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.

APPENDIX A

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 ***Immunological:*** decreased resistance to bacterial infection	Rosenthal and Snyder 1985
		10	30		
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.

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

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.

APPENDIX A

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

		NOAEL/LOAEL (ppm)			
Species (number)	Duration	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) x 8 hours/24 hours x 6 days/7 days
$LOAEL_{ADJ}$ = 0.16 ppm

Uncertainty Factor: 100

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

Provisional chronic-duration inhalation MRL = $LOAEL_{ADJ}$ (0.16 ppm) ÷ total UF (100)
= 0.0016 ppm ≈ 0.002 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 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:

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

Provisional intermediate-duration oral MRL = $NOAEL_{ADJ}$ ÷ UFs
= 0.09 mg/kg/day ÷ (10x10)
= 0.0009 mg/kg/day ($9x10^{-4}$ mg/kg/day)

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.

APPENDIX A

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).

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

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.

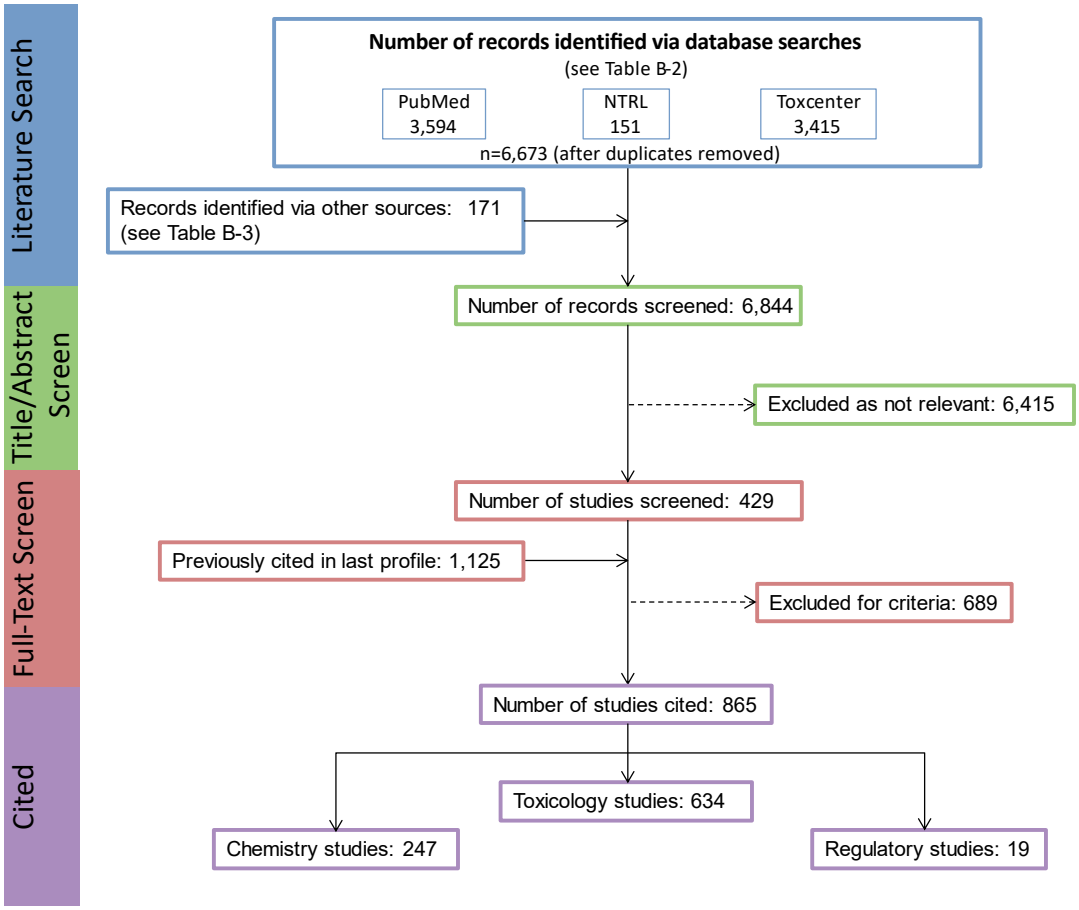
- Number of titles and abstracts screened: 6,844
- Number of studies considered relevant and moved to the next step: 429

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

APPENDIX C

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

Reference	Risk of bias criteria and ratings						
	Selection bias	Confounding bias	Attrition / exclusion bias	Detection bias		Selective reporting bias	
	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

APPENDIX C

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

APPENDIX C

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

APPENDIX C

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?	
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				Initial study confidence
	Concurrent control group	Sufficient number of animals per group	Appropriate parameters to assess potential effect	Adequate data for statistical analysis	
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	Key feature: Sufficient number of animals per group	Key feature: Appropriate parameters to assess potential effect	Key feature: 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	High
Fan 1992	Moderate	
Heijne et al. 2005	High	
Hsieh et al. 1988	High	
Hsieh et al. 1990	High	
NTP 1986); rat	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	High
NTP 1986; mouse	High	
Maltoni et al. 1983, 1985	High	
Outcome: Immunological Effects		
Inhalation acute information		
Animal studies		
Aoyama 1986; 7 days	High	High
Aoyama 1986; 14 days	High	
Dempster and Snyder 1991	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

	Confidence in body of evidence	
Outcome	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:

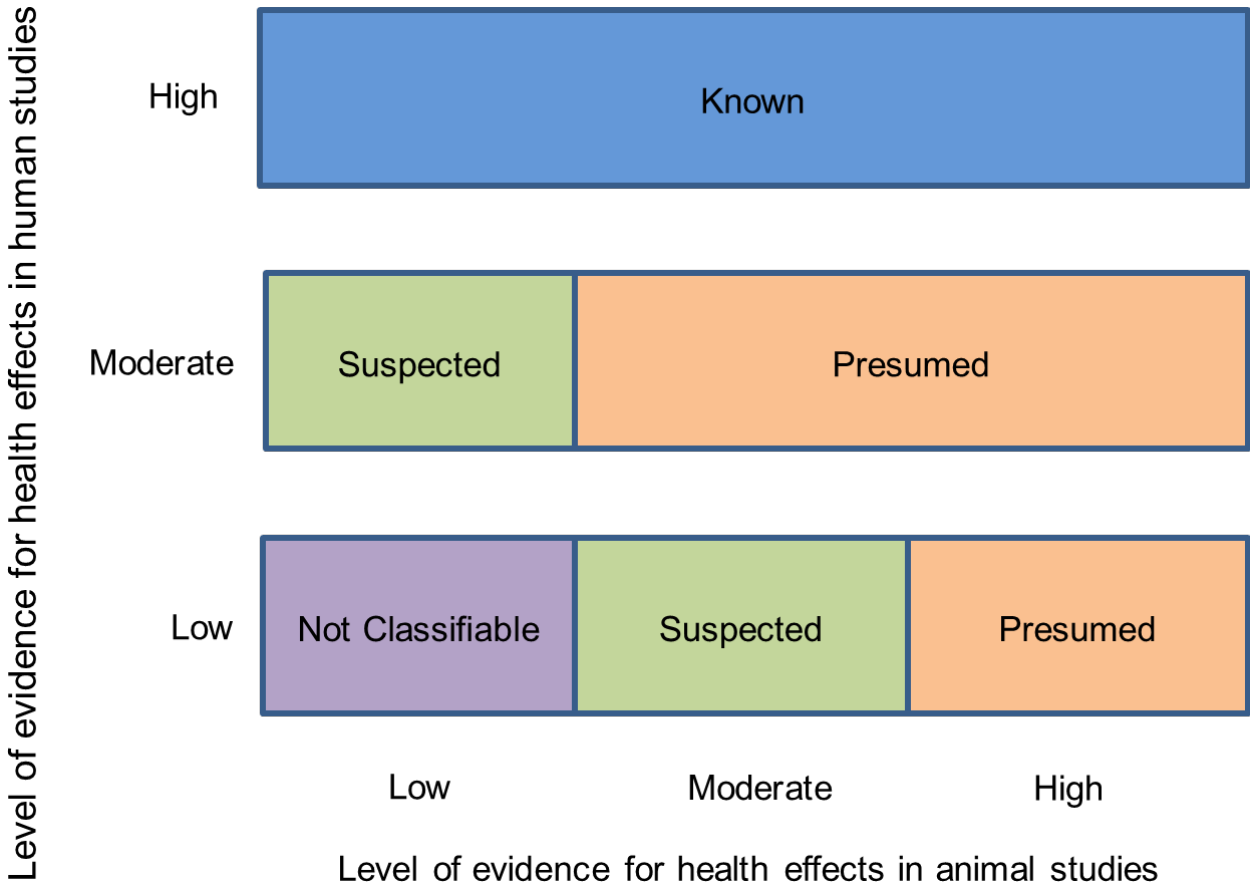
- **Known** to be a hazard to humans
- **Presumed** to be a hazard to humans
- **Suspected** to be a hazard to humans
- **Not classifiable** as to the hazard to humans

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)

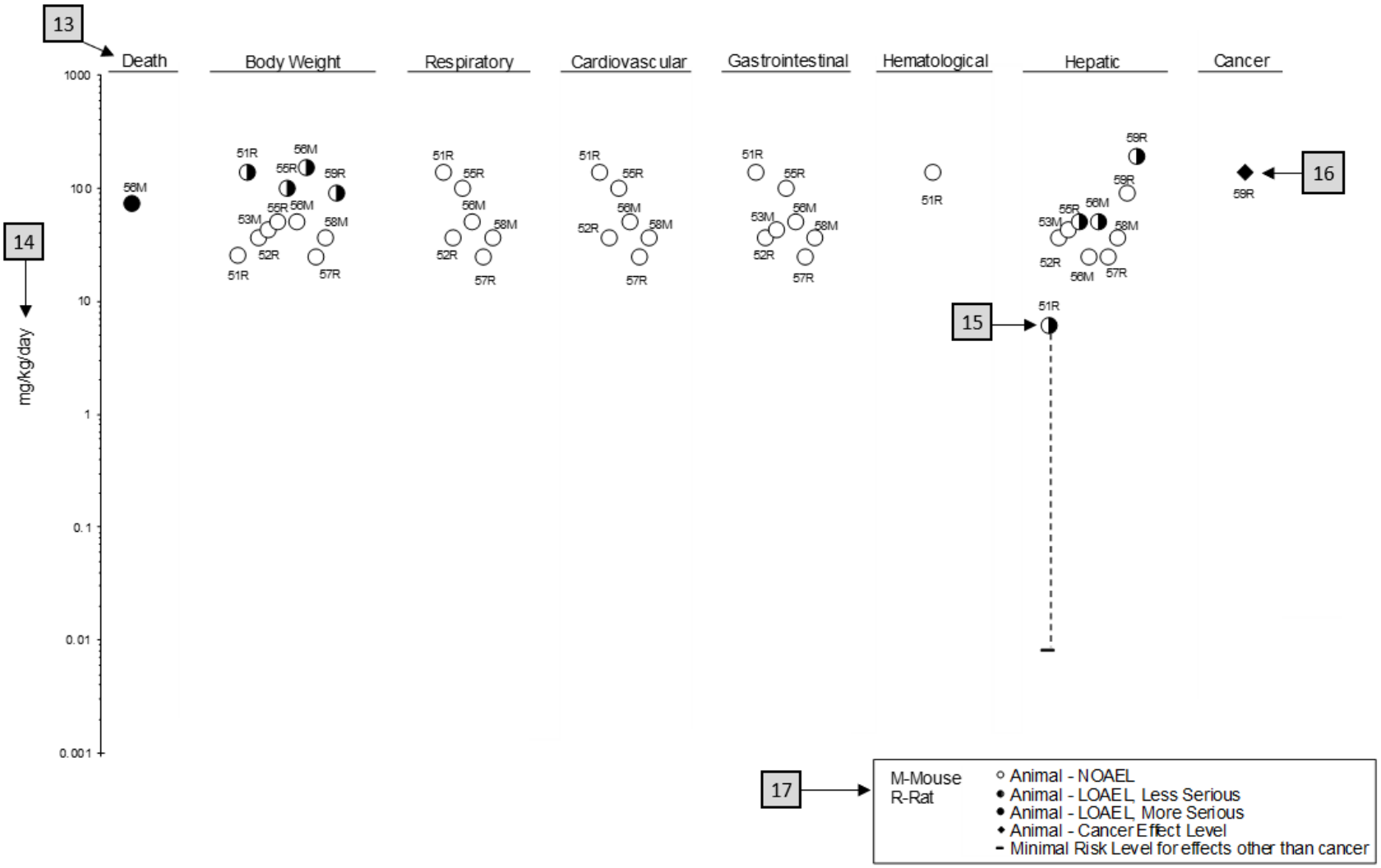
Figure key[a] (3)	Species (strain) No./group (4)	Exposure parameters (5)	Doses (mg/kg/day) (5)	Parameters monitored (6)	Endpoint (7)	NOAEL (mg/kg/day) (8)	Less serious LOAEL (mg/kg/day) (9)	Serious LOAEL (mg/kg/day) (9)	Effect
CHRONIC EXPOSURE (2)									
51	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 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



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