2021b; Ward et al. 1996)

- High level of evidence for hematology changes (decreased WBCs, lymphocytes, neutrophils, decreased bone marrow cellularity, decreased splenic cellularity and granulocytes, decreased granulopoietic stem cells, decreased spleen weight, decreased thymus weight, decreased hematocrit, decreased MCV) in rats and mice exposed by inhalation for acute, intermediate, and chronic durations (Dow 1992; Baarson et al. 1984; Li et al. 1986; Ward et al. 1985; Aoyama 1986; Chertkov et al. 1992; Cronkite 1986; Cronkite et al. 1982, 1985; Dempster and Snyder 1991; Farris et al. 1997a, 1997b; Gill et al. 1980; Green et al. 1981a, 1981b; Mukhopadhyay and Nath 2014; Neun et al. 1992; Plappert et al. 1994a, 1994b; Robinson et al. 1997; Rosenthal and Snyder 1985, 1987; Rozen et al. 1984; Seidel et al. 1989; Snyder et al. 1978, 1980, 1982, 1984, 1988; Toft et al. 1982; Vacha et al. 1990; Wells and Nerland 1991)
- Evidence for hematology changes (decreased WBCs, lymphocytes, neutrophils, and RBCs, decreased splenic cellularity, decreased spleen weight, decreased thymus weight, decreased MCV) in rats and mice by oral exposure for acute, intermediate, and chronic durations (Bahadar et al. 2015b; Cui et al. 2022; Fan 1992; Heijne et al. 2005; Hsieh et al. 1988, 1990; Huang et al. 2013; Karaulov et al. 2017; Li et al. 2018; Maltoni et al. 1983, 1985; NTP 1986; Shell 1992; Wolf et al. 1956).
- An abundance of mechanistic evidence supports a mode of action for hematological effects of benzene that involves marrow cytotoxicity and genotoxicity of reactive metabolites of benzene (see Section 2.20).

Presumed Health Effects

- Immunological
  - Human studies of immunological endpoints are lacking but hematology findings are supportive of immune effects (decreased circulating immune cells).
  - High level of evidence for immune system effects (altered production of interleukins by splenic lymphocytes; altered splenic lymphocyte proliferative response to mitogens; altered splenic lymphocyte cytotoxic response to tumor cells; decreased splenic lymphocyte antibody production; decreased response of marrow CFU-E to erythropoietin; decreased resistance to bacterial infection; decreased mitogen-induced blastogenesis of marrow lymphocytes; decreased antibody response to fluid tetanus toxoid; lymph node histopathology changes) in rats and mice exposed by inhalation for acute and intermediate durations (Aoyama 1986; Dempster and Snyder 1991; Robinson et al. 1997; Rosenthal and Snyder 1985, 1987; Rozen et al. 1984; Stoner et al. 1981; Ward et al. 1985) and by oral administration for acute and intermediate durations (Fan 1992; Hsieh et al. 1988, 1990, 1991; Karaulov et al. 2017). Immune system effects are supported by results of hematology studies; immunosuppression is a secondary effect of hematological effects (decreased circulating immune cells).

Table C-19. Hazard Identification Conclusions for Benzene

Outcome	Hazard identification
Hematological effects	Known
Immunological effects	Presumed

APPENDIX D. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page D-5)

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), food intake (FI), gross necropsy (GN), hematology (HE), histopathology (HP), immune function (IX), lethality (LE), neurological function (NX), organ function (OF), ophthalmology (OP), organ weight (OW), reproductive function (RX), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

FIGURE LEGEND

See Sample LSE Figure (page D-6)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(12) Exposure period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the chronic exposure period are illustrated.

(13) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

(14) Levels of exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(15) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(16) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

(17) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral (1)

Figure key[a] (3)	Species (strain) No./group (4)	Exposure parameters (5)	Doses (mg/kg/day) (5)	Parameters monitored (6)	Endpoint (7)	NOAEL (mg/kg/day) (8)	Less serious LOAEL (mg/kg/day) (9)	Serious LOAEL (mg/kg/day) (9)	Effect
CHRONIC EXPOSURE (2)									
51	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4	CS, WI, BW, OW, HE, BC, HP	Bd wt	25.5	138.0		Decreased body weight gain in males (23–25%) and females (31–39%)
					Hemato	138.0			
					Hepatic		6.1[c]		Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure
Aida et al. 1992 (10)									
52	Rat (F344) 78 M	104 weeks (W)	0, 3.9, 20.6, 36.3	CS, BW, FI, BC, OW, HP	Hepatic	36.3			
					Renal	20.6	36.3		Increased incidence of renal tubular cell hyperplasia
					Endocr	36.3			
George et al. 2002									
59	Rat (Wistar) 58M, 58F	Lifetime (W)	M: 0, 90 F: 0, 190	BW, HP	Cancer		190 F		Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided
Tumasonis et al. 1985									

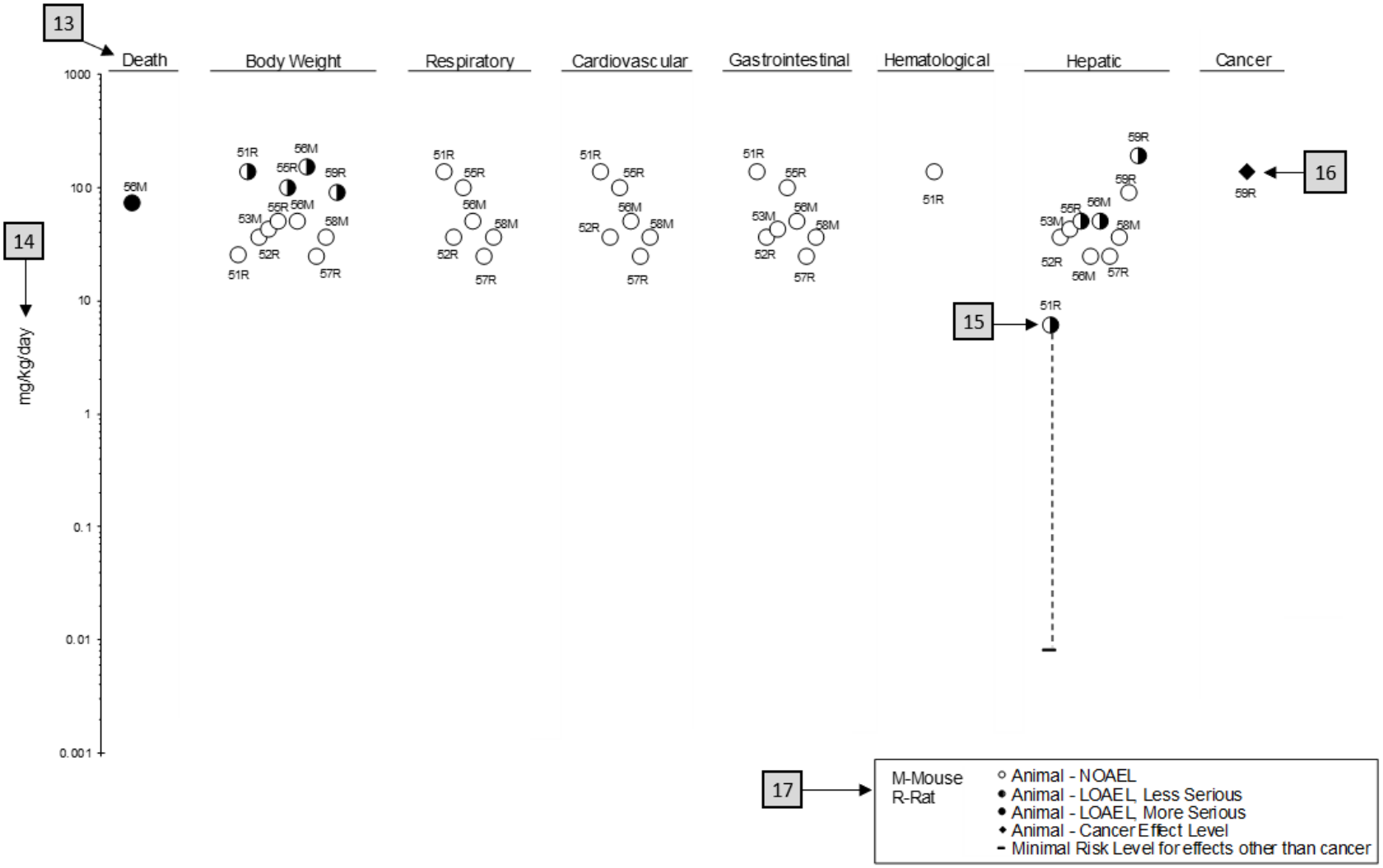
[a]The number corresponds to entries in Figure 2-x.

(11) [b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral

12 → Chronic (≥365 days)



APPENDIX E. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Relevance to Public Health: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

Chapter 2: Health Effects: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting.

Pediatrics:
Section 3.2 Children and Other Populations that are Unusually Susceptible
Section 3.3 Biomarkers of Exposure and Effect

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

ATSDR develops educational and informational materials for health care providers categorized by hazardous substance, clinical condition, and/or by susceptible population. The following additional materials are available online:

Clinician Briefs and Overviews discuss health effects and approaches to patient management in a brief/factsheet style. They are narrated PowerPoint presentations with Continuing Education credit available (see https://www.atsdr.cdc.gov/emes/health_professionals/clinician-briefs-overviews.html).

Managing Hazardous Materials Incidents is a set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.html).

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 400 7th Street, S.W., Suite 5W, Washington, DC 20024 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

APPENDIX F. GLOSSARY

Absorption—The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD) or Benchmark Concentration (BMC)—is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a BMD_{10} would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

Cancer Effect Level (CEL)—The lowest dose of a chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or malignant tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

Clastogen—A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

Epidemiology—The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Excretion—The process by which metabolic waste products are removed from the body.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

Health Advisory—An estimate of acceptable drinking water levels for a chemical substance derived by EPA and based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunotoxicity—Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Metabolism—Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

Minimal LOAEL—Indicates a minimal adverse effect or a reduced capacity of an organ or system to absorb additional toxic stress that does not necessarily lead to the inability of the organ or system to function normally.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

Mortality—Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

No-Observed-Adverse-Effect Level (NOAEL)—The exposure level of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Although effects may be produced at this exposure level, they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) ≥1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio/Relative Risk—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Serious LOAEL—A dose that evokes failure in a biological system and can lead to morbidity or mortality.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxicokinetic—The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

Toxics Release Inventory (TRI)—The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX G. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AAPCC	American Association of Poison Control Centers
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ACMT	American College of Medical Toxicology
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Level
AIC	Akaike's information criterion
AIHA	American Industrial Hygiene Association
ALT	alanine aminotransferase
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BCF	bioconcentration factor
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
C	centigrade
CAA	Clean Air Act
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
cm	centimeter
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DWEL	drinking water exposure level
EAFUS	Everything Added to Food in the United States
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
ERPG	emergency response planning guidelines
F	Fahrenheit
F1	first-filial generation
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	Federal Register

FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GGT	γ-glutamyl transferase
GRAS	generally recognized as safe
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substances Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Level of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
MRL	Minimal Risk Level
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
Mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NCEH	National Center for Environmental Health
ND	not detected
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences

NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEHSU	Pediatric Environmental Health Specialty Unit
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
pg	picogram
PND	postnatal day
POD	point of departure
ppb	parts per billion
ppbv	parts per billion by volume
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure limit
REL-C	recommended exposure limit-ceiling value
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification
SLOAEL	serious lowest-observed-adverse-effect level
SMR	standardized mortality ratio
sRBC	sheep red blood cell
STEL	short term exposure limit
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey

USNRC	U.S. Nuclear Regulatory Commission
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result