Toxicological Profile for Nitrobenzene

January 2024

U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

CS274127-A

DISCLAIMER

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FOREWORD

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

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

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

Each profile includes the following:

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

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

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

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

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

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*Legislative Background

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

VERSION HISTORY

Date	Description
January 2024	Final toxicological profile released
April 2022	Draft for public comment toxicological profile released
December 1990	Final toxicological profile released

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

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

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CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

1.1 OVERVIEW AND U.S. EXPOSURES

Nitrobenzene ($C_6H_5NO_2$; Chemical Abstracts Service Registry Number [CASRN] 98-95-3) is a synthetic chemical mainly used to produce aniline, quinolone antibiotics, azobenzene, and trinitrotoluene to make explosives, rubbers, pesticides, herbicides, insecticides, pharmaceuticals, and dyes (Dai et al. 2010b; Dong et al. 2010). Nitrobenzene is also used to manufacture cellulose ethers and acetates, dinitrobenzene, dichloroaniline, and acetaminophen (Dasgupta et al. 2018). It is also used as a solvent for petroleum refining, coating materials, and dyes (Dai et al. 2010a; Dasgupta et al. 2018). Nitrobenzene does not occur naturally.

Human exposure to nitrobenzene results from releases to air and wastewater from industrial sources. The general public may be exposed to nitrobenzene from inhalation of ambient air and possibly in drinking water. Occupational exposure occurs from both dermal and inhalation routes. Nitrobenzene has been detected in surface waters and effluents from both wastewater treatment plants and industrial sources (Gatermann et al. 1995; Li et al. 2010; Staples et al. 1985). Nitrobenzene has been detected infrequently in public water supplies but has been detected in groundwater. Nitrobenzene is detected infrequently in soils and sediments (Harkov et al. 1985; LaRegina et al. 1986; Nelson and Hites 1980).

When released to the environment, nitrobenzene has the potential to volatilize from water and soil surfaces. Based on measured soil adsorption coefficients, nitrobenzene possesses low to moderate adsorption to soil and may leach into groundwater. Nitrobenzene is susceptible to direct photolysis in both air and surface water (Bao et al. 2012). It is expected to undergo biodegradation in soil and water under both aerobic and anaerobic conditions (Piwoni et al. 1986). Other abiotic degradation mechanisms, such as hydrolysis, are not expected to be important environmental fate processes. Based upon experimental bioconcentration studies, nitrobenzene is not expected to bioconcentrate in aquatic organisms.

Biomonitoring data of nitrobenzene blood levels in the U.S general population were generally below the detection limits (CDC 2021b). Biomonitoring can also be performed for nitrobenzene in urine; however, this will only reflect recent exposures. p-Nitrophenol and p-aminophenol are two metabolites of nitrobenzene that may be present in urine following exposure to nitrobenzene. However, these metabolites are not specific to nitrobenzene. p-Nitrophenol is also a common metabolite of

organophosphate insecticides such as parathion, methyl parathion, and O-ethyl-O-(4-nitrophenyl) phenylphosphonothioate (EPN) (Chang et al. 1993; Kao et al. 1978; McCarthy et al. 1985; Parke 1956; Robinson et al. 1951).

1.2 SUMMARY OF HEALTH EFFECTS

There are a limited number of epidemiological studies on the health effects of nitrobenzene exposure in humans. There are many case studies in humans exposed by intentional ingestion with suicidal intent. There are studies of animals exposed by inhalation, oral, and dermal routes in which comprehensive endpoints were evaluated. As illustrated in Figures 1-1 and 1-2, the most sensitive noncancer effects of nitrobenzene are hematological, respiratory, hepatic, renal, endocrine, and reproductive.

Hematological Effects. In human case studies of both inhalation and ingestion exposure to nitrobenzene, methemoglobinemia was the most common finding (Agrawal et al. 2011; Gupta et al. 2012; Ikeda and Kita 1964; Lee et al. 2013; Perera et al. 2009; Saxena and Prakash Saxena 2010). Methemoglobinemia is a condition in which the iron in hemoglobin is oxidized, disrupting the ability of hemoglobin to bind oxygen and leading to reduced oxygen delivery to tissues. Additionally, experimental animal studies have demonstrated increased methemoglobin levels in mice and rats of both sexes exposed through any exposure route with acute, intermediate, and chronic (≥365 days) exposure durations (Biodynamics 1984; Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983b). A variety of related adverse effects in the hematologic system, including hemolytic anemia, extramedullary hematopoiesis, hemosiderosis, congestion and lymphoid depletion of the spleen, and changes in bone marrow in response to anemia have also been observed with inhalation, oral, and dermal exposures in B6C3F1 mice, F344 rats, and Sprague-Dawley rats (Burns et al. 1994; Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b).

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

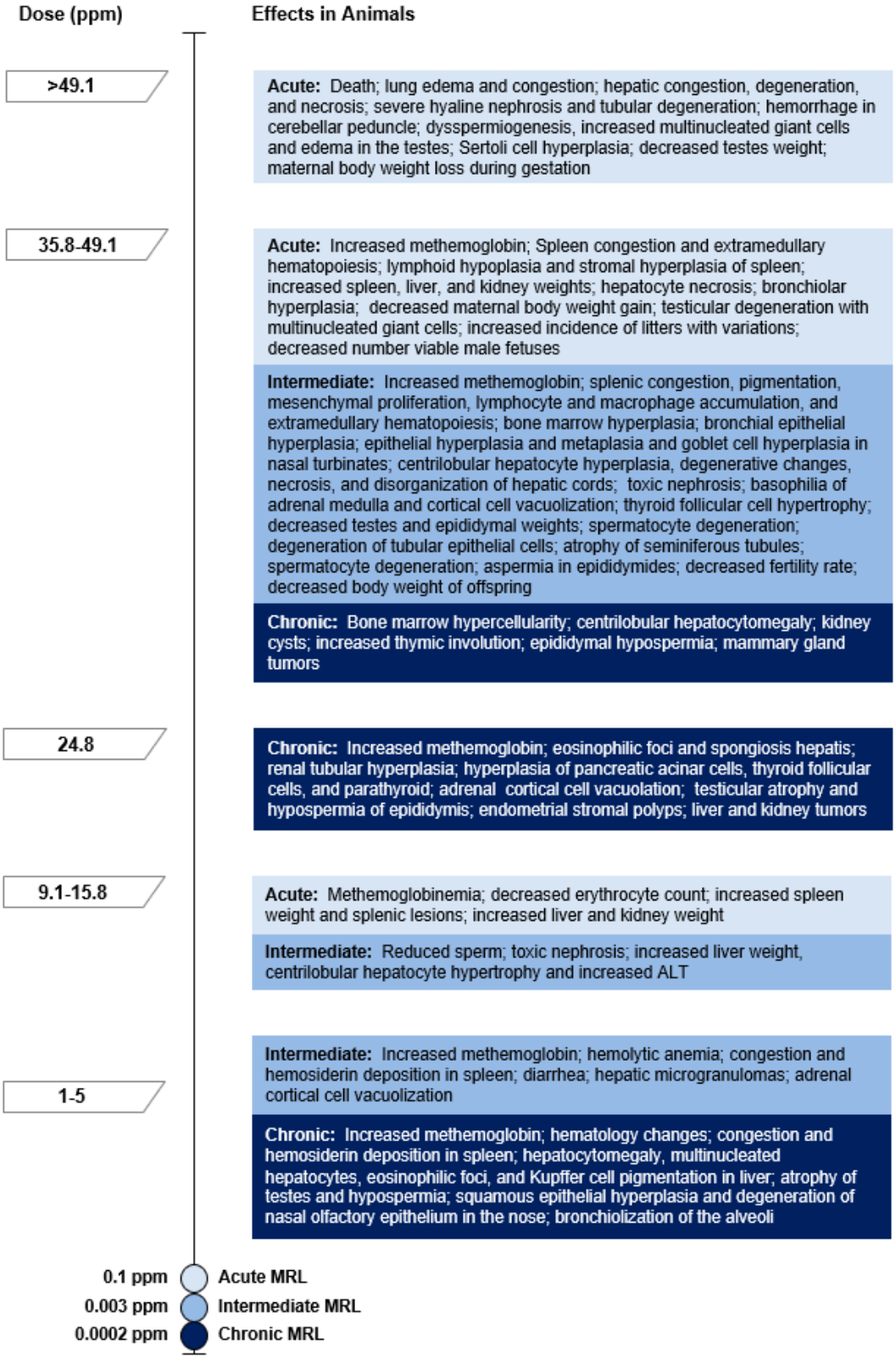
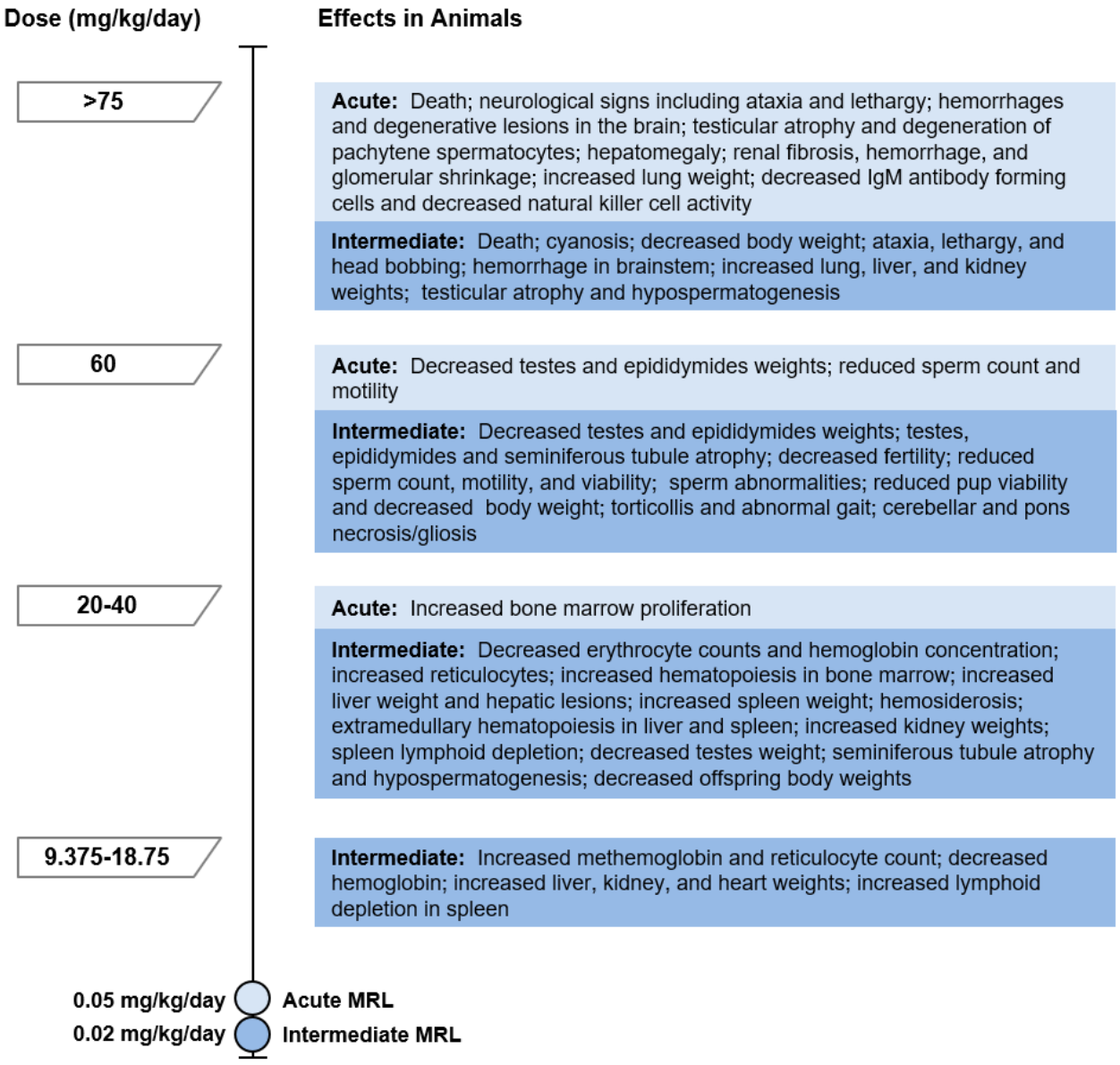


Figure 1-2. Health Effects Found in Animals Following Oral Exposure to Nitrobenzene



Respiratory Effects. Results from inhalation studies indicate effects of nitrobenzene exposure on the nasal passages of rats and mice and the lungs of mice. In chronic-duration studies, mice had degeneration of the nasal olfactory epithelium and glandularization of respiratory epithelium and rats had squamous epithelial hyperplasia, pigment deposition in the olfactory epithelium, and inflammatory changes (Cattley et al. 1994, 1995; CIIT 1993). In the same study, increases in bronchiolization of the alveoli and alveolar/bronchiolar hyperplasia were observed in mice (Cattley et al. 1994, 1995; CIIT 1993). Acute- and intermediate-duration dermal exposure studies have demonstrated lung congestion after nitrobenzene exposure in F344 rats (NTP 1982).

Hepatic Effects. Two human case studies (Gupta et al. 2012; Ikeda and Kita 1964) reported hepatic effects evidenced by an increase in the retention of bromosulphthalein (BSP), a dye used in liver function tests, and an increase in icterus index (i.e., jaundice) and indirect bilirubin levels (Ikeda and Kita 1964), and pathological observations of hepatic centrilobular necrosis (Gupta et al. 2012). There are several experimental animal studies that reported adverse liver effects (Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b). Experimental animal studies with inhalation and oral exposures displayed a range of adverse liver effects, with the most common effects being necrosis and hepatomegaly in the centrilobular region (Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; NTP 1983a).

Renal Effects. The kidney also appears to be a target for nitrobenzene toxicity. In one case study, renal tubular necrosis was seen following the death of the subject who ingested nitrobenzene (Gupta et al. 2012). Several experimental animal studies have demonstrated increases in kidney weight, hemosiderin deposition, increased nephrosis, congestion, and degenerative changes or hyperplasia in the cortical tubules (Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b).

Endocrine Effects. Adrenal effects have been observed in rats and mice exposed to nitrobenzene. In female mice exposed by inhalation, oral, and dermal routes, cortical cell vacuolization and fatty changes were seen (Hamm et al. 1984; Cattley et al. 1994, 1995; CIIT 1993; NTP 1983a, 1983b). F344 and CD rats exposed by inhalation exhibited increased basophilia of the adrenal medullary cells (Hamm et al. 1984). Some studies have also reported effects on the thyroid gland in rats and mice (Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Oladele et al. 2020a) and on the parathyroid glands in rats (Cattley et al. 1994, 1995; CIIT 1993).

Reproductive Effects. Nitrobenzene is a known male reproductive toxicant and has been used as a positive control in animal studies evaluating effects on spermatogenesis (Allenby et al. 1990, 1991; Linder et al. 1992). Common effects seen after exposure to nitrobenzene include decreases in testes weight, atrophy of the seminiferous tubules, hypospermatogenesis, Sertoli cell hyperplasia, and dysfunctional spermiogenesis. These effects have been demonstrated in a variety of rodent species after acute-, intermediate-, and chronic-duration exposure via all exposure routes (Cattley et al. 1994, 1995; CIIT 1993; Dodd et al. 1987; Hamm et al. 1984; Iida et al. 1997; Kato et al. 2002; Kawaguchi et al. 2004; Kawashima et al. 1995; Levin et al. 1988; Linder et al. 1992; McLaren et al. 1993a; Medinsky and Irons 1985; Mitsumori et al. 1994; Oladele et al. 2020c; NTP 1982, 1983a, 1983b; Shinoda et al. 1998). The effects on the male reproductive system observed in intermediate-duration inhalation and oral studies of Sprague-Dawley rats included decreased fertility indices (Dodd et al. 1987; Kawashima et al. 1995).

Cancer. There are no reliable human data pertaining to the carcinogenicity of nitrobenzene. The carcinogenicity of nitrobenzene was evaluated in a 2-year inhalation study of rats and mice. Exposure to nitrobenzene resulted in increased incidences of hepatocellular adenomas or carcinomas in male F344 and CD rats; renal tubular adenomas or carcinomas in male F344 rats; lung alveolar/bronchiolar adenomas or carcinomas and thyroid follicular cell adenomas in male mice; and mammary gland adenocarcinomas in female mice. The U.S. Environmental Protection Agency (EPA) has deemed nitrobenzene to be "likely to be carcinogenic to humans" by any route of exposure (EPA 2009a). The Department of Health and Human Services (HHS) of the National Toxicology Program (NTP) has determined that nitrobenzene is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in studies of animals (NTP 2021). The International Agency for Research on Cancer (IARC) concluded nitrobenzene is possibly carcinogenic in humans (Group 2B) based on inadequate evidence in humans and sufficient evidence in animals (IARC 1996, 2019).

1.3 MINIMAL RISK LEVELS (MRLs)

Available information on the toxicity of inhaled nitrobenzene was considered adequate data for derivation of acute-, intermediate-, and chronic-duration inhalation MRLs. As illustrated in Figure 1-3, hematological, hepatic, renal, respiratory, and endocrine effects appear to be the most sensitive targets of nitrobenzene inhalation. Data on effects of oral exposure to nitrobenzene were considered adequate to derive acute- and intermediate-duration oral MRLs, but the absence of chronic-duration oral studies precludes derivation of a chronic-duration oral MRL. Hematological, hepatic, renal, and reproductive effects appear to be the most sensitive targets of ingested nitrobenzene (Figure 1-4).

The MRL values are summarized in Table 1-1 and discussed in greater detail in Appendix A.

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

The hematological, hepatic, renal, respiratory, and endocrine endpoints are the most sensitive targets of nitrobenzene inhalation exposure.
Numbers in circles are the lowest LOAELs among health effects in animals.

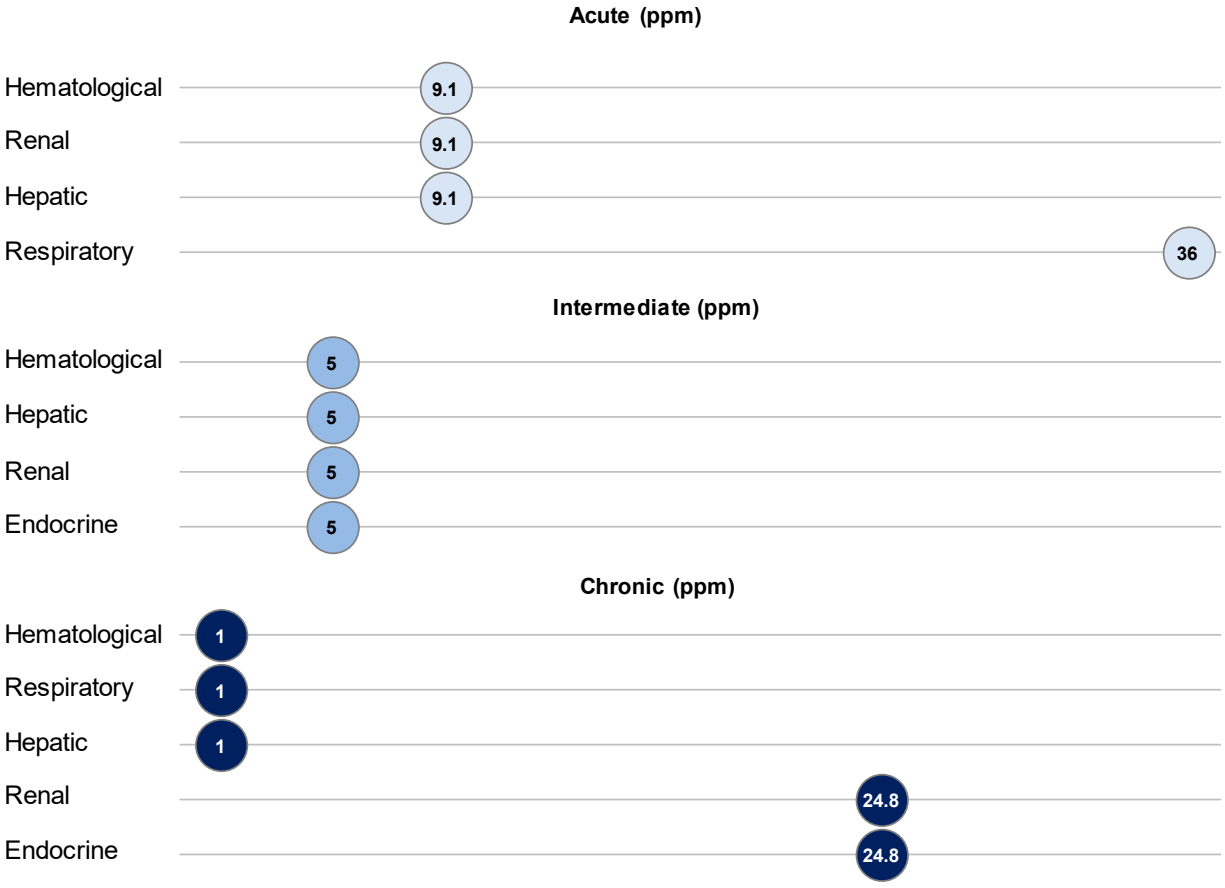


Figure 1-4. Summary of Sensitive Targets of Nitrobenzene – Oral

The hematological, hepatic, renal, and reproductive endpoints are the most sensitive targets of nitrobenzene oral exposure.
Numbers in circles are the lowest LOAELs among health effects in animals.

Table 1-1. Minimal Risk Levels (MRLs) for Nitrobenzene

Exposure route	Exposure duration	MRL	Critical effect	POD type	POD value	Uncertainty/ modifying factor	Reference
Inhalation	Acute	**0.1 ppm** (0.5 mg/m^3)	Increased methemoglobin	$BMCL_{HEC}$	2.91 ppm	UF: 30	Medinsky and Irons 1985
	Intermediate	**0.003 ppm** (0.02 mg/m^3)	Hematological, renal, hepatic, and endocrine effects	$LOAEL_{HEC}$	0.89 ppm	UF: 300	Hamm et al. 1984
	Chronic	**0.0002 ppm** (0.001 mg/m^3)	Hyperplasia of the nasal squamous epithelium	$LOAEL_{HEC}$	0.054 ppm	UF: 300	Cattley et al. 1994, 1995; CIIT 1993
Oral	Acute	**0.05 mg/kg/day**	Proliferative changes in the bone marrow	$BMDL_{1SD}$	4.7 mg/kg/day	UF: 100	Burns et al. 1994
	Intermediate	**0.02 mg/kg/day**	Increased methemoglobin	$BMDL_{1SD}$	1.8 mg/kg/day	UF: 100	NTP 1983a
	Chronic	None	–	–	–	–	–

[a]See Appendix A for additional information.

BMCL = lower confidence limit on the benchmark concentration; BMDL = lower confidence limit on the benchmark dose; HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; POD = point of departure; SD = standard deviation; UF = uncertainty factors

CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of nitrobenzene. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile. To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to nitrobenzene, but may not be inclusive of the entire body of literature. Animal inhalation studies are presented in Table 2-1 and Figure 2-2, animal oral studies are presented in Table 2-2 and Figure 2-3; animal dermal studies are presented in Table 2-3.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the

Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of nitrobenzene are indicated in Table 2-2 and Figure 2-3.

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

As illustrated in Figure 2-1, most of the health effects data for nitrobenzene comes from experimental animal studies. Even though there were more than 30 human studies, the majority of these were case studies. There were studies of comprehensive noncancer endpoints in animals exposed by inhalation and oral routes, and cancer was assessed in animals exposed by inhalation. The effects examined in most studies included reproductive, body weight, hematology, hepatic, renal, and neurological endpoints.

The human and animal studies suggest that the hematological, respiratory, hepatic, renal, endocrine, and reproductive systems are the most sensitive targets of nitrobenzene’s toxicity.

- ***Hematological:*** Nitrobenzene has induced methemoglobinemia in humans and animals through all exposure routes. In addition, animal studies of all exposure routes and durations have shown related effects including hemolytic anemia, large increases in spleen weight, and histopathology changes in the spleen (congestion, extramedullary hematopoiesis, hemosiderosis, lymphoid depletion) and bone marrow (hypercellularity).

- ***Respiratory:*** Nitrobenzene exposure has been associated with histopathology changes in the nasal cavity of rats (hyperplasia, inflammatory changes, suppurative exudate) and mice (olfactory epithelial degeneration, glandularization of respiratory epithelium) and in the lungs (alveolar/ bronchiolar hyperplasia and alveolar bronchiolization) of mice exposed by inhalation for intermediate and chronic durations.

- ***Hepatic:*** The liver is a target for nitrobenzene toxicity as evidenced by experimental animal studies that reported increased liver weights, degenerative changes in hepatocytes, hepatocytomegaly, and centrilobular necrosis. Some histopathology changes observed in the

livers of exposed animals (e.g., extramedullary hematopoiesis and hemosiderosis) are attributable to the hematologic effects of nitrobenzene.

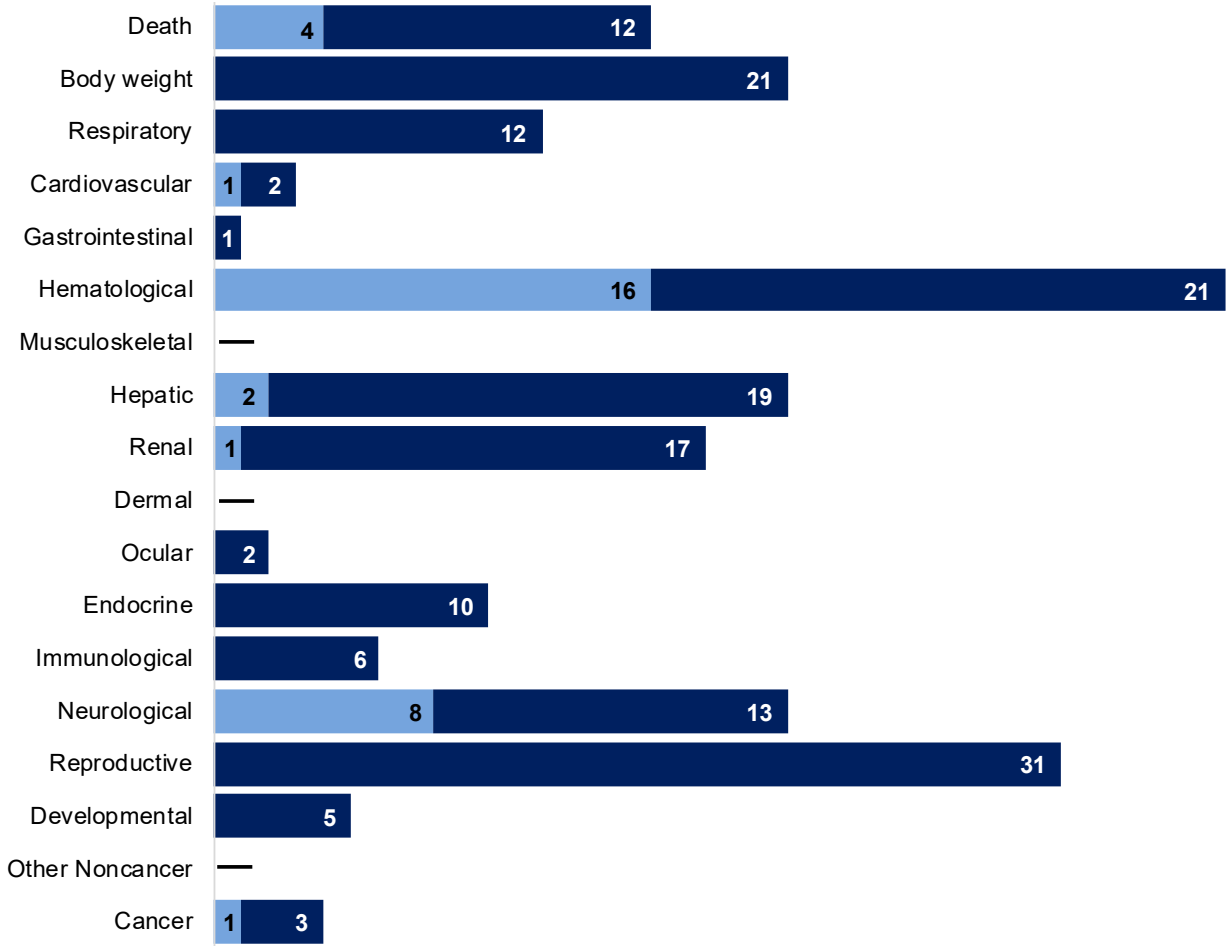
- ***Renal:*** Renal effects of nitrobenzene in animals include increases in kidney weight, increased incidence or severity of nephrosis, renal tubular hyperplasia, cysts, fibrosis, glomerular shrinkage, and degenerative changes in the cortical tubules.

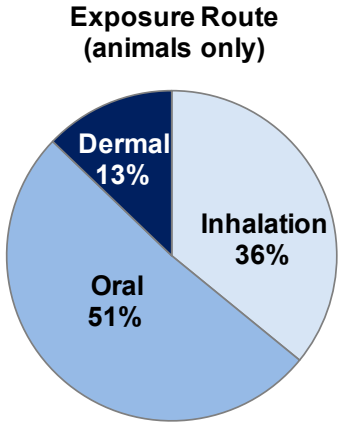
- ***Endocrine:*** Nitrobenzene effects on endocrine organs of laboratory rodents included increased incidences of adrenal cortical cell vacuolization and/or fatty changes in mice; increased basophilia of the adrenal medullary cells in rats; thyroid follicular cell hypertrophy, hyperplasia, and/or decreased serum thyroid-stimulating hormone (TSH) in rats and mice; and diffuse hyperplasia of the parathyroid glands in rats.

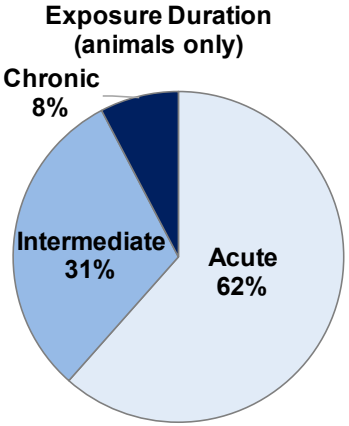
- ***Reproductive:*** Nitrobenzene is a known male reproductive toxicant and is often used as a positive control in studies of testicular toxicity. Rats and mice exposed to nitrobenzene via inhalation, oral, and dermal routes have exhibited decreases in testes and/or epididymal weights; atrophy of the testes and seminiferous tubules; hypospermatogenesis; Leydig cell hyperplasia; and dysfunctional spermiogenesis. Inhalation and oral studies have also shown that the effects on the male reproductive organs were associated with decreased fertility indices.

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

Most studies examined the potential reproductive, body weight, hematology, hepatic, renal, and neurological effects of nitrobenzene.
Fewer studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)







*Includes studies discussed in Chapter 2. A total of 68 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
1	Rat (F344) 10 M, 10 F	14 days, 5 days/week, 6 hours/day (WB)	0, 9.1, 35.8, 124.5	LE, CS, BC, HE, OW, GN, HP	Resp	124.5			
					Hemato		9.1		Splenic lesions (not specified); increased relative spleen weight (33%); at 35.8 ppm, effects included sinusoidal congestion, hemosiderosis, extramedullary hematopoiesis in spleen; and markedly increased relative spleen weight (89–111%)
					Hepatic	9.1 F	9.1 M 35.8 F		Increased relative liver weight (13% in males and 27% in females)
					Renal	9.1F	9.1 M 35.8 F	124.5	LOAEL: Increased relative kidney weight (15% in males and 23% in females) SLOAEL: Severe hyaline nephrosis
					Neuro	124.5			
					Repro	35.8 M		124.5 M	Severe dysfunctional spermiogenesis; increased multinucleated giant cells and interstitial edema in testes; Sertoli cell hyperplasia; 44% reduction in testes weight

Medinsky and Irons 1985

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
2	Rat (CD) 10 M, 10 F	5–14 days, 5 days/week, 6 hours/day (WB)	0, 9.1, 35.8, 124.5	LE, CS, BC, HE, OW, GN, HP	Death			124.5	5/10 male and 3/10 females found dead on 4th exposure day; remainder sacrificed moribund at end of week 1
					Resp		124.5		Perivascular edema and vascular congestion in lungs
					Hemato		9.1[b]		Decreased RBC count; increased relative spleen weight (44% in females); splenic lesions (not specified); increased methemoglobin in females (6.3 versus 4.8% in controls) ($BMCL_{1SD}$ = 16.3 ppm). At 35.8 ppm, effects included sinusoidal congestion, hemosiderosis and extramedullary hematopoiesis (both sexes); lymphoid hypoplasia and stromal hyperplasia (males) of spleen; markedly increased relative spleen weights (74–94%); and increased methemoglobin in males (8.7 versus 6.9% in controls).
					Hepatic	9.1	35.8		Mild hepatocyte necrosis; at 124.5 ppm, effects included sinusoidal congestion, basophilic and centrilobular hepatocyte degeneration, and hepatocyte necrosis

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Renal		124.5		Moderate to severe hydropic degeneration of cortical tubular cells
					Neuro	35.8		124.5	Perivascular hemorrhage in cerebellar peduncle; edema and malacia
					Repro			124.5 M	Moderate dysfunctional spermiogenesis
Medinsky and Irons 1985									
3	Rat (CD) 26 F	GDs 6–15, 6 hours/day (WB)	0, 1.06, 9.8, 39.4	LE, CS, BW, OW, GN, DX	Bd wt	9.8	39.4		Decreased maternal weight gain (19% compared to control during exposure)
					Hemato	1.06	9.8		Increased absolute and relative maternal spleen weights (13–15%)
					Develop	9.8	39.4		Increased incidences of litters with variations (hole in parietal bone and ecchymosis on trunk)
Tyl et al. 1987									
4	Mouse (B6C3F1) 10 M, 10 F	2-14 days, 5 days/week, 6 hours/day (WB)	0, 9.1, 35.8, 124.5	LE, CS, BC, HE, OW, GN, HP	Death			124.5	All mice were prostrate with bradypnea and dyspnea, and therefore sacrificed humanely between days 2 and 4 of exposure
					Resp		35.8		Mild bronchiolar hyperplasia
					Hemato		9.1		Splenic lesions (not specified); at 35.8 ppm effects included splenic sinusoidal congestion, extramedullary hematopoiesis, hemosiderosis, lymphoid hypoplasia, and stromal hypoplasia

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic		124.5 M		Centrilobular necrosis and severe hydropic degeneration
					Neuro	35.8		124.5	Perivascular hemorrhage in the cerebellar peduncle
					Repro		35.8 M	124.5 M	LOAEL: Testicular degeneration and increased multinucleated giant cells SLOAEL: Absence of spermatozoa in seminiferous tubules; degeneration of tubular epithelial cells; spermatocyte maturation arrest
Medinsky and Irons 1985									
5	Rabbit (New Zealand) 12 F	GDs 7–19, 6 hours/day (WB)	0, 10, 40, 81	LE, CS, BW, HE, OW, GN, DX	Bd wt	81			
					Hemato	10	40		Increased maternal methemoglobin (1.7 versus 1.0% in controls)
					Develop	81			No effect on numbers of corpora lutea, implantations, resorptions, or fetuses
Biodynamics 1983									
6	Rabbit (New Zealand) 22 F	GDs 7–19, 6 hours/day (WB)	0, 9.9, 41, 104	LE, CS, BW, HE, OW, GN, DX	Bd wt	41		104	Maternal body weight loss during gestation (10 g loss from GD 7 to GD 19 versus gain of 34 g in controls)
					Hemato	9.9	41		Increased maternal methemoglobin (1.4 versus 1.0% in controls)
					Develop	9.9	41		Decreased mean number viable male fetuses

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Biodynamics 1984									
INTERMEDIATE EXPOSURE									
7	Rat (CD) 120 M, 120 F	10 weeks (2 generations), 5 days/week, 6 hours/day (WB)	0, 1, 10, 40	BW, LE, RX, GN, OW	Repro	10		40	Decreased fertility rate; atrophy of seminiferous tubules; spermatocyte degeneration; reduced testicular and epididymal weights
					Develop	10	40		12% decrease in mean body weight of F1 offspring on PND 21
Dodd et al. 1987									
8	Rat (Fischer-344) 10 M, 10 F	90 days, 5 days/week, 6 hours/day (WB)	0, 5, 15.8, 48.7	CS, HP, BW, BC, HE, GN, UR, OW, BI	Bd wt	48.7			
					Resp	15.8 M 48.7 F	48.7 M		Minimal to slight hyperplasia of the bronchial epithelium in males
					Gastro	48.7 M	5 F		Diarrhea in females
					Hemato		5[c]		

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
									Increased methemoglobin in males (3.0 versus 1.2% in controls); hematology changes indicative of hemolytic anemia in females; minimal to slight acute sinusoidal congestion and moderate to marked hemosiderin deposition in spleen in both sexes. At 48.7 ppm, effects included moderate sinusoidal congestion, proliferation of mesenchymal cells, fibroblastic hyperplasia of the splenic capsule, accumulation of lymphocytes and macrophages, and extramedullary hematopoiesis in spleen; and bone marrow hyperplasia.
					Hepatic		15.8	48.7	LOAEL: Increased liver weights SLOAEL: Disorganized hepatic cords, vascular ectasia, centrilobular hepatocyte degeneration, and periportal hepatocyte basophilia (males), focal necrosis (females)
					Renal	15.8 F	5 M 48.7 F		Minimal nephrosis in males, slight to moderate nephrosis in females
					Endocr	15.8	48.7		Increased basophilia of medullary cells in adrenal glands

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	15.8 M		48.7 M	Moderate to severe degeneration of tubular epithelial cells, absence of mature sperm in epididymis; slight to moderate interstitial edema in testes; minimal to slight interstitial cell hyperplasia in testes; 33% decrease in testes weight
Hamm et al. 1984									
9	Rat (CD) 10 M, 10 F	90 days, 5 days/week, 6 hours/day (WB)	0, 5, 15.8, 48.7	CS, HP, BW, BC, HE, GN, UR, OW, BI	Bd wt	48.7			
					Resp	15.8 M 48.7 F	48.7 M		Epithelial hyperplasia/metaplasia and goblet cell hyperplasia in nasal turbinates of males
					Hemato		5		Moderate hemosiderin pigmentation (males) and slight to moderate sinusoidal congestion (both sexes) in spleen
					Hepatic	5 F	5 M 15.8 F		Microgranulomas in males; centrilobular hepatocyte hypertrophy, increased liver weight, and 4-fold increase in serum ALT in females
					Renal	5 M 48.7 F	15.8 M	48.7 M	LOAEL: Minimal to slight toxic nephrosis in males SLOAEL: Moderate to marked toxic nephrosis in males
					Endocr	15.8 M 48.7 F	48.7 M		Basophilia of adrenal medullary cells and slight thyroid follicular cell hypertrophy in males

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	5 M	15.8 M	48.7 M	LOAEL: slight reduction in mature sperm in two animals SLOAEL: Bilateral testicular atrophy, complete loss of seminiferous epithelium, and absence of mature sperm in lumen of epididymis in all animals; increased interstitial cell hyperplasia, interstitial testicular atrophy, and multinucleate giant cells; 55% reduction in testes weight
Hamm et al. 1984									
10	Mouse (B6C3F1) 10 M, 10 F	90 days, 5 days/week, 6 hours/day (WB)	0, 5, 15.8, 48.7	CS, BW, BC, HE, UR, OW, GN, HP	Bd wt	48.7			
					Resp	15.8	48.7		Minimal to slight hyperplasia of the bronchial mucosa
					Hemato		5		Minimal to slight hemosiderin deposition (both sexes) and slight to moderate sinusoidal congestion (females) in spleen; at 48.7 ppm, effects included 45–66% increase in spleen weights and bone marrow hyperplasia
					Hepatic	15.8	48.7		Centrilobular hepatocyte hyperplasia with some cord disorganization (females) and basophilic hepatocytes (males); increased liver weight; increased ALT (males)
					Renal	48.7			
					Endocr	48.7 M	5 F	48.7 F	

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
									LOAEL: Minimal to slight cortical cell vacuolization in zona reticularis of adrenal glands in females SLOAEL: Marked to very severe cortical cell vacuolization in adrenal glands of females
					Repro	48.7			No significant histopathology changes in reproductive organs
Hamm et al. 1984									
CHRONIC EXPOSURE									
11	Rat (Fischer-344) 70 M, 70 F	2 years, 5 days/week, 6 hours/day (WB)	0, 1, 5, 24.8	LE, CS, BW, HE, GN, HP	Bd wt	24.8			
					Resp		1		Increased pigmented olfactory epithelium in nose
					Gastro	5 M 24.8 F	24.8 M		Increased focal pancreatic acinar cell hyperplasia in males
					Hemato		1		Increased spleen congestion and pigmentation in the spleen
					Hepatic	1 M 5 F	5 M 24.8 F		Increased eosinophilic foci and centrilobular hepatocytomegaly (males) and eosinophilic foci and spongiosis hepatis (females)
					Renal	5 M 24.8 F	24.8 M		Increased renal tubular hyperplasia in males
					Endocr	5 M 24.8 F	24.8 M		Increased diffuse hyperplasia of the parathyroid glands in males
					Repro	24.8			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Cancer			24.8	CEL: Increased incidences of endometrial stromal polyps in the uterus (females), hepatocellular adenoma or carcinoma (males), and renal tubular adenoma or carcinoma (males)
Cattley et al. 1994, 1995; CIIT 1993									
12	Rat (CD) 70 M	2 years, 5 days/week, 6 hours/day (WB)	0, 1, 5, 24.8	LE, CS, BW, HE, GN, HP	Bd wt	24.8			
					Resp		1[d]		Increased squamous epithelial hyperplasia in nose
					Hemato		1		Increased methemoglobin at 15-month sacrifice (4.08 versus 1.18% in controls); increased splenic congestion
					Hepatic		1		Increased Kupffer cell pigmentation
					Renal	24.8			
					Endocr	24.8			
					Repro	5		24.8	Increased bilateral atrophy of the testes and bilateral hypospermia in the epididymis
					Cancer			24.8	CEL: Increased incidences of hepatocellular adenoma and adenoma or carcinoma
Cattley et al. 1994, 1995; CIIT 1993									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
13	Mouse (B6C3F1) 70 M, 70 F	2 years, 5 days/week, 6 hours/day (WB)	0, 5, 24.8, 49.1	LE, CS, BW, HE, GN, HP	Bd wt	49.1			
					Resp		5		Bronchiolization of the alveoli and pigment deposition in olfactory epithelium (both sexes); degeneration of nasal olfactory epithelium in females
					Hemato	5 F 24.8 M	24.8 F 49.1 M		Increased methemoglobin in males (3.97 versus 1.97% in controls) and females (2.22 versus 1.39% in controls); increased bone marrow hypercellularity (males)
					Hepatic	24.8 F	5 M 49.1 F		Increased centrilobular hepatocytomegaly and multinucleated hepatocytes in males and centrilobular hepatocytomegaly in females
					Renal	49.1 F 24.8 M	49.1 M		Increased kidney cysts in males
					Endocr	5	24.8		Increased thyroid follicular cell hyperplasia in males; increased adrenal gland cortical cell vacuolization in females
					Immuno	24.8 F 49.1 M	49.1 F		Increased thymic involution in females
					Repro	24.8 M		49.1 M	Increased hypospermia of the epididymis

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Cancer			5 M 49.1 F	CEL: lung adenoma or carcinoma (males); mammary gland adenocarcinoma (females)

Cattley et al. 1994, 1995; CIIT 1993

[a]The number corresponds to the entries in Figure 2-2; differences in levels of health effects and cancer between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an acute-duration inhalation MRL using benchmark dose analysis. The $BMCL_{1SD}$ of 16.3 ppm was adjusted to continuous exposure and converted to a $BMCL_{HEC}$ of 2.91 ppm. The $BMCL_{HEC}$ was divided by an uncertainty factor of 30 (10 for human variability and 3 for animal to human extrapolation after dosimetric adjustment), resulting in an MRL of 0.1 ppm.
[c]Used to derive an intermediate-duration inhalation MRL. The LOAEL of 5 ppm was adjusted to continuous exposure and converted to a $LOAEL_{HEC}$ of 0.89 ppm. The $LOAEL_{HEC}$ was divided by an uncertainty factor of 300 (10 for human variability, 3 for animal to human extrapolation after dosimetric adjustment, and 10 for use of a LOAEL), resulting in an MRL of 0.003 ppm.
[d]Used to derive a chronic-duration inhalation MRL. The LOAEL of 1 ppm was adjusted to continuous exposure and converted to a $LOAEL_{HEC}$ of 0.054 ppm. The $LOAEL_{HEC}$ was divided by an uncertainty factor of 300 (10 for human variability, 3 for animal to human extrapolation after dosimetric adjustment, and 10 for use of a LOAEL), resulting in an MRL of 0.0002 ppm.

ALT = alanine aminotransferase; BI = biochemical changes; BC = blood chemistry; Bd wt or BW = body weight; BMCL = benchmark concentration lower confidence limit; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); GN = gross necropsy; HE = hematology; HEC = human equivalent concentration; Hemato = hematological; HP = histopathological; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Neuro = neurological; NOAEL = no-observed-adverse-effect level; OW = organ weight; PND = postnatal day; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; SD = standard deviation; SLOAEL = serious LOAEL; UR = urinalysis; (WB) = whole body

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

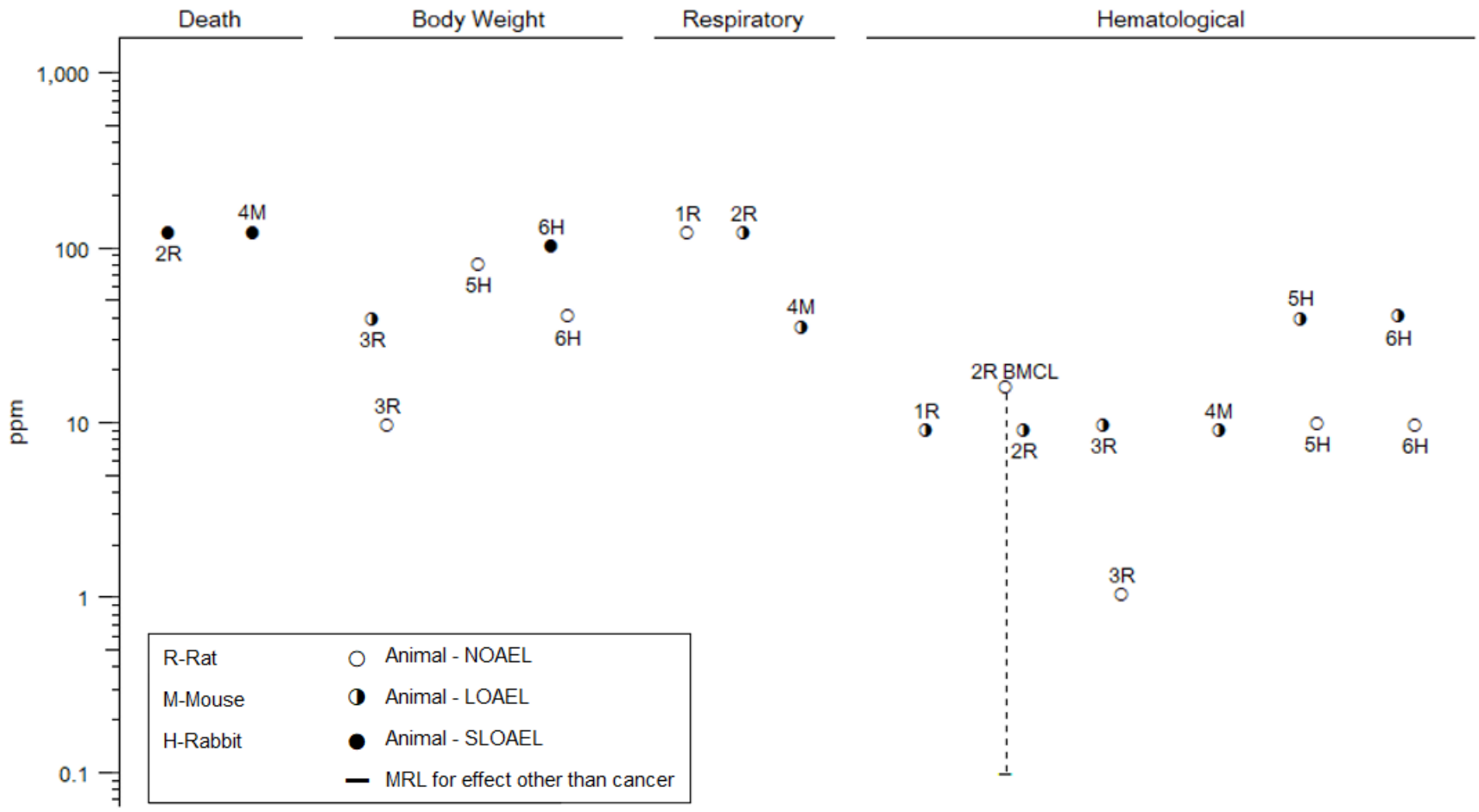


Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Acute (≤14 days)

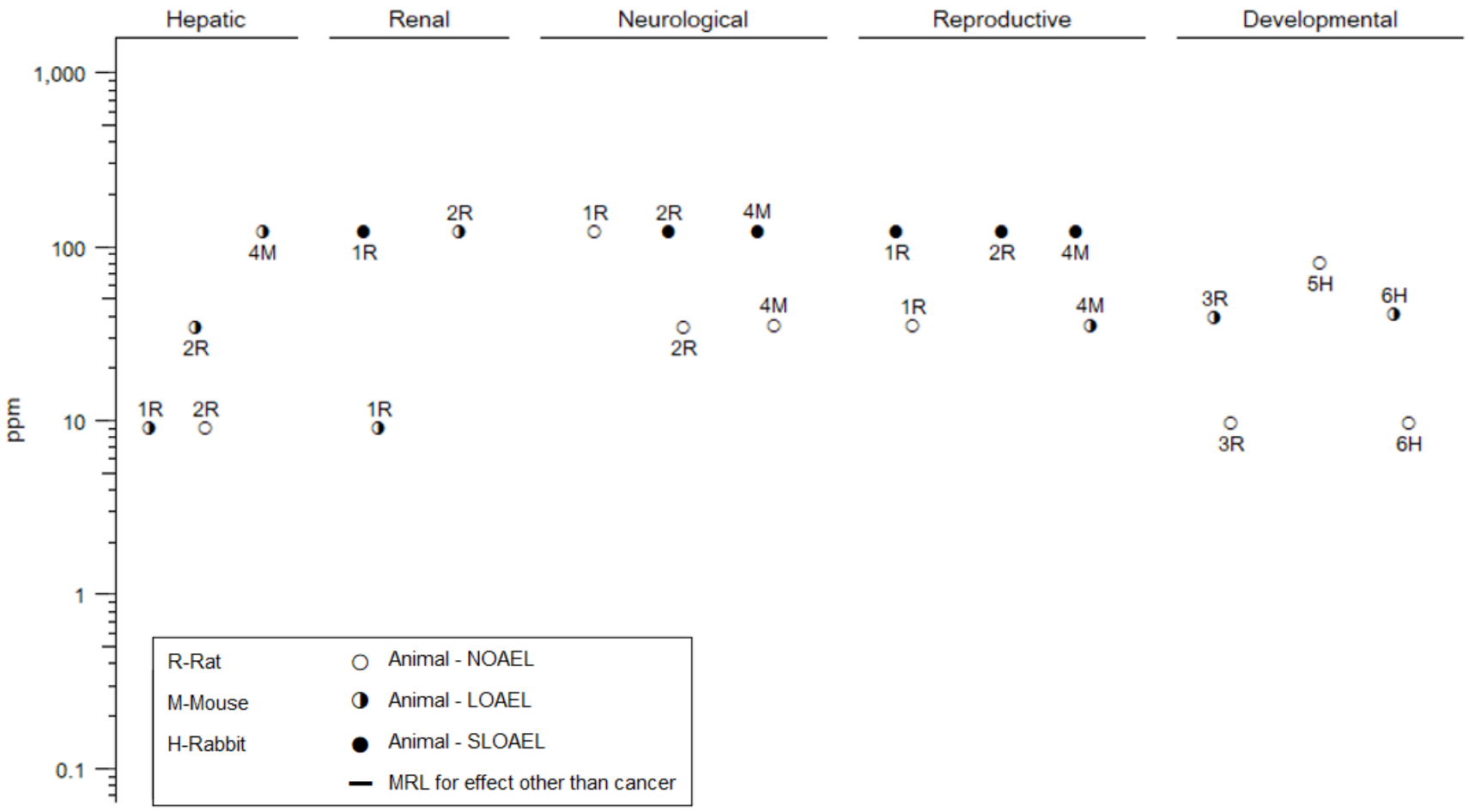


Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Intermediate (15-364 days)

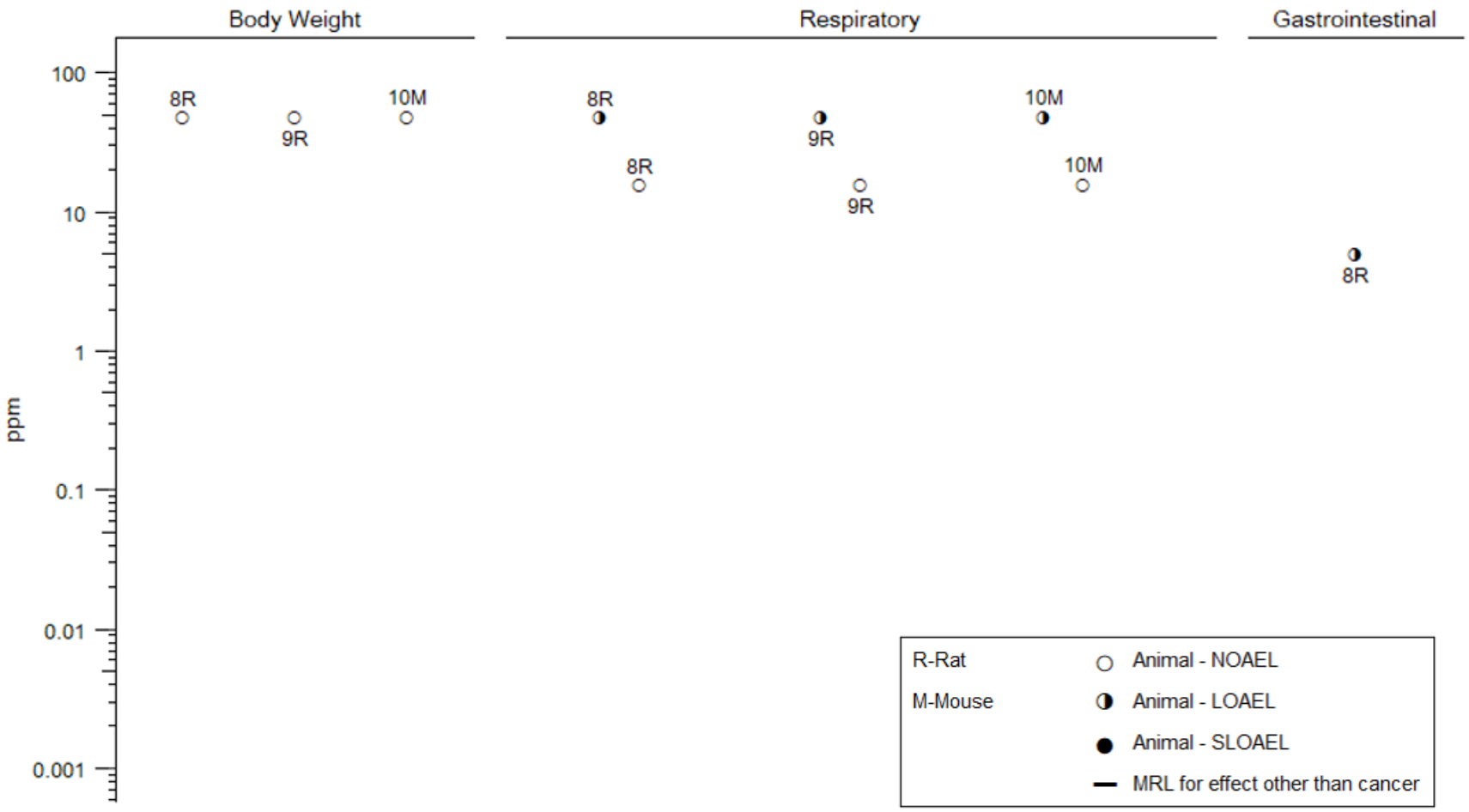


Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Intermediate (15-364 days)

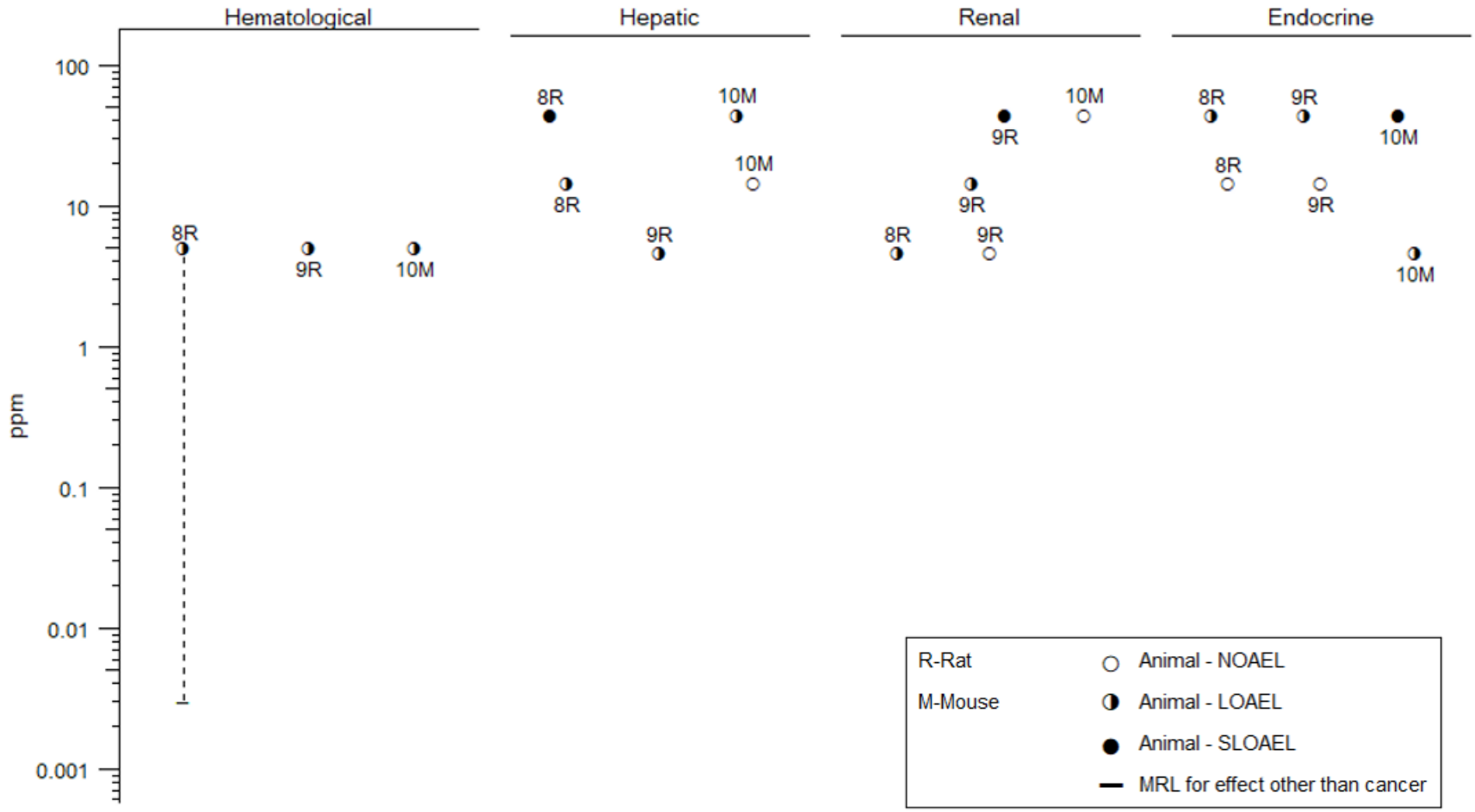


Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Intermediate (15-364 days)

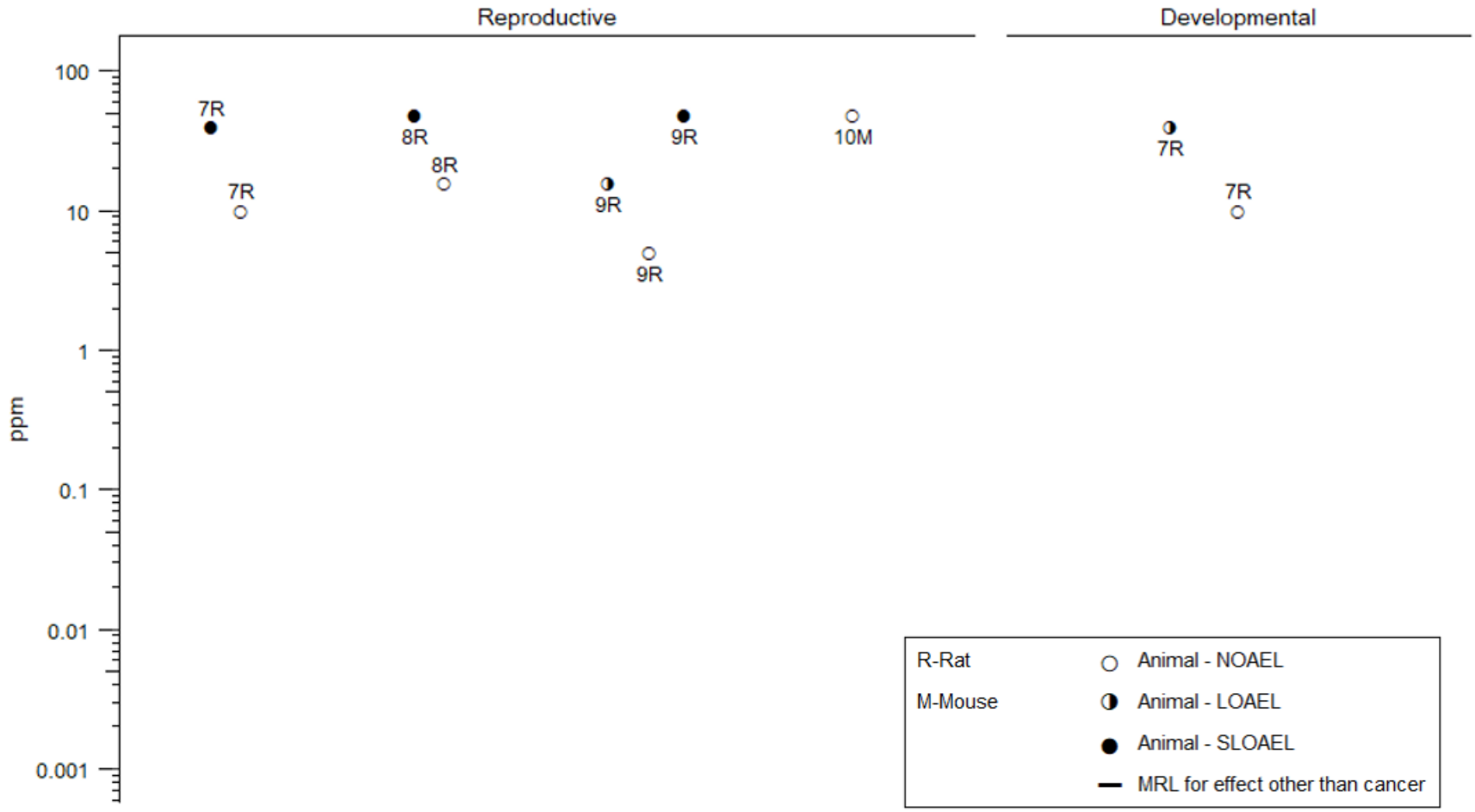


Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Chronic (≥365 days)

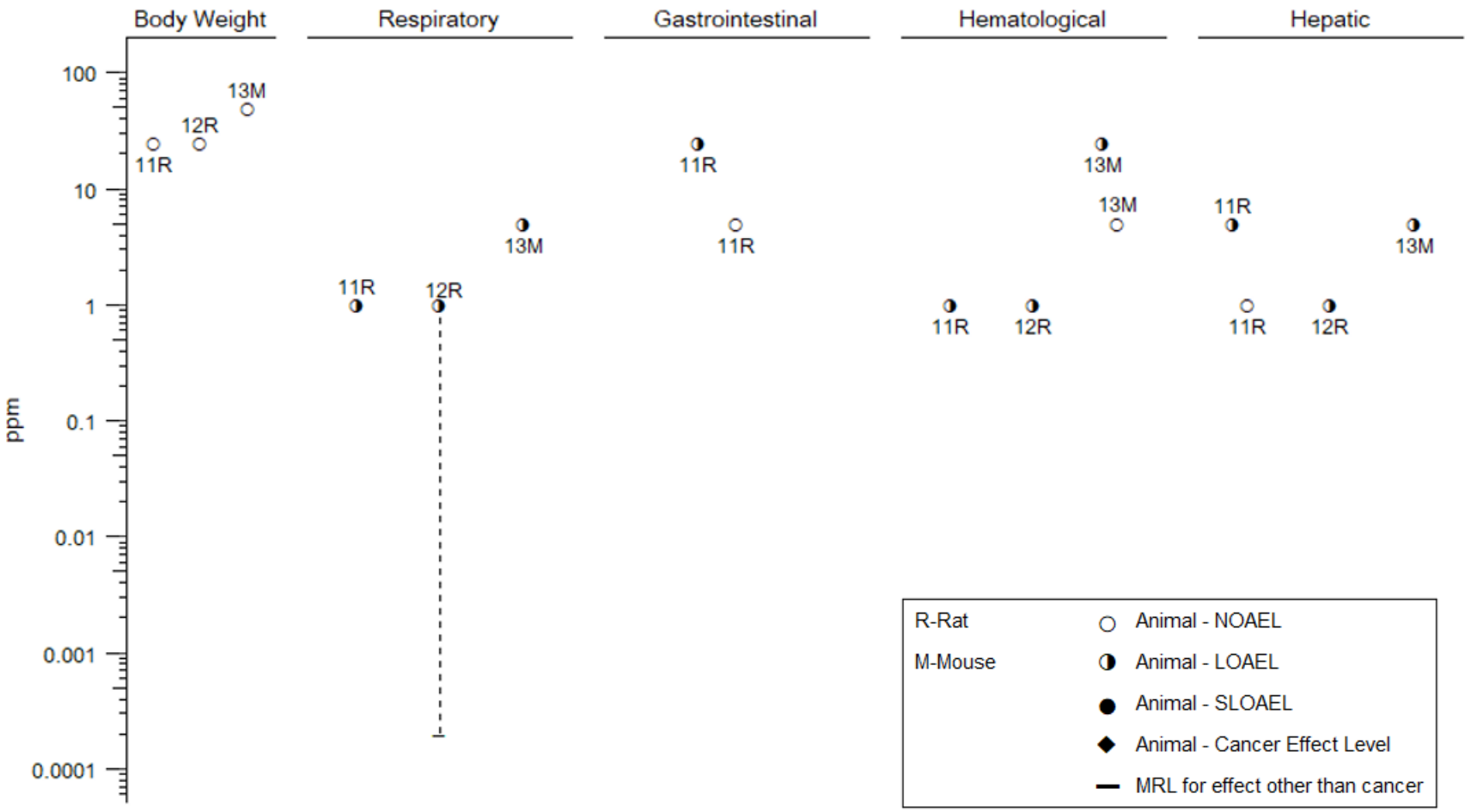


Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Chronic (≥365 days)

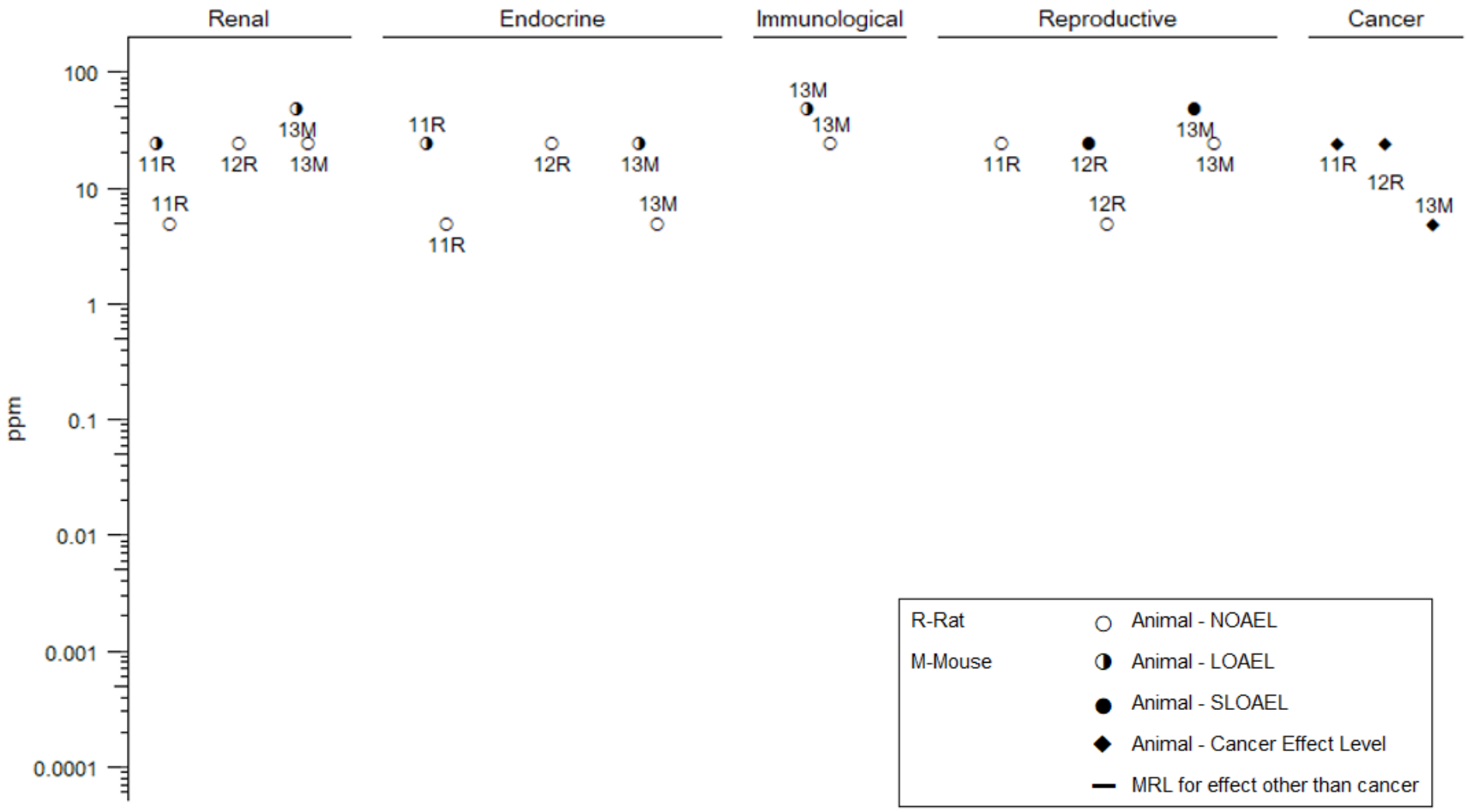


Table 2-2. Levels of Significant Exposure to Nitrobenzene – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
ACUTE EXPOSURE									
1	Rat (Sprague-Dawley) 5 M	2 weeks, daily (GO)	0, 60	OW	Repro		60		Decreased relative testes weight
Iida et al. 1997									
2	Rat (Sprague-Dawley) 8 M	3 days, daily (G)	0, 60	CS, BW, OW, HP	Bd wt Repro	60 60			
Kawaguchi et al. 2004									
3	Rat (Sprague-Dawley) 10 M	14 days, daily (GO)	0, 60	RX, OW	Repro		60		Significantly decreased testes and epididymal weights, 34% decrease in sperm count, and significant decrease in sperm motility with no effect on copulation or fertility rate
Kawashima et al. 1995									
4	Rat (Fischer-344) 45 M	Once (GO)	0, 300	HP	Repro			300	Marked degeneration of the seminiferous epithelium with presence of multinucleated giant cells and loss of mature spermatids
Levin et al. 1988									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
5	Rat (Sprague-Dawley) 6 M	Once (GO)	0, 300	OW, RX, HP	Repro			300	Degenerating and missing pachytene spermatocytes; immature germ cells and debris in epididymis; maturation depletion of spermatids; some multinucleated giant cells; decreased weights of testes and epididymis; decreased cauda and caput sperm counts and abnormal sperm morphology
Linder et al. 1992									
6	Rat (Wistar) 36 M	Once (GO)	0, 300	OW, RX, BI	Repro			300	13 and 23% decreases in testicular weight 1 and 3 days post-treatment, respectively
McLaren et al. 1993a									
7	Rat (Fischer-344) 10 M	Once (GO)	0, 550	CS, HP	Neuro			550	Moderate to severe ataxia, loss of righting reflex, unresponsive to stimuli; hemorrhages in the brain stem and cerebellum, bilateral symmetric degeneration in the cerebellum and cerebellar peduncles
Morgan et al. 1985									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
8	Rat (Wistar) 6 M	14 days, daily (G)	0, 100	BW, BC, BI, NX, OW, HP	Bd wt	100			
					Renal		100		Increased serum urea and creatinine; renal lesions including mild fibrosis and hemorrhage and marked glomerular shrinkage
					Neuro			100	Neurobehavioral changes (decreased exploratory behavior and increased defecation); increased acetylcholinesterase activity in brain; degenerative lesions in the cerebellum, cerebrum, and hippocampus
					Endocr		100		Decreased serum TSH
					Repro			100	Decreased testicular and epididymal weights; decreased serum prolactin, luteinizing hormone, follicle stimulating hormone, and testosterone; atrophic and degenerated seminiferous tubules
Oladele et al. 2020a, 2020b, 2021									
9	Rat (Sprague-Dawley) 3 M	Once (GO)	0, 250	HP	Repro			250	Degeneration of late pachytene spermatocytes; spermatid degeneration and formation of multinucleated giant cells; sloughing of cells into tubular lumen; loss of round and elongate spermatids
Shinoda et al. 1998									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
10	Mouse B6C3F1 8 F	14 days, Daily (GO)	0, 30, 100, 300	LE, CS, BW, BC, BI, HE, OW, GN, HP, IX	Death			300	8.5% of mice across several experiments died prematurely at this dose
					Bd wt	300			
					Resp		300		Increased lung weight
					Hemato		30[b]		Increased DNA synthesis, number of cells and number of granulocyte-monocyte progenitor cells in bone marrow ($BMDL_{1SD}$ = 4.7 mg/kg/day)
					Hepatic	30	100		Increased liver weight, hepatomegaly
					Renal	100	300		Increased kidney weight
					Immuno	30	100		Decreased IgM AFC in spleen cells; decreased natural killer cell activity
					Neuro			300	Ataxia, lethargy, and circling behavior
Burns et al. 1994									
INTERMEDIATE EXPOSURE									
11	Rat (Sprague-Dawley) 5 M	3 weeks, daily (GO)	0, 60	OW, HP	Repro			60	Decreased testes weight (50%); atrophy of the epididymis
Iida et al. 1997									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
12	Rat (Sprague-Dawley) 4 M	49 days, daily (GO)	0, 20, 40, 60	RX	Repro	20		40	Absence of motile sperm
Kato et al. 2002									
13	Rat (Sprague-Dawley) 8 M	18 days, daily (G)		CS, BW, OW, HP, RX	Bd wt Repro	60		60	Decreased weights of testes and epididymides; severe atrophy of seminiferous tubules; decrease in sperm concentration and cell debris in tubular lumina of caput/corpus and cauda epididymides; increased percent detached sperm heads and decreased percentage motile sperm, sperm velocities, and amplitudes of sperm heads
Kawaguchi et al. 2004									
14	Rat (Sprague-Dawley) 10 M	21–70 days, daily (GO)	0, 60	RX, OW	Repro			60	Fertility index decreased to <20% (0% after 28 days); significantly decreased testes weight (>50%) and epididymal weights; sperm count decreased to 10% of controls; decreased sperm motility and viability; and increased % abnormal sperm
Kawashima et al. 1995									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
15	Rat (Sprague-Dawley) 10 M, 10 F	54 days, daily (GO)	0, 20, 60, 100	LE, BW, HE, BC, RX, DX, GN, OW, HP	Death			100	2/10 males and 9/10 females died prior to study termination
					Bd wt	60 M	100 M		17% decrease in body weight of males
					Hemato		20 M		Decreased erythrocytes, hemoglobin, and packed cell volume; increased hematopoiesis in bone marrow; increased spleen weight; extramedullary hematopoiesis and hemosiderin deposition in the spleen in males
					Hepatic		20 M		Increased liver weights; centrilobular swelling of hepatocytes, hemosiderin deposition in Kupffer cells, extramedullary hematopoiesis in males
					Renal		20 M		Hemosiderin deposition in proximal tubules in males
					Neuro	20		60	LOAEL: Necrosis/gliosis in cerebellar medulla and pons (3/10 males); torticollis and abnormal gait (females)
					Repro	20 M		60 M	~60% decrease in testes weight and ~20% decrease in epididymides weight; atrophy of seminiferous tubules (all males), Leydig cell hyperplasia, loss of intraluminal sperm or cell debris in epididymis
					Develop		20	60	

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
									LOAEL: decreased male pup weight on PND 4 (6% relative to controls) SLOAEL: decreased pup viability and >20% reduction in pup weights on PND 4
Mitsumori et al. 1994									
16	Rat (Fischer-344) 10 M, 10 F	90 days daily (GO)	0, 9.375, 18.75, 37.5, 75, 150	LE, CS, BW, FI, HE, GN, OW, HP	Death			150	9/10 males and 3/10 females died between weeks 6 and 13
					Bd wt	75 M 150 F		150 M	30% lower final body weight in the surviving male
					Resp	37.5 F 75 M	75 F		Increased lung weight in females
					Cardio	75 M	9.375 F		Increased heart weight in females
					Hemato		9.375[c]	75	LOAEL: increased absolute reticulocyte count and decreased hemoglobin; increased methemoglobin in males (2.752 versus 1.131% in controls) and females (2.059 versus 0.941% in controls) ($BMDL_{1SD}$ = 1.8 mg/kg/day) SLOAEL: cyanosis
					Hepatic		9.375		Increased liver weights
					Renal	37.5 F	9.375 M 75 F		Increased kidney weights (both sexes); pigment deposition in kidney (females)
					Immuno	9.375		18.75 F	Increased lymphoid depletion in spleen
					Neuro			75	Ataxia (females); hemorrhage in brainstem (males)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
					Repro	18.75 M	37.5 M 150 F	75 M	LOAEL: Atrophic seminiferous tubules in one male; atrophied uteri in 2 females SLOAEL: atrophy of the testes in 9/10 males; hypospermatogenesis; ~40% decreased testes weight
NTP 1983a									
17	Mouse (B6C3F1) 10 M, 10 F	90 days, daily (GO)	0, 18.75, 37.5, 75, 150, 300	LE, CS, BW, FI, HE, GN, OW, HP	Death			300 M	3/10 males died between weeks 4 and 5; 2/10 males were sacrificed moribund
					Bd wt	150			
					Hemato		18.75		Increased methemoglobin in males (2.162 versus 1.074% in controls) and females (1.198 versus 0.871% in controls); increased reticulocytes in females
					Hepatic	75 M	18.75 F 150 M		Increased liver weights
					Renal	150 M 300 F	300 M		Increased kidney weight in males
					Endocr	150 F	300 F		Fatty change in zona reticularis of adrenal glands of females
					Immuno			150 F	Lymphoid depletion in females

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
					Neuro	37.5 M 75 F	75 M	300	LOAEL: Lethargy in 1/10 males SLOAEL: Ataxia, lethargy (males); irritability, ataxia, and head bobbing behavior (females)
					Repro	75 M		150 M	Testicular atrophy
NTP 1983a									

[a]The number corresponds to the entries in Figure 2-3; differences in levels of health effects and cancer between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an acute-duration oral MRL. The data on increased DNA synthesis in bone marrow were subjected to benchmark dose modeling, resulting in a $BMDL_{1SD}$ of 4.7 mg/kg/day. The $BMDL_{1SD}$ was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability), resulting in an MRL of 0.05 mg/kg/day.
[c]Used to derive an intermediate-duration oral MRL. The data on increased methemoglobin were subjected to BMD modeling, resulting in a $BMDL_{1SD}$ of 1.8 mg/kg/day. The $BMDL_{1SD}$ was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability), resulting in a MRL of 0.02 mg/kg/day.

AFC = antibody forming cell; BI = biochemical changes; BC = blood chemistry; Bd wt or BW = body weight; BMD = benchmark dose; BMDL = lower confidence limit on the benchmark dose; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DNA = deoxyribonucleic acid; DX = developmental toxicity; Endocr = endocrine; F = female(s); FI = food intake; (G) = gavage; GN = gross necropsy; (GO) = gavage in oil vehicle; HE = hematology; Hemato = hematological; HP = histopathological; IgM = immunoglobin M; Immuno = immunological; IX = immune effects; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NX = neurological function; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; SD = standard deviation; SLOAEL = serious LOAEL; TSH = thyroid-stimulating hormone

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

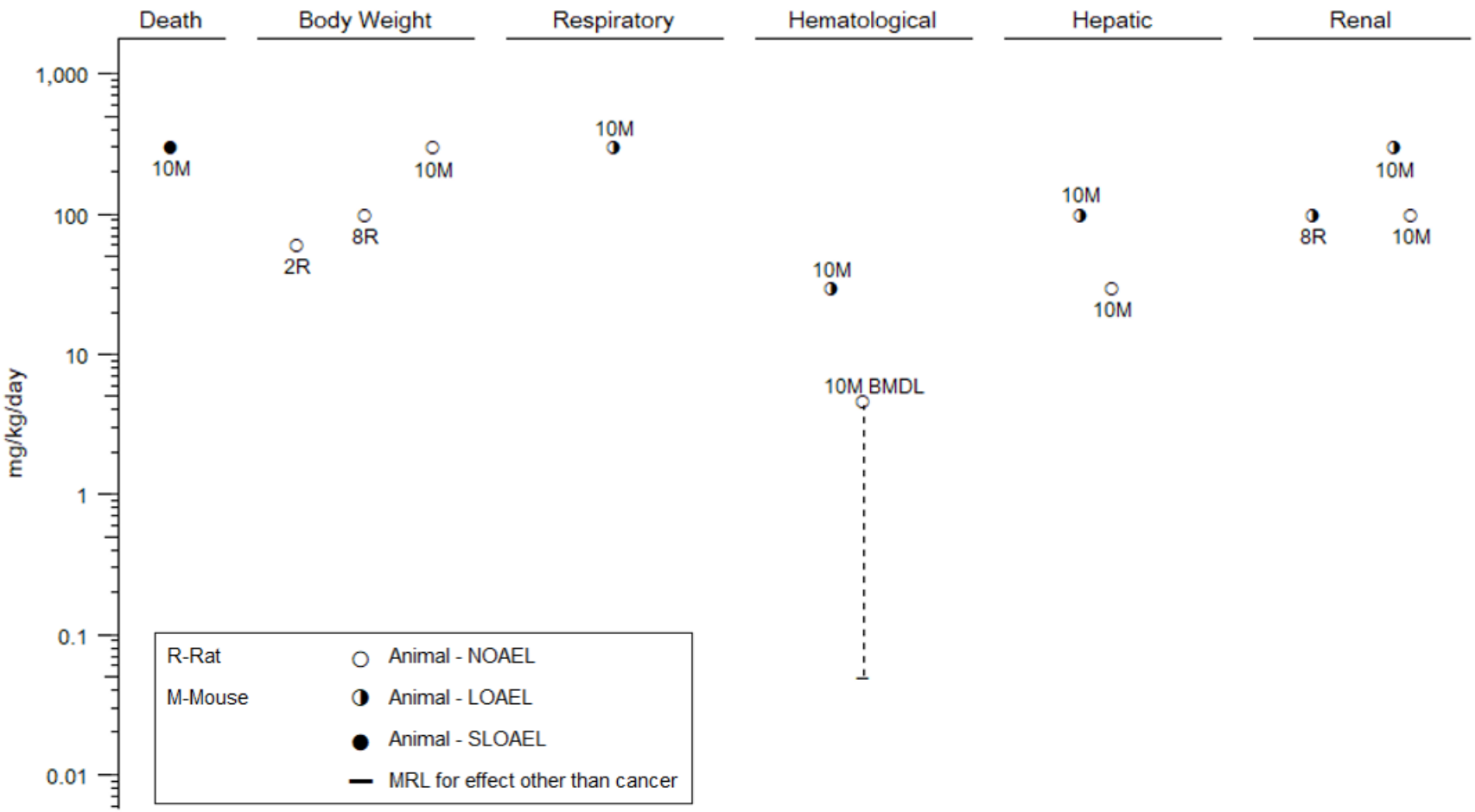


Figure 2-3. Levels of Significant Exposure to Nitrobenzene – Oral
Acute (≤14 days)

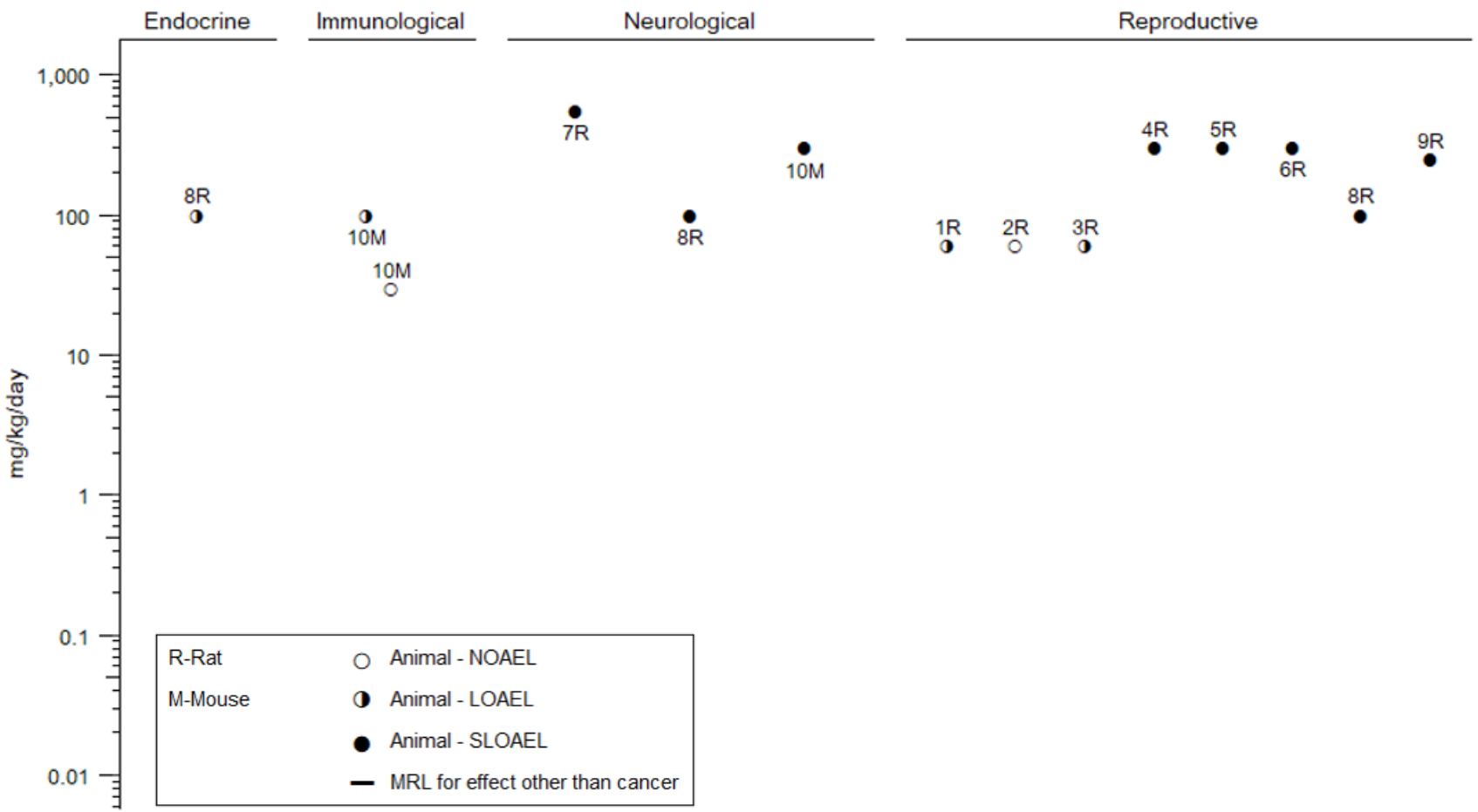


Figure 2-3. Levels of Significant Exposure to Nitrobenzene – Oral
Intermediate (15-364 days)

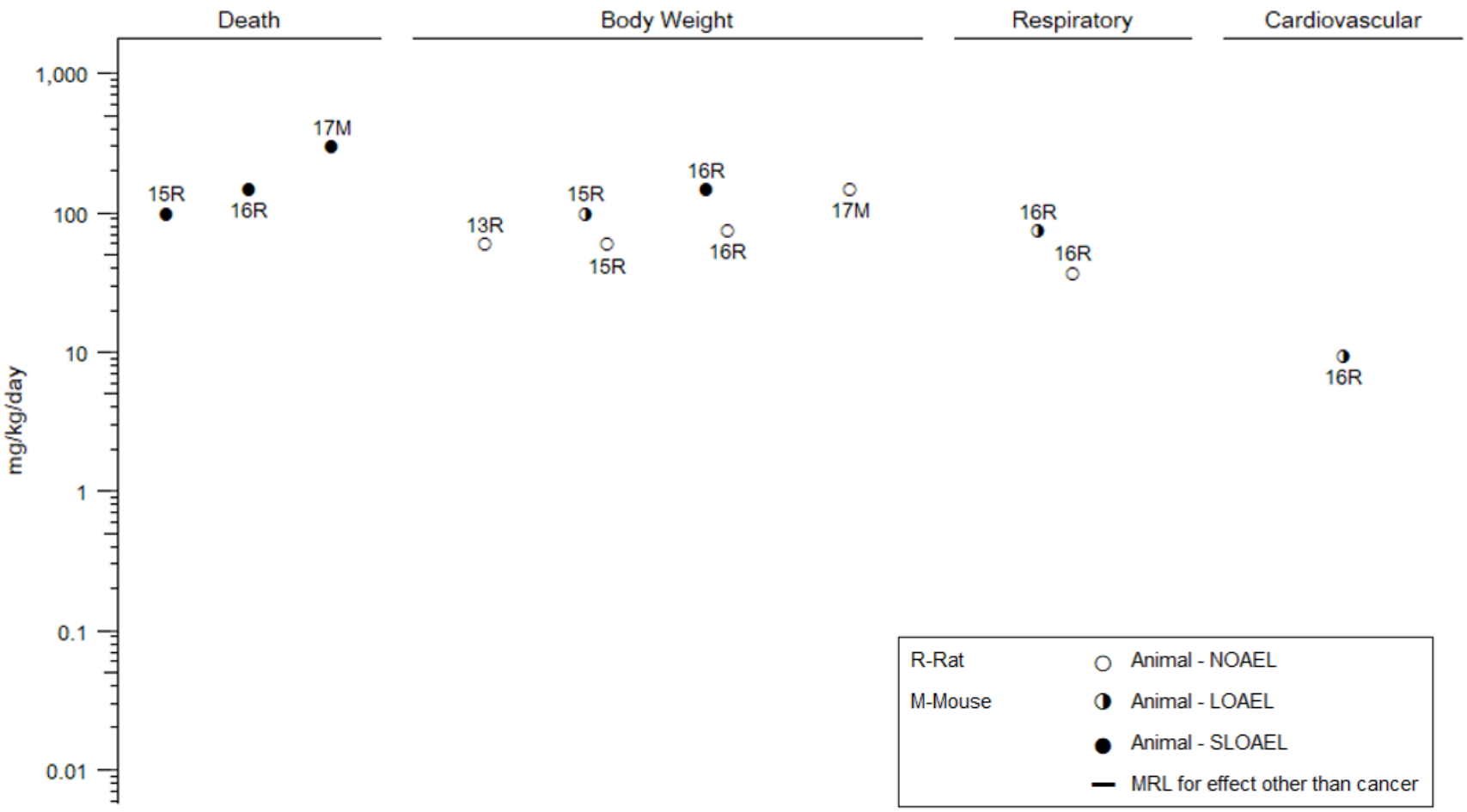


Figure 2-3. Levels of Significant Exposure to Nitrobenzene – Oral
Intermediate (15-364 days)

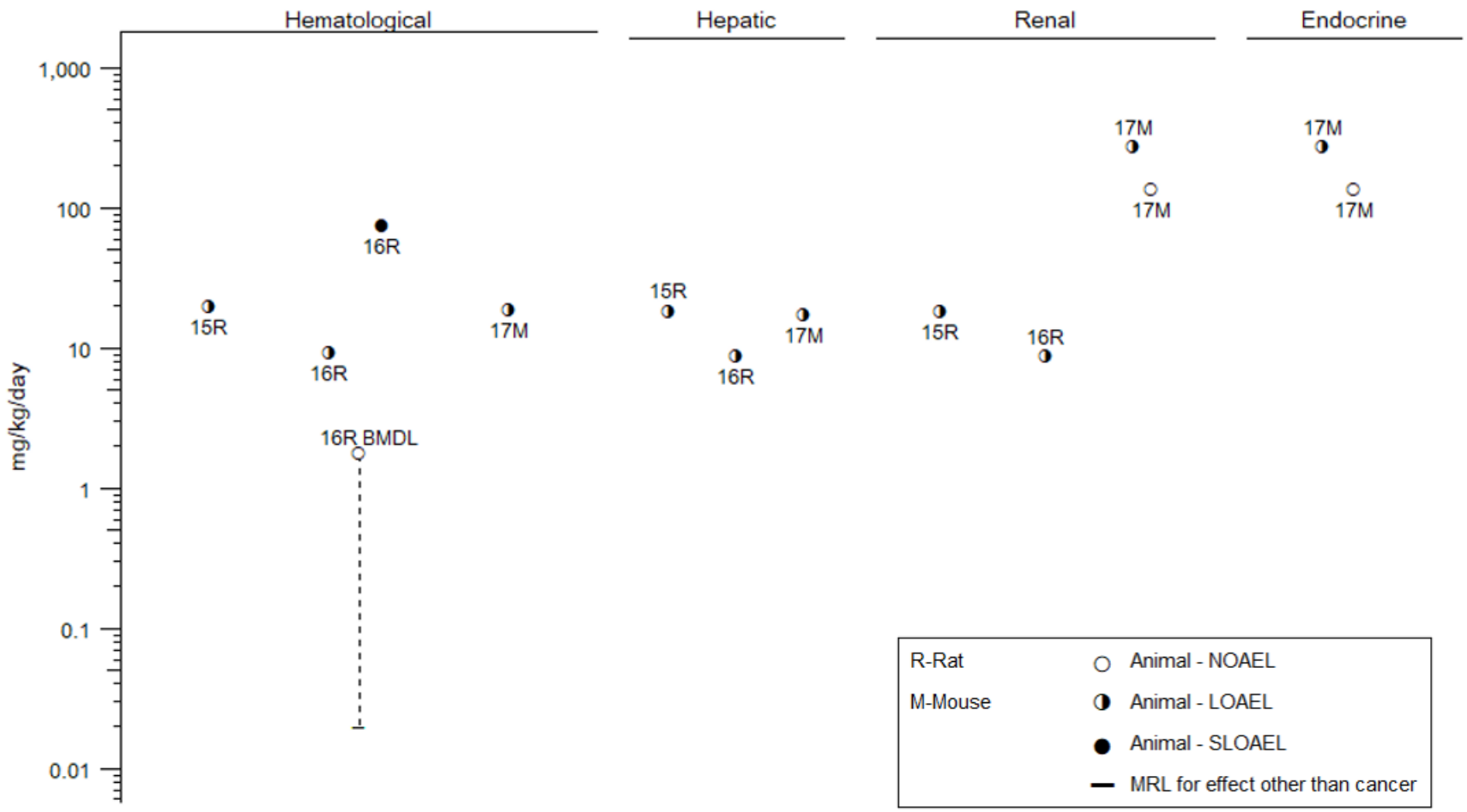


Figure 2-3. Levels of Significant Exposure to Nitrobenzene – Oral
Intermediate (15-364 days)

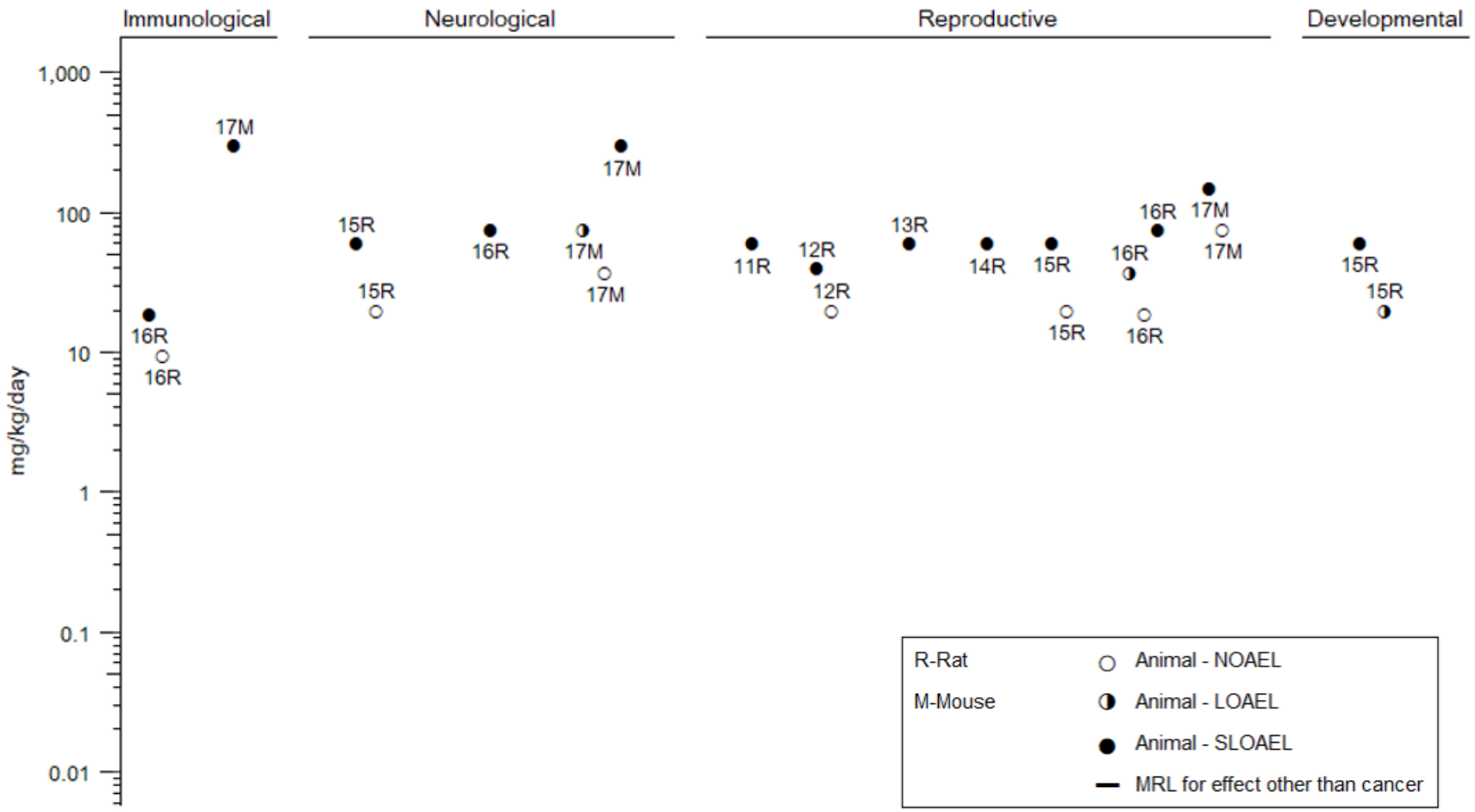


Table 2-3. Levels of Significant Exposure for Nitrobenzene – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Rat (Fischer-344) 5 M, 5 F	12 days, daily	0, 0.2, 0.4, 0.8, 1.6, 3.2 g/kg	LE, CS, BW, HE, OW, GN, HP	Death			1.6	All animals died or were sacrificed moribund at ≥1.6 g/kg/day
				Bd wt	0.8			
				Resp	0.8	1.6		Lung congestion
				Hemato		0.2	0.4 M	LOAEL: Decreased hemoglobin, hematocrit, and erythrocyte counts (both sexes); increased WBCs (males); increased MCV and MCH (females); increased reticulocytes (both sexes); increased methemoglobin in males (7.672 versus 0.580% in controls) and females (6.924 versus. 0.644% in controls); minimal to mild spleen congestion SLOAEL: Cyanosis in males
				Hepatic		0.2		Increased liver weight
				Renal	0.8 F	1.6 F		Renal cortical tubule degeneration and cytoplasmic vacuolization in females
				Immuno			0.4	Lymphoid atrophy in males
				Repro	0.4 M		0.8 M	Seminiferous tubule atrophy; absence of spermatids and spermatozoa; multinucleated giant cells; decreased testes weight
NTP 1982								
Mouse (B6C3F1) 5 M, 5 F	12 days, daily	0, 0.2, 0.4, 0.8, 1.6, 3.2 g/kg	LE, CS, BW, HE, OW, GN, HP	Death			1.6	All animals died or were sacrificed moribund at ≥1.6 g/kg/day
				Bd wt	0.8			
				Hemato	0.2 M	0.2 F 0.4 M		Increased absolute and relative reticulocyte counts (both sexes); decreased hemoglobin concentration and erythrocyte counts (males)
				Hepatic	0.2 F 0.8 M	0.4 F		Increased liver weight
				Renal	0.8			
NTP 1982								

Table 2-3. Levels of Significant Exposure for Nitrobenzene – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
INTERMEDIATE EXPOSURE								
Rat (Fischer-344) 60 M, 60 F	90 days	0, 0.05, 0.1, 0.2, 0.4, 0.8 g/kg	BW, CS, GN, HP, LE, RX, FI, HE	Death			0.8	All animals died by week 11
				Bd wt	0.4			
				Resp	0.2 F 0.4 M	0.4 F		Lung congestion in females
				Cardio	0.2 M 0.4 F	0.4 M		Increased heart weight
				Hemato		0.05		Increased methemoglobin in males (0.985 versus 0.571% in controls) and females (0.995 versus 0.684% in controls); decreased erythrocyte count (females); spleen congestion
				Hepatic	0.1 F	0.1 M 0.2 F		Increased relative liver weight
				Renal	0.2	0.4		Kidney congestion
				Immuno			0.2	Lymphoid atrophy of spleen
				Neuro	0.05 F 0.4 M		0.1 F	Hemorrhages in the brain and brain stem; vacuolization in white matter, cerebellar white matter, and brain stem of females
				Repro	0.2 M		0.4 M	Markedly atrophic seminiferous tubules; hypospermatogenesis; multinucleate giant cells; >60% decrease in testes weight
NTP 1983b								
Mouse (B6C3F1) 60 M, 60 F	90 days	0, 0.05, 0.1, 0.2, 0.4, 0.8 g/kg	BW, CS, GN, HP, LE, RX, FI, HE	Death			0.8	9/10 males, 8/10 females died between weeks 3 and 10
				Bd wt	0.4			
				Hemato		0.05		Increased reticulocytes in males; increased methemoglobin in females (2.361 versus 1.357% in controls)
				Hepatic	0.2	0.4		Increased liver weight in females; cytomegaly in males
				Renal	0.1 M 0.4 F	0.2 M		Increased kidney weight in males

Table 2-3. Levels of Significant Exposure for Nitrobenzene – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
				Endocr	0.4 M	0.05 F		Fatty change in the adrenal cortex of females
				Immuno			0.8	Thymic atrophy
				Neuro			0.8	Brain stem hemorrhage and degeneration
				Repro	0.2 M	0.4 M 0.2 F		Decreased relative testes weight; testicular atrophy and hypospermatogenesis; uterine atrophy
NTP 1983b								

Bd wt or BW = body weight; Cardio = cardiovascular; CS = clinical signs; F = female(s); FI = food intake; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; Neuro = neurological; NOAEL = no-observed-adverse-effect level; OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; WBC = white blood cell

2.2 DEATH

No studies were located regarding lethal effects of nitrobenzene in humans after inhalation or dermal exposure. However, several case studies have reported deaths after nitrobenzene ingestion verified by blood analysis (Martínez et al. 2003) or where the substance was known to contain nitrobenzene (Gupta et al. 2000, 2012; Kumar et al. 2017). In the case reported by Martínez et al. (2003), an 82-year-old man ingested 250 mL of an unknown substance and developed severe (70%) methemoglobinemia; he died 4 days later. An analysis of blood collected 48 hours after ingestion showed that the nitrobenzene level was 3.2 µg/mL. Gupta et al. (2012) reported the case of a 17-year-old girl who was admitted to the hospital 6 hours after ingestion of an unknown quantity of nitrobenzene. The patient was unconscious, and her methemoglobin level was 63%. She was treated with oral methylene blue and intravenous vitamin C but died 4 days later (Gupta et al. 2012). In a case presented by Kumar et al. (2017), a 17-year-old girl who had accidentally ingested nitrobenzene 7 days prior to admittance to the hospital was treated with methylene blue for suspected acute methemoglobinemia. Sixteen days after she was hospitalized, she died of secondary aspiration pneumonitis, sepsis, and toxic brain injury due to nitrobenzene ingestion. Kumar et al. (2017) did not report the amount of nitrobenzene ingested or blood levels of nitrobenzene or methemoglobin. Gupta et al. (2000) briefly described the death of a 5-year-old boy who had ingested screen-printing material that contained nitrobenzene. Upon hospitalization, the boy was cyanotic, and was treated with gastric lavage and vitamin C. His condition deteriorated and the child expired about 26 hours after admission (Gupta et al. 2000). No information on the dose or blood levels of methemoglobin or nitrobenzene was reported.

Deaths have been reported in laboratory animals following acute-, intermediate-, and chronic-duration studies by several exposure routes. The available data suggest a steep dose-response curve for nitrobenzene lethality. For example, significant mortality was seen in rats and mice exposed by inhalation for 2 weeks to 124.5 ppm nitrobenzene, while there were no deaths at 35.8 ppm (Medinsky and Irons 1985). Similarly, in an intermediate-duration study of oral exposure, most male rats and male mice died prematurely at a dose of 150 mg/kg/day, while there were no deaths at 75 mg/kg/day (NTP 1983a). In acute- and intermediate-duration dermal exposure studies, the dose-response relationship for mortality was similar (NTP 1982, 1983b). All animals of both species died with dermal exposure to 1.6 g/kg but survived 0.8 g/kg in the acute-duration study (NTP 1982), and most animals of both species died with exposure to 0.8 g/kg but survived 0.4 g/kg in the intermediate-duration study (NTP 1983b).

In an unpublished acute lethality study, male Sprague-Dawley rats were exposed to 439, 514, 542, 555, 578, and 714 ppm of nitrobenzene 4 hours at a time (Dupont 1981). The following mortality incidences were reported: 0/10, 0/10, 1/10, 7/10, 8/10, and 10/10 rats for the low to high exposure concentrations, respectively. Most of the deaths occurred 1–2 days post exposure; one death occurred 7 days after exposure. The LC_{50} was calculated to be 556 ppm (Dupont 1981).

In a 14-day inhalation study comparing effects in Fischer 344 rats, CD rats, and B6C3F1 mice, species and strain differences in lethality were observed (Medinsky and Irons 1985). While all male and female Fischer-344 rats survived 2 weeks of exposure to 124.5 ppm, five male and three female CD rats and all B6C3F1 mice of both sexes exposed to this concentration died or were humanely sacrificed by the 4th day of exposure. The deaths of CD rats and B6C3F1 mice were attributed to perivascular hemorrhage in the cerebellar peduncle (Medinsky and Irons 1985). Pregnant CD rats survived acute-duration exposure on gestation days (GDs) 6–15 (6 hours/day) to concentrations up to 39.4 ppm (Tyl et al. 1987). Similarly, no treatment-related mortalities were observed when pregnant New Zealand white rabbits were exposed to nitrobenzene concentrations up to 104 ppm for 6 hours/day during GDs 7–19 (Biodynamics 1983, 1984).

In intermediate-duration inhalation studies, F344 and CD rats and B6C3F1 mice of both sexes survived exposure to nitrobenzene concentrations up to 48.7 ppm for 90 days (6 hours/day, 5 days/week). In addition, 2 years of inhalation exposure to concentrations up to 24.8 ppm (F344 and CD rats) or 49.1 ppm (B6C3F1 mice) did not alter survival rates of either species (Cattley et al. 1994, 1995; CIIT 1993).

The oral LD_{50} of nitrobenzene in female albino rats exposed by gavage was estimated to be 600 mg/kg (Smyth et al. (1969). Only one other acute-duration oral study reported animal deaths; in a study reporting multiple experiments, the study authors reported that 8.5% of female B6C3F1 mice (across several experiments) given 300 mg/kg/day nitrobenzene in corn oil for 14 days died (Burns et al. 1994). No deaths were reported among male Sprague-Dawley rats exposed for 14 days to oral doses of 60 mg/kg/day (Kawashima et al. 1995).

In intermediate-duration oral studies, significant mortality was reported at doses ≥100 mg/kg/day in rats or 300 mg/kg/day in mice. Mitsumori et al. (1994) reported that 2/10 males and 9/10 female Sprague-Dawley rats exposed to 100 mg/kg/day by gavage died before scheduled termination in a combined repeat-dose and reproductive/developmental screening study. The causes of death were not reported. The study authors also reported the deaths of 1/10 females in each of the 20 and 60 mg/kg/day dose groups during the lactation period. It is not clear whether the deaths at the latter doses were related to treatment,

but females in this group were noted to exhibit anemia (6/10) and neurological signs of toxicity (ataxia and torticollis, 1/10) (Mitsumori et al. (1994). In a 90-day gavage study, 9/10 male and 3/10 female F344 rats given doses of 150 mg/kg/day died prior to study completion (NTP 1983a). In the same study, exposure to doses of 300 mg/kg/day resulted in premature death of 3/10 male mice and moribund sacrifice of another 2/10 male mice (NTP 1983a).

In an acute-duration dermal study, Fischer 344 rats and B6C3F1 mice were exposed to nitrobenzene via daily dermal application for 12 days. All rats and mice of both sexes exposed to doses of 1.6 and 3.2 g/kg of nitrobenzene either died or were sacrificed moribund (NTP 1982). When Fischer 344 rats and B6C3F1 mice were exposed by dermal application in a 13-week intermediate-duration study, all rats, 9/10 male mice, and 8/10 female mice exposed to 0.80 g/kg died by week 11 (NTP 1983b).

2.3 BODY WEIGHT

No human studies were located evaluating body weight effects of nitrobenzene exposure following inhalation, oral, or dermal exposure. Few animal studies have reported changes in body weight associated with nitrobenzene exposure. Male Sprague-Dawley rats exposed to 439–714 ppm of nitrobenzene for a single 4-hour period in an acute lethality study experienced weight losses of 8–21% of initial body weight 1–4 days after exposure (Dupont 1981). In gestational exposure studies, pregnant rats and rabbits exhibited reduced weight gain or weight loss. Decreased maternal weight gain was observed in CD rats exposed to 39.4 ppm for 6 hours/day on GDs 6–15 (Tyl et al. 1987). In pregnant rabbits exposed to 40 ppm nitrobenzene for 6 hours/day during GDs 7–19, there was a slight mean weight loss during gestation; the change was not statistically significant relative to controls (Biodynamics 1984). Body weights were not measured in the 14-day inhalation experiments in F344 rats, CD rats, and B6C3F1 mice conducted by Medinsky and Irons (1985).

No effects on body weights were reported in B6C3F1 mice or F344 or CD rats exposed to concentrations of up to 48.7 ppm for 90 days (Hamm et al. 1984). In a 2-year inhalation study of B6C3F1 mice and Fischer 344 and CD rats, no effects on body weight were observed at concentrations up to 49.1 ppm in mice or 24.8 ppm in rats (Cattley et al. 1994, 1995; CIIT 1993). Statistically significant fluctuations in mean body weights occurred over the course of the 2-year study but they were not exposure-related, and differences from control body weights did not reach 10%.

Female B6C3F1 mice administered 300 mg/kg/day nitrobenzene for 14 days via gavage displayed a 12% increase in body weight (compared with controls), which the study authors attributed to fluid retention (Burns et al. 1994). Female mice receiving 100 mg/kg/day in the same study did not have any body weight differences from controls (Burns et al. 1994). Male Sprague-Dawley rats given 100 mg/kg/day nitrobenzene orally in a combined repeat-dose and reproductive/developmental screening study exhibited a 17% decrease in body weight at termination (Mitsumori et al. 1994). For females in this study, body weight changes were reported qualitatively. The study authors reported that females given 100 mg/kg/day consumed less food prior to mating and during pregnancy, with a consequent decrease in body weight gain on day 21 of pregnancy. Maternal body weight gain was also decreased during the lactation period at this dose. Females given 60 mg/kg/day also showed an inhibition of body weight gain during the lactation period, accompanied by a decrease in food consumption (Mitsumori et al. 1994). In 90-day studies of Fischer 344 rats and B6C3F1 mice exposed orally, mean final body weight was not significantly different in rats exposed to doses up to 75 mg/kg/day or mice exposed to doses up to 300 mg/kg/day when compared to controls. The only surviving male rat in the 150 mg/kg/day group did have a significant decrease in body weight (>10% compared to control) (NTP 1983a).

In studies using dermal application of nitrobenzene, Fischer 344 rats and B6C3F1 mice exposed to doses up to 0.8 g/kg for 12 days or up to 0.4 g/kg for 90 days showed no significant changes in body weight (NTP 1982, 1983b).

2.4 RESPIRATORY

No studies were located in humans evaluating the respiratory effects of nitrobenzene through inhalation, oral, or dermal exposure routes.

In an acute-duration, 2-week inhalation study in male and female F344 and CD rats and B6C3F1 mice (Medinsky and Irons 1985), mice exhibited moderate bronchiolar epithelial hyperplasia after exposure to 124.5 ppm nitrobenzene (a concentration that resulted in death or humane sacrifice within the first 4 days of exposure) and mild bronchiolar hyperplasia after exposure to 35.8 ppm (all mice survived to termination). Male and female CD rats exposed to 124.5 ppm nitrobenzene also died or were sacrificed moribund within the first exposure week; in these animals, perivascular edema and vascular congestion in the lungs were found (Medinsky and Irons 1985). In a developmental toxicity study, Biodynamics (1984) reported a high incidence of discolored lungs in New Zealand White rabbits in all exposure groups (concentrations from 10 to 81 ppm were administered on GDs 7–19).

Hamm et al. (1984) observed treatment-related lesions in the lungs of F344 and CD rats and B6C3F1 mice exposed to nitrobenzene by inhalation for 90 days. There was a minimal to slight hyperplasia in the bronchial epithelium in male F344 rats and in both male and female mice after exposure to 48.7 ppm. Female rats did not show this effect of treatment. In male CD rats, rhinitis associated with epithelial and goblet cell hyperplasia was observed in nasal turbinates after exposure to 48.7 ppm nitrobenzene (Hamm et al. 1984).

In a 2-year study of the toxicity of inhaled nitrobenzene, Cattley et al. (1994, 1995; CIIT 1993) observed lesions in the nasal turbinates and lungs of mice and in the nasal turbinates of male CD rats. In mice, a marked increase in bronchiolization of alveolar walls at all exposure concentrations (≥5 ppm), and males showed increased alveolar/bronchiolar hyperplasia at ≥24.8 ppm (Cattley et al. 1994, 1995; CIIT 1993).

Nasal lesions occurred at all exposure concentrations (≥1 ppm) in rats including olfactory epithelial pigment deposition (F344 and CD rats) and squamous epithelial hyperplasia (CD rats). At higher exposures, nasal findings in the rats of both strains included inflammation, sometimes with submucosal gland hypertrophy, and suppurative exudate. Mice exhibited more severe lesions in the nasal passages, including the following findings that were seen at significantly increased incidences at all exposure levels (≥5 ppm): glandularization of the respiratory epithelium, olfactory epithelial degeneration, increased secretory product in the respiratory epithelium, olfactory epithelial pigment deposition, and dilatation of the submucosal glands.

A significant increase in absolute lung weight was reported when B6C3F1 mice were gavaged with ≥30 mg/kg/day nitrobenzene for 14 days; however, relative lung weight was increased only at the high dose (300 mg/kg/day) (Burns et al. 1994). Increased lung weights were also reported in female F344 rats after 90 days of oral exposure to ≥75 mg/kg/day (NTP 1983a).

Dermal exposure to nitrobenzene for 12 or 90 days resulted in lung congestion in F344 rats, but not B6C3F1 mice (NTP 1982, 1983b). In rats, 12-day exposure to 1.6 g/kg induced this effect in both sexes, while a significant increase was seen only in females exposed for 90 days to 0.4 g/kg (NTP 1982, 1983b).

2.5 CARDIOVASCULAR

No relevant studies were located regarding cardiovascular effects in humans or animals after inhalation exposure to nitrobenzene. In a case study of a 32-year-old male exposed orally to nitrobenzene, cardiogenic pulmonary edema was noted (Agrawal et al. 2011). The amount of nitrobenzene ingested was not known.

Increased incidences of mineralization of the aorta and myocardium were noted in male CD rats exposed for 2 years to 24.8 ppm nitrobenzene; however, these lesions were considered secondary effects of severe chronic nephropathy at this exposure level (Cattley et al. 1994, 1995; CIIT 1993). No cardiovascular effects were noted in rats or mice exposed to nitrobenzene by inhalation in other acute-, intermediate-, or chronic-duration studies (Medinsky and Irons 1985; Hamm et al. 1984; Cattley et al. 1994, 1995; CIIT 1993). No acute-duration oral studies included assessment of cardiovascular tissues. Of the available oral studies, only the 90-day studies by NTP (1983a) reported results of endpoints relevant to cardiovascular health. Increased absolute and relative heart weights were observed in F344 female rats at all dose levels (≥9.375 mg/kg/day) after 90 days of exposure via gavage (NTP 1983a). There were no histopathology correlates in the hearts of female rats, and no treatment-related changes in heart weights or histopathology were observed in male rats or in male or female mice (NTP 1983a).

In the 90-day dermal exposure study of rats and mice, increased absolute and relative heart weights were noted in male rats exposed to 0.4 g/kg (the highest dose that animals survived), but not in female rats or in male or female mice at the same dose (NTP 1983b). No treatment-related lesions were observed in the hearts of rats or mice in this study.

2.6 GASTROINTESTINAL

No studies were located regarding gastrointestinal effects in humans after inhalation, oral, or dermal exposure to nitrobenzene. Female F344 rats exposed to concentrations ≥5 ppm nitrobenzene for 90 days by inhalation exhibited diarrhea, but male rats and mice did not exhibit this effect (Hamm et al. 1984). Chronic inhalation exposure to 24.8 ppm nitrobenzene resulted in an increased incidence of focal pancreatic acinar cell hyperplasia in male F344 rats (Cattley et al. 1994, 1995; CIIT 1993). No other reports of gastrointestinal effects in animals exposed to nitrobenzene were located.

2.7 HEMATOLOGICAL

Overview. A key event in the mechanism by which nitrobenzene induces many of its toxic effects is via conversion of the iron component of hemoglobin from the ferrous state to the ferric state (oxidized), forming methemoglobin. Methemoglobin is not capable of binding and transporting oxygen to the tissues of the body, leading to tissue hypoxia. Methemoglobin occurs naturally in people, at levels around 1–4% in blood (Smith and McHale 2018). However, increases in the amount of methemoglobin cause cyanosis (slate blue coloration), fatigue, weakness, dyspnea, headache, and dizziness. The severity of effects of methemoglobinemia in humans increase with the percentage in the blood (Ludlow et al. 2021):

- At ≥10%, cyanosis may be evident in a healthy person.
- At ≥15%, the characteristic "chocolate brown blood" may be present.
- As the level approaches 20%, symptoms of anxiety, light-headedness, and headaches may occur.
- At levels in the range of 30–50%, there may be tachypnea, confusion, and loss of consciousness.
- As the level approaches 50%, there is risk of seizures, dysrhythmias, metabolic acidosis, and coma.
- Levels above 70% are often fatal in humans.

The mechanism by which nitrobenzene induces methemoglobinemia is well-studied and is a result of redox cycling of its metabolites (see Metabolic Mechanisms in Section 3.1.3). The action of bacteria normally present in the small intestine and gut is an important element in the formation of methemoglobin resulting from nitrobenzene exposure (Goldstein et al. 1984; Reddy et al. 1976). Germ-free rats do not develop methemoglobinemia when exposed to nitrobenzene by oral administration (Reddy et al. 1976).

Many of the effects induced by nitrobenzene result from production of methemoglobin, along with the destruction of erythrocytes and oxidative stress. Hematology changes include decreases in red blood cell counts and decreases in hemoglobin concentration. These effects trigger stimulation of hematopoiesis in the bone marrow, increases in reticulocyte counts, and extramedullary hematopoiesis in the spleen and liver. As the spleen is the primary site where damaged erythrocytes are scavenged, splenic congestion and hemosiderosis are common findings, and may result in relative depletion of lymphoid cells. The liver may also participate in phagocytosis of damaged red blood cells, and this organ is where heme is recycled, leading to increases in bilirubin as the heme molecule is broken down. Some renal effects of nitrobenzene may also be attributable to its hematotoxicity, as free hemoglobin from lysed erythrocytes

can form nephrotoxic dimers. In addition, due to their roles in elimination of damaged red blood cells, the spleen, liver, and kidneys are also exposed to oxidants released from these cells. As detailed below and in Tables 2-1, 2-2, and 2-3, studies in laboratory animals exposed by inhalation, oral, and dermal routes have demonstrated all of these effects.

Human Studies. Case studies reported elevated methemoglobin levels in workers exposed to nitrobenzene via inhalation in the workplace (Ikeda and Kita 1964; Lee et al. 2013). Airborne concentrations of nitrobenzene to which the workers were exposed were not reported. In addition, numerous cases of methemoglobinemia in humans have been reported due to oral exposure to nitrobenzene (Agrawal et al. 2011; Balwani et al. 2017; Boukobza et al. 2015; Chongtham et al. 1997; D'sa et al. 2014; Kumar et al. 1990; Perera et al. 2009; Saxena and Prakash Saxena 2010), including several that resulted in fatalities (Gupta et al. 2000, 2012; Kumar et al. 2017; Martínez et al. 2003). Mallouh and Sarette (1993) reported the case of a 2-month-old infant who was exposed by dermal contact with hair oil containing 1% nitrobenzene. The infant had a methemoglobin level of 31.5%, which gradually dropped over 3 days without treatment (Mallouh and Sarette). Additionally, Ewert et al. (1998) described methemoglobinemia and related symptoms occurring in a 33-year-old man who attempted suicide by intravenously injecting "India ink," which contained nitrobenzene. Blood and urine samples confirmed the presence of nitrobenzene.

Animal Studies. As with humans, dose-related increases in the amount of methemoglobin in the blood have been shown in studies of rats, mice, and rabbits after acute-, intermediate-, and/or chronic-duration exposures to nitrobenzene (Biodynamics 1983, 1984; Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b).

In acute-duration, 2-week inhalation exposure studies in rats and mice (Medinsky and Irons 1985), hematology, spleen weight, and splenic histopathology changes attributable to nitrobenzene exposure were observed at all exposure levels (9.1, 35.8, and 124.5 ppm). In this study, half of each group (5/species/sex/exposure level) were sacrificed 3 days after the end of exposure and the other half were sacrificed 14 days after exposure termination. Blood samples were collected for hematology and methemoglobin analysis at sacrifice. Methemoglobin levels were statistically significantly increased only in female CD rats exposed to 124.5 ppm from the group scheduled to be sacrificed three days after the end of exposure (Medinsky and Irons 1985). However, all CD rats exposed to 124.5 ppm were reported to have died or been humanely sacrificed at the end of the first week of exposure; thus, the timing of the blood sample collection and methemoglobin analysis is uncertain. The lack of effect on methemoglobin

in surviving animals of other exposure groups could have resulted from recovery occurring during the post-exposure period, or from a delay in analysis after sampling.

Hematologic effects seen in the exposed animals included decreased red blood counts in F344 and CD rats at all exposure levels; other hematology changes were restricted to the highest exposure group (124.5 ppm) in which all CD rats and all mice died or were euthanized early (Medinsky and Irons 1985). Concentration-dependent increases in relative spleen weight were observed in rats, with marked increases at higher exposure concentrations; organ weights were not reported for mice. At the lowest exposure level (9.1 ppm), spleen weights measured 3 days after the end of exposure were increased by 33% in female F344 rats and 44% in female CD rats. Male rats of both strains exhibited significant increases at ≥35.8 ppm of at least 74% relative to controls. The study authors stated that "splenic lesions were evident in all animals and dose groups exposed to nitrobenzene;" however, Medinsky and Irons (1985) reported quantitative histopathology data only for the 35.8 and 124.5 ppm groups and not for the control or 9.1 ppm exposure groups. As a result, the specific nature and incidences of splenic lesions in the 9.1 ppm groups are not known. At 35.8 ppm, nearly all animals of all sexes and strains (9/10 or 10/10 per group) showed extramedullary hematopoiesis in the spleen. In addition, all rats of both strains and sexes had hemosiderosis, while all F344 rats and about half of the CD rats, along with half of the female mice showed sinusoidal congestion. At 124.5 ppm, 90–100% of rats and mice examined demonstrated hemosiderin-laden macrophages in red pulp, extramedullary hematopoiesis, and acute congestion of the spleen (Medinsky and Irons 1985).

Many of the same effects were also seen with 90 days of inhalation exposure to nitrobenzene in mice (B6C31) and rats (Sprague-Dawley and Fischer 344) (Hamm et al. 1984). Increased serum methemoglobin was observed at all exposure levels (≥5 ppm) in male F344 rats; at ≥15.8 ppm in female F344 and male CD rats; and at 48.7 ppm in female CD rats and mice of both sexes. Hematology changes indicative of hemolytic anemia were seen in rats but not mice. Decreased erythrocyte counts, hematocrit, and/or hemoglobin, and increased erythrocyte width were evident at all exposure concentrations (≥5 ppm) in female F344 rats, at ≥15.8 ppm in male and female CD rats, and at 48.7 ppm in male F344 rats. Male and female F344 rats exhibited increased absolute spleen weights at ≥15.8 ppm; similarly, male and female CD rats had increased absolute and relative spleen weights (≥15.8 ppm in females and at 48.7 ppm in males). Spleen weights were increased in male and female mice exposed to 48.7 ppm (Hamm et al. 1984). Increased incidences of splenic lesions were observed at all levels in both rats and mice of both sexes. Apart from male mice, all animals in all treatment groups exhibited sinusoidal congestion in the spleen, while this finding was absent in all control groups. Male and female F344 rats and male CD rats

also exhibited increased severity of hemosiderin deposition at all exposure concentrations (with severity score generally increasing from slight in controls to moderate at 5 ppm). In F344 rats, additional treatment-related effects seen at the highest concentration (48.7 ppm) included proliferation of mesenchymal cells in the spleen, fibroblastic hyperplasia of the splenic capsule, accumulation of lymphocytes and macrophages in the spleen, extramedullary hematopoiesis in the spleen, and bone marrow hyperplasia. Male and female mice of all treatment groups showed increased incidences (but not severity) of hemosiderin deposition in the spleen. At the highest exposure level in B6C3F1 mice, increased bone marrow hyperplasia was observed (Hamm et al. 1984).

The hematology and histopathology changes observed in the acute- and intermediate- duration inhalation studies were also observed in a chronic-duration, 2-year study of the same species. In this study, F344 (both sexes) and CD (male) rats were exposed to 0, 1, 5, and 24.8 ppm of nitrobenzene and B6C3F1mice (both sexes) to 0, 5, 24.8, and 49.1 ppm nitrobenzene (Cattley et al. 1994, 1995; CIIT 1993). Male and female F344 rats exhibited decreased erythrocyte counts, hematocrit, and hemoglobin as well as increased methemoglobin at 24.8 ppm; these changes were evident at both the interim and final sacrifices in F344 rats. At the interim sacrifice, male CD rats exhibited increased methemoglobin at all exposure levels (≥1 ppm), while at termination the difference from control was significant only at 24.8 ppm. Spleen weights were not altered by exposure at any concentration in rats. Increased incidences of spleen congestion were reported in male and female F344 rats and male CD rats at all exposure levels (≥1 ppm), and increased spleen pigmentation was reported at all exposure levels in male F344 rats. In male mice, the hematology changes consisted of decreased erythrocyte counts and hematocrit, and increased methemoglobin at 49.1 ppm. In female mice, methemoglobin was increased at ≥24.8 ppm (Cattley et al. 1994, 1995; CIIT 1993). No changes in spleen weights were observed in mice. In addition, splenic lesions were not observed in male mice, but females showed an increased incidence of lymphoid hyperplasia in the spleen at 49.1 ppm. Finally, an increased incidence of bone marrow hypercellularity was reported for male mice exposed to 49.1 ppm (Cattley et al. 1994, 1995; CIIT 1993).

In a 14-day immunotoxicity study of oral exposure to nitrobenzene in female B6C3F1 mice, exposure to nitrobenzene at all doses (≥30 mg/kg/day) resulted in proliferative effects on the bone marrow, measured as increased deoxyribonucleic acid (DNA) synthesis and increased numbers of cells per femur (Burns et al. 1994). The numbers of granulocyte-monocyte colony-forming units (CFUs) were increased when expressed as number per femur, but not when expressed as number per 10^5 bone marrow cells. The study authors noted that the assays did not distinguish the specific cell population(s) responsible for the increase in DNA synthesis (Burns et al. 1994). However, a dose-dependent increase in the numbers of peripheral

blood reticulocytes was also observed in exposed mice, with increases of >3-fold (relative to controls) in the 100 and 300 mg/kg/day groups, suggesting stimulation of bone marrow erythropoiesis. The immature erythrocytes (reticulocyte) resulted in compensatory increases in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) starting at doses of 100 mg/kg. Hepatomegaly and splenomegaly were observed by the researchers, as were pathological observations consistent with extramedullary hematopoiesis.

In a repeat-dose and reproductive/developmental toxicity screening study, Mitsumori et al. (1994) observed significant hematological effects in male Sprague-Dawley rats with 54 days of oral exposure to nitrobenzene at doses ranging from 20 to 100 mg/kg/day. Dose-dependent increases in methemoglobin and decreases in erythrocyte count, hemoglobin concentration, and hematocrit were seen at all doses (≥20 mg/kg/day). At doses ≥60 mg/kg/day, there were significant increases in MCV, mean erythrocyte hemoglobin concentration, reticulocytes, and erythroblasts. Absolute and relative spleen weights were increased at all dose levels (≥60% relative to controls). Mitsumori et al. (1994) also observed extramedullary hematopoiesis and hemosiderin deposition in the spleen and increased hematopoiesis in bone marrow in all treated rats at all dose levels (≥20 mg/kg/day).

In intermediate-duration oral toxicity studies in rats and mice conducted by NTP (1983a), B6C3F1 mice and F344 rats of both sexes were exposed to doses ranging from 9.375 to 150 mg/kg/day for rats and from 18.75 to 300 mg/kg/day for mice. Because only one male rat survived in the 150 mg/kg/day dose group, results from this group are not informative. Mice and rats of both sexes had increased methemoglobin levels at all doses. Rats of both sexes had increased reticulocytes and decreased hemoglobin at ≥9.375 mg/kg/day. In addition, male rats had decreased MCV and MCH, while female rats had decreased hematocrit at all doses. Increased reticulocytes were observed in female mice at ≥18.75 mg/kg/day and in males at ≥37.5 mg/kg/day; decreases in red blood cells, hematocrit, and hemoglobin were seen in both sexes at ≥150 mg/kg/day. Male mice exhibited anisocytosis and polychromasia at 300 mg/kg/day. Histological effects included congestion of the spleen in most of the treated rats of both sexes. Hemosiderin pigment was observed in the red pulp of the spleen, while lymphoid depletion was noted in the white pulp. In mice, lymphoid depletion of the spleen was noted at ≥150 mg/kg/day.

Dermal exposure studies have demonstrated effects very similar to those seen in inhalation and oral studies. NTP (1982) noted increased methemoglobin; decreased hemoglobin, hematocrit, and erythrocyte counts; increased MCV, MCH, reticulocytes; and spleen congestion at doses of 0.2 g/kg when nitrobenzene was applied to the skin of the intrascapular region of F344 rats for 12 days. B6C3F1 mice

exhibited decreased hemoglobin concentrations and erythrocyte counts, along with increased reticulocytes at ≥0.2 g/kg (NTP 1982). In a 90-day dermal exposure study with both F344 rats and B6C3F1 mice, increased methemoglobin was seen in both species and sexes, and changes consistent with hemolytic anemia (decreased hemoglobin and erythrocyte counts, increased reticulocytes) were observed at all doses (≥0.05 g/kg) (NTP 1983b). Spleen congestion was seen in all groups of treated rats but only in mice exposed to the highest dose (0.8 g/kg).

2.8 MUSCULOSKELETAL

No studies were located regarding musculoskeletal effects in humans or animals after inhalation, oral, or dermal exposure to nitrobenzene. In the chronic-duration inhalation study of nitrobenzene (Cattley et al. 1994, 1995; CIIT 1993), increased incidences of mineralization of the stomach muscle and fibrous osteodystrophy of the nose and bone were observed in male CD rats at the highest exposure concentration (24.8 ppm). These changes were considered secondary effects of the severe chronic nephropathy observed at this exposure level (CIIT 1993).

2.9 HEPATIC

There is some evidence that the human liver is affected after exposure to nitrobenzene. Ikeda and Kita (1964) reported that a woman who was occupationally exposed via inhalation to nitrobenzene for 17 months had an enlarged and tender liver; liver function tests showed marked retention of BSP and slight increases in icterus index and indirect bilirubin level (Ikeda and Kita 1964). Nitrobenzene exposure levels were not measured or estimated. Gupta et al. (2012) presented a case of a 17-year-old female who died by suicide after consuming an unknown quantity of nitrobenzene, resulting in severe methemoglobinemia. At autopsy, hepatic centrilobular necrosis was observed in the patient (Gupta et al. 2012). No studies were located regarding hepatic effects in humans after dermal exposure to nitrobenzene.

Treatment-related liver effects, including increased liver weights, clinical chemistry changes, and a wide range of histopathology changes (including necrosis, degeneration, eosinophilic foci, basophilia, spongiosis hepatis, and hepatocellular hypertrophy) have been observed in rats and mice exposed by inhalation, oral, and dermal routes. In addition to these changes, some studies have reported hepatic effects such as Kupffer cell pigmentation that likely stem from nitrobenzene-induced erythrocyte

destruction. Studies in rats and mice show species, strain, and sex differences in the nature and severity of liver effects induced by nitrobenzene.

In acute-duration studies of F344 rats, CD rats, and B6C3F1 mice exposed by inhalation for 14 days, Medinsky and Irons (1985) observed increased liver weights in male F344 rats exposed to nitrobenzene concentrations ≥9.1 ppm and in female F344 rats exposed to concentrations ≥35.8 ppm. Upon microscopic examination, however, there were no histopathology changes in the livers of F344 rats. In contrast, no liver weight changes were seen in CD rats of either sex at the same exposure levels, but male CD rats exhibited mild hepatocyte necrosis at 35.8 ppm. At the highest exposure level (124.5 ppm), all male and female CD rats died or were sacrificed moribund by the end of the first week; these rats exhibited sinusoidal congestion, along with centrilobular hydropic degeneration and periportal basophilic hepatocyte degeneration (Medinsky and Irons 1985).

In developmental toxicity studies, maternal liver weight did not differ significantly from controls when pregnant CD rats were exposed to concentrations up to 39.4 ppm on GDs 6–15 (Tyl et al. 1987) or when pregnant New Zealand white rabbits were exposed to concentrations up to 104 ppm on GDs 7–19 (Biodynamics 1983, 1984). No other maternal hepatic endpoints were evaluated in the developmental toxicity studies.

Hamm et al. (1984) conducted a 90-day inhalation exposure study in B6C3F1 mice and CD and F344 rats exposed to nitrobenzene concentrations of 5, 15.8, and 48.7 ppm. Increased absolute and relative liver weights were observed at ≥15.8 ppm in male and female F344 rats. Histopathology changes in F344 rats were restricted to the highest exposure group; both males and females exposed to 48.7 ppm exhibited disorganized hepatic cords, vascular ectasia in the liver, and centrilobular hepatocyte degeneration. Male F344 rats also had increased incidences of periportal hepatocyte basophilia while females had increased incidences of focal necrosis. CD rats were somewhat more sensitive to the hepatic effects of nitrobenzene. In male CD rats, increased incidences of microgranulomas in the liver were noted at all exposure concentrations (≥5 ppm), without changes in liver weight. Female CD rats showed increased absolute and relative liver weights as well as increased incidences of centrilobular hepatocyte hypertrophy ≥15.8 ppm (Hamm et al. 1984). Intermediate-duration inhalation exposure to nitrobenzene at concentrations up to 15.8 ppm did not result in liver effects in mice (Hamm et al. 1984). At the highest concentration (48.7 ppm), liver weights were increased in both male and female mice; serum alanine aminotransferase (ALT) was increased by ~2-fold in male mice; and increased incidences of hepatic

lesions were seen (centrilobular hepatocyte hyperplasia with some cord disorganization in females and basophilic hepatocytes in males).

In the 2-year study of inhalation exposure, the liver was a target organ for nitrobenzene in both rats and mice (Cattley et al. 1994, 1995; CIIT 1993). In male F344 rats, increased incidences of eosinophilic foci and centrilobular hepatocytomegaly were observed at concentrations ≥5 ppm, while increases in liver weight and an increased incidence of spongiosis hepatis were seen at 24.8 ppm. Increased liver weights and increased incidences of eosinophilic foci and spongiosis hepatis were noted in female F344 rats exposed to 24.8 ppm; no hepatic effects were seen at lower exposure levels in the females. Male CD rats showed increased incidences of pigmentation in the Kupffer cells at all exposure levels (≥1 ppm) (Cattley et al. 1994, 1995; CIIT 1993). In addition, increased centrilobular hepatocytomegaly and increased spongiosis hepatis occurred in male CD rats at ≥5 and 24.8 ppm, respectively. In mice, increased incidences of centrilobular hepatocytomegaly and multinucleated hepatocytes were reported in males at all exposure levels (≥5); females exhibited an increased incidence of centrilobular hepatocytomegaly only at the highest exposure (49.1 ppm) (Cattley et al. 1994, 1995; CIIT 1993). In rats and female mice, nitrobenzene exposure also resulted in dose-related trends or significant increases in the incidences of liver tumors, while liver tumor incidences were not altered by exposure in male mice (see Section 2.19, Cancer).

Female B6C3F1 mice exposed to nitrobenzene by gavage for 14 days in a series of immunotoxicity experiments showed increased liver weight and hepatomegaly at doses ≥100 mg/kg/day (Burns et al. 1994). Further, at 300 mg/kg/day (a dose at which 8.5% of mice died), serum ALT was significantly increased by >2-fold, and minor histopathological changes including mild hydropic degeneration around the focal central veins were seen in the female mice (Burns et al. 1994). In a repeated-dose and reproductive/developmental toxicity screening study (Mitsumori et al. 1994), male Sprague-Dawley rats exposed for 54 days to doses ≥20 mg/kg/day exhibited increased liver weights and centrilobular hepatocyte swelling, along with increased incidences of microscopic lesions indicative of hemolysis (hemosiderin deposition in Kupffer cells and extramedullary hematopoiesis). Liver weights and histopathology were not evaluated in females in this study (Mitsumori et al. 1994).

With longer (90-day) oral exposure, hepatic effects were seen at lower doses. NTP (1983a) reported increases in liver weights at all doses in F344 rats (≥9.375 mg/kg/day); however, no treatment-related increases in the incidences of histopathology findings were observed at these doses. At the highest dose (150 mg/kg/day), which resulted in premature mortalities in 9/10 males and 3/10 females, congestion in

the liver was seen (NTP 1983a). Increases in liver weight without histopathology changes were observed in female mice at all doses (≥18.75 mg/kg/day) in the 90-day gavage study (NTP 1983a). In male mice, liver weight increased at 150 and 300 mg/kg/day, and there was a significant increase in the incidence of hepatocellular cytomegaly at 300 mg/kg/day. Mortalities among male mice at 300 mg/kg/day limits interpretation of hepatic effects at that dose (NTP 1983a). Cytomegaly was observed in one and two male mice each in the 75 and 150 mg/kg/day groups, respectively (NTP 1983a).

In acute-duration (12 days) and intermediate-duration (90 days) studies of dermal exposure to nitrobenzene, similar hepatic effects were reported. Both F344 rats and female B6C3F1 mice exhibited increased liver weights after 12 days of dermal exposure (NTP 1982). Rats of both sexes showed higher liver weights at doses of ≥0.2 g/kg. The only dose-related histopathology finding in the liver of rats was an increase in the incidence of mild hematopoiesis in males. In female mice, increased liver weights occurred at doses ≥0.4 g/kg, while male mice showed a significant increase in relative (to body weight) liver weight at 0.8 g/kg (NTP 1982). Histopathology examination in the mice was limited to the 1.6 g/kg group, and all mice died or were sacrificed moribund prior to study termination in this group. Hepatic congestion was reported in a few of the decedents at this dose (NTP 1982).

After 90 days of dermal exposure to nitrobenzene, male and female F344 rats exhibited increased relative liver at ≥0.1 and ≥0.2 g/kg, respectively (NTP 1983b). Histopathology findings in the liver were confined to the high dose of 0.8 g/kg, a dose at which all rats of both sexes died prematurely. Findings in the livers of the decedents included hepatic congestion with blood pooled in the hepatic artery, portal and collecting veins, and sinusoids (NTP 1983b). In mice exposed for 90 days by dermal application of 0.4 g/kg, increased absolute and relative liver weights were seen in females and increased absolute (but not relative) liver weight was observed in males (NTP 1983b). No microscopic lesions were observed in the livers of female mice at 0.4 g/kg, but all male mice exposed to this dose exhibited hepatic cytomegaly. At the high dose of 0.8 g/kg, which also resulted in significant mortality in mice, 8/10 female mice showed hepatocellular cytomegaly, and 6/10 male mice had yellow pigmented cells in the liver (NTP 1983b).

2.10 RENAL

No studies were located regarding renal effects in humans after inhalation or dermal exposure to nitrobenzene. One publication (Gupta et al. 2012) reported a case of renal tubular necrosis identified at autopsy following the death of a 17-year-old girl by nitrobenzene ingestion. The amount of nitrobenzene ingested was unknown.

In developmental toxicity studies of rats and rabbits exposed by inhalation during gestation, no effects on maternal kidney weights were observed at concentrations up to 39.4 and 104 ppm, respectively (Tyl et al. 1987; Biodynamics 1983, 1984). Renal histopathology was not evaluated in these studies.

Dose-related increases in kidney weights were observed in F344 rats of both sexes exposed to nitrobenzene via inhalation for 14 days (Medinsky and Irons 1985). The increases were statistically significant at concentrations ≥9.1 ppm in males and at ≥35.8 ppm in females in animals sacrificed 3 days after the end of exposure; the changes were no longer evident in the groups sacrificed 14 days after the end of exposure (Medinsky and Irons 1985). All (10/10) male and two (2/10) female F344 rats exposed to 124.5 ppm nitrobenzene exhibited moderate to severe hyaline nephrosis that also regressed after the recovery period. Kidney weight changes were not observed at any exposure concentration in male or female CD rats in this study (Medinsky and Irons 1985). At 124.5 ppm, all CD rats died or were sacrificed moribund by the end of the first week of exposure; in these animals, moderate to severe hydropic degeneration of the cortical tubular cells was a frequent finding. Male CD rats that died or were humanely sacrificed after exposure to this concentration also exhibited degenerative changes in the kidneys (focal hyalinosis and basophilic degeneration of tubular epithelial cells) (Medinsky and Irons 1985). Renal effects were also reported in B6C3F1 mice in this study; however, due to limitations in the information reported, neither a NOAEL nor a LOAEL could be determined. The study authors indicated that degenerative changes were observed in the renal tubular epithelium of "a small number of mice and were less severe than those described for CD male rats," and that males exposed to 35.8 ppm exhibited this finding. The report did not tabulate these results or report quantitative results. It should be noted that the highest exposure level of 124.5 ppm was also lethal to all mice before the end of the planned exposure period.

Using the same three animal models exposed to nitrobenzene at 5–48.7 ppm for 90 days, Hamm et al. (1984) observed dose-related renal lesions, characterized only as nephrosis, in both rat strains but not in mice. Male F344 rats appeared to be slightly more susceptible, with significant increases in the incidence of nephrosis occurring at concentrations ≥5 ppm, compared with 48.7 ppm in female F344 rats and male CD rats. Female CD rats did not exhibit this finding at any concentration (Hamm et al. 1984). The only group of rats that exhibited a treatment-related change in kidney weight was male CD rats exposed to 48.7 ppm; in this group, a significant increase in kidney weight was noted (Hamm et al. 1984). Neither male nor female B6C3F1 mice exposed to nitrobenzene showed changes in kidney histology (Hamm et al. 1984). Male mice exposed to 47.7 ppm showed increases in absolute and relative-to-brain-weight kidney weight, but not relative-to-body-weight kidney weight.

In the chronic-duration study of nitrobenzene inhalation exposure (Cattley et al. 1994, 1995; CIIT 1993), organ weight changes in both male and female F344 rats included increased absolute and relative kidney weights at 24.8 ppm (the highest concentration tested). In F344 rats, increases in renal tubular hyperplasia, cysts (in males) and chronic nephropathy (females) were reported at 24.8 ppm, as were increases in renal tubular suppurative inflammation in both sexes. Male CD rats showed no significant changes in kidney weights at any exposure level; the only renal effect observed in these animals was mineralization at the highest concentration of 24.8 ppm. CIIT (1993) noted, however, that the male CD rats in the highest exposure group exhibited changes associated with secondary hyperparathyroidism (mineralization and fibrous osteodystrophy in various organs) that suggested that the severity of nephropathy was increased by nitrobenzene treatment (without a change in incidence of the finding).

In female mice, absolute and relative kidney weights were increased at the highest exposure level (49.1 ppm); no biologically relevant organ weight changes were observed in male mice (Cattley et al. 1994, 1995; CIIT 1993). Male, but not female mice showed a higher incidence of kidney cysts at 49.1 ppm (Cattley et al. 1994, 1995; CIIT 1993). No other renal lesions were noted in mice.

A 14-day study of male Wistar rats exposed to nitrobenzene by gavage demonstrated renal effects at the one dose level tested, 100 mg/kg/day (Oladele et al. 2021). The findings included increases in serum urea and creatinine, as well as lesions consisting of mild fibrosis, hemorrhage, and marked glomerular shrinkage (Oladele et al. 2021). In an acute-duration immunotoxicity study that included assessment of kidney weights, female B6C3F1 mice exposed to nitrobenzene for 14 days via gavage had increases in absolute, but not relative kidney weight with 300 mg/kg/day exposure (Burns et al. 1994). There was not a significant increase in kidney weight at 100 mg/kg/day in that study (Burns et al. 1994). In addition, histopathology examination showed no renal effects in the female mice at any dose (Burns et al. 1994).

Intermediate-duration oral exposure to nitrobenzene induced significant increases in kidney weights in male Sprague-Dawley rats after 54 days of exposure to doses ≥60 mg/kg/day (Mitsumori et al. 1994) and in male and female F344 rats after 90 days of exposure to ≥9.375 and ≥75 mg/kg/day, respectively (NTP 1983a). At the same doses, male Sprague-Dawley and female F344 rats also exhibited histopathology changes that were suggestive of hemolysis (hemosiderin or pigment deposition). Additionally, NTP (1983a) reported the observation of pale green hyaline globules in renal cortical tubular cells in rats of the 75 and 150 mg/kg/day dose groups; no further description of these findings was provided. In male mice in the 90-day gavage study, kidney weights were increased at 300 mg/kg/day nitrobenzene, a dose at

which half of the male mice died prematurely (NTP 1983a). In female mice, relative (to body weight) kidney weight was significantly increased at the 300 mg/kg/day dose level, but the difference from controls was not significant for absolute kidney weight or kidney weight relative to brain weight (NTP 1983a). No microscopic kidney lesions were seen in the mice (NTP 1983a).

In studies of dermal exposure, kidney effects were observed after acute- and intermediate-duration exposures to nitrobenzene. In F344 rats exposed for 12 days, kidney weights were increased in males at 0.8 g/kg, while female rats exhibited no treatment-related change in kidney weight. Renal histopathology changes were seen only at the highest dose (1.6 g/kg), a dose that was lethal to all animals. Females, but not males, that died prematurely at this dose showed evidence of cytoplasmic vacuolation and degeneration in renal cortical tubules (NTP 1982). In B6C3F1 mice in the same study, no change in kidney weights were seen in either males or females (NTP 1982). As with rats, all mice exposed to 1.6 g/kg died or were sacrificed moribund prior to study termination, and in mice, this was the only group examined for histopathology. There were no microscopic kidney findings in this group (NTP 1982).

In the 90-day dermal application study, F344 rats exposed to 0.4 g/kg (the highest dose at which any rats survived to study termination) exhibited congestion in the kidneys (NTP 1983b). Kidney weights were not altered by exposure in female rats, but there were small increases in relative (to body weight) kidney weights in males exposed to 0.2 and 0.4 g/kg (NTP 1983b). Neither absolute kidney weights nor kidney weights relative to brain weight were altered at either of these doses in the male rats (NTP 1983b). Male mice exposed to nitrobenzene by dermal application exhibited dose-related increases in kidney weights at ≥0.2 g/kg (NTP 1983b). No changes to kidney weight were seen at doses up to 0.4 g/kg in female mice (the highest dose of 0.8 g/kg resulted in deaths of nearly all mice prior to study termination). No treatment-related renal lesions were observed in the mice (NTP 1983b).

Mechanisms. Nitrobenzene induced oxidative stress in the kidneys of rats given 100 mg/kg/day nitrobenzene by gavage for 14 days (Oladele et al. 2021). The study authors reported increases in kidney levels of malondialdehyde, nitric oxide, myeloperoxidase, and hydrogen peroxide along with concurrent reductions in renal concentrations of reduced glutathione, superoxide dismutase, and catalase at the end of the exposure period. No other data on potential mechanisms of nitrobenzene-induced kidney changes were located.

2.11 DERMAL

No human studies that assessed the dermal toxicity of nitrobenzene from inhalation, oral, or dermal exposure were located. Additionally, no studies were located that described any dermal effects in experimental animals after inhalation or oral ingestion of nitrobenzene. Studies in which nitrobenzene was applied directly to the skin of the animal did not report significant dermal effects (NTP 1982, 1983b).

2.12 OCULAR

No human studies have been located that assessed the ocular toxicity of nitrobenzene from inhalation, oral, or dermal exposure. Additionally, no studies were located that described any ocular effects after oral ingestion or dermal exposure to nitrobenzene in experimental animals.

Slight corneal clouding was observed in Crl:CD rats after 4 hours of inhalation exposure at a concentration of 514 ppm in an acute lethality study (Dupont 1981).

2.13 ENDOCRINE

No studies were located that evaluated the endocrine effects from nitrobenzene exposure in humans by any route of exposure.

In experimental animal studies, effects on the adrenal glands have been observed in F344 and CD rats exposed by inhalation (Hamm et al. 1984), and in mice exposed by inhalation (Hamm et al. 1984; Cattley et al. 1994, 1995; CIIT 1993), oral (NTP 1983a), and dermal (NTP 1983b) exposure routes. Both male and female F344 rats and male CD rats exhibited increased basophilia of adrenal medullary cells after 90 days of exposure to 48.7 ppm (Hamm et al. 1984), but no adrenal effects were observed after 2 years of exposure to nitrobenzene concentrations up to 24.8 ppm. Female mice exhibited adrenal gland cortical cell vacuolization or fatty change after 90 days of inhalation exposure to ≥5 ppm (Hamm et al. 1984), after 2 years of inhalation exposure to ≥24.8 ppm (Cattley et al. 1994, 1995; CIIT 1993), after 90 days of oral exposure to 300 mg/kg/day (NTP 1983a), and after 90 days of dermal exposure to ≥0.05 g/kg (NTP 1983b). Male mice did not show evidence of nitrobenzene-induced effects on the adrenal glands.

Thyroid changes associated with nitrobenzene exposure were observed in male CD rats and male B6C3F1 mice exposed by inhalation. Slight thyroid follicular cell hypertrophy was noted in male CD rats after

90 days of exposure to 48.7 ppm nitrobenzene (Hamm et al. 1984). Male mice exhibited thyroid follicular cell hyperplasia after 2 years of exposure to concentrations ≥24.8 ppm (Cattley et al. 1994, 1995; CIIT 1993). No thyroid effects were noted in male or female F344 rats, female CD rats, or female B6C3F1 mice exposed to nitrobenzene by inhalation (Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984).

Chronic-duration inhalation exposure to nitrobenzene resulted in microscopic changes in the pancreas of female B6C3F1 mice and the parathyroid gland of male F344 rats. In female mice exposed to 49.1 ppm nitrobenzene for 2 years, an increase in the incidence of mononuclear cell infiltrate in the pancreas was observed (Cattley et al. 1994, 1995; CIIT 1993). Male F344 rats showed an increase in the incidence of diffuse hyperplasia of the parathyroid gland after exposure to 24.8 ppm nitrobenzene (Cattley et al. 1994, 1995; CIIT 1993). There were no effects on the pancreas or parathyroid gland noted in female F344 rats, female CD rats, or female B6C3F1 mice exposed to nitrobenzene by inhalation (Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984) or in rats or mice exposed by oral or dermal routes (NTP 1983a, 1983b).

In an acute-duration oral study of nitrobenzene in male Wistar rats, exposure to 100 mg/kg/day for 14 days resulted in decreased serum levels of thyroid-stimulating hormone (TSH); other thyroid hormone levels or related endpoints were not evaluated in this study (Oladele et al. 2020a). No other thyroid effects were observed in rats or mice exposed by oral or dermal routes (NTP 1983a, 1983b).

Mechanisms. Data on potential mechanisms of the adrenal, thyroid, and parathyroid effects of nitrobenzene are limited to a single study of oxidative stress in thyroid cells. In an *in vitro* study using porcine thyroid cells, Zasada and Karbownik-Lewinska (2015) showed that exposure to nitrobenzene resulted in concentration-related increases in lipid peroxidation measured as malondialdehyde and 4-hydroxy alkenals. Concurrent treatment with antioxidants (melatonin and propylthiouracil) mitigated the nitrobenzene effects on oxidative stress (Zasada and Karbownik-Lewinska 2015).

2.14 IMMUNOLOGICAL

No studies were located that examined potential immunologic effects of nitrobenzene exposure on humans via any route of exposure.

After intermediate-duration inhalation exposure to nitrobenzene, proliferative changes in the lymph nodes were seen in F344 and CD rats, but not in mice (Hamm et al. 1984). These findings were described as

proliferation of plasma cells containing mitotic figures and extending as clusters or sheets from the subcapsular sinusoids, sometimes in conjunction with increased numbers of mast cells and macrophage infiltration at the margins. The incidences of these findings did not reach statistical significance (compared with control incidence) at any exposure level. In a chronic-duration inhalation study, involution of the thymus occurred at increased incidence in female B6C3F1 mice exposed to 49.1 ppm nitrobenzene (Cattley et al. 1994, 1995; CIIT 1993). There were no changes in the thymus of male mice or in rats in the same study exposed to concentrations up to 24.5 ppm nitrobenzene (Cattley et al. 1994, 1995; CIIT 1993). The thymus is known to shrink with age, so this finding should be interpreted within that context (Aspinall and Andrew 2000).

Only one study of nitrobenzene specifically focused on assessment of immune system effects. In this acute-duration oral exposure study (Burns et al. 1994), female B6C3F1 mice were administered 0, 30, 100, or 300 mg/kg of nitrobenzene in corn oil via gavage for 14 days in several different experiments. Thymus weight was measured, and the following immune function assays were assessed: spleen IgM and IgG antibody response following stimulation with sheep erythrocytes (sRBC), spleen cell proliferation following stimulation with mitogens, mixed leukocyte response to allogeneic spleen cells, delayed hypersensitivity response to keyhole limpet hemocyanin (KLH), measurement of serum complement proteins, reticuloendothelial system clearance of sRBCs, peritoneal cell number count, macrophage phagocytic activity, natural killer cell activity, and host resistance to various microbes and a tumor cell line.

There were mortalities (8.5% of animals) among the mice across several experiments at the 300 mg/kg/day dose (Burns et al. 1994). No treatment-related changes in thymus weight were observed, and differential leukocyte counts in peripheral blood were unchanged with the exception of a significant dose-related trend for decrease in eosinophil count. Nitrobenzene treatment did not significantly affect serum complement levels or delayed hypersensitivity response (Burns et al. 1994).

In the sRBC assay performed 4 days after the end of nitrobenzene exposure, there were significant increases in spleen weight and spleen cell number at 300 mg/kg/day and significantly reduced numbers of IgM antibody-forming cells (AFCs) at ≥100 mg/kg/day. On the 5th day after exposure, there were significant increases in spleen weight and spleen cell number at ≥100 mg/kg/day. No effect of treatment on IgG AFCs was detected. The effects on spleen weight and IgM AFC counts did not persist after a 20-day recovery period.

The spleen cell mitogenic response assay was confounded by an increase in background (in the absence of mitogen) 3H-thymidine incorporation induced by nitrobenzene doses of 100 and 300 mg/kg/day, evident also when measured as spleen cell number (Burns et al. 1994). When expressed as ^{3}H-thymidine incorporation, T-cell mitogen (phytohemagglutinin and concanavalin A) response appeared to be suppressed at ≥100 mg/kg/day nitrobenzene and there was no effect of treatment on B-cell mitogen (lipopolysaccharide) response. However, when expressed as stimulation indices, T-cell mitogenic response was stimulated and B-cell mitogenic response was inhibited at doses ≥100 mg/kg/day (Burns et al. 1994). The mixed lymphocyte response to allogeneic spleen cells from DBA/2 mice was suppressed with nitrobenzene exposure; however, this assay was also influenced by the background spleen cell proliferation induced by nitrobenzene. When expressed as stimulation index, a decrease was observed at ≥100 mg/kg/day, indicating that nitrobenzene reduced the ability of splenic T cells to recognize and respond to alloantigens. The ratio of responding and nonresponding cells in the spleen was likely altered by nitrobenzene-induced increase in numbers of non-immune cells. Finally, natural killer cell activity in the spleen, measured as the cells' ability to lyse the YAC-1 cell *in vitro* was decreased at ≥100 mg/kg/day (Burns et al. 1994). The study authors suggested that the percentage of natural killer cells in the spleen could have decreased due to increases in other cell types in the spleen.

Measurement of peritoneal cell numbers and their macrophage function showed increases in both at 300 mg/kg/day nitrobenzene (Burns et al. 1994). In assays of radiolabelled sRBC clearance by the fixed mononuclear phagocyte system, overall phagocytic activity was increased with dose, and macrophage activity was increased in the liver at 300 mg/kg/day and decreased in the spleen and lungs at ≥100 mg/kg/day. The study authors suggested that the decreases in spleen and lungs resulted from the increases in uptake by the liver (fewer particles available for uptake by other organs), and that the increased hepatic uptake was partly attributable to liver enlargement (Burns et al. 1994).

The host-resistance assays showed little effect of nitrobenzene, except when the mice were challenged with *Listeria monocytogenes* (Burns et al. 1994). Exposed mice were more susceptible (e.g., exhibited higher mortality) to *L. monocytogenes* at 100 and 300 mg/kg/day nitrobenzene. The study authors noted that resistance to *L. monocytogenes* is mediated by T-lymphocytes, macrophages, and complement activity (Burns et al. 1994).

Other studies provide limited data on immune system endpoints. F344 rats treated by gavage for 90 days showed dose-related increases in the incidence of lymphoid depletion in the spleen at doses ≥37.5 (males) and ≥18.75 mg/kg/day (females) (NTP 1983a). In female mice exposed by gavage for 90 days, lymphoid

depletion in the spleen was observed at doses ≥150 mg/kg/day (NTP 1983a). Only one male mouse at the 300 mg/kg/day dose exhibited this effect.

Lymphoid atrophy in the spleen was also seen in acute- and intermediate-duration studies of rats exposed by dermal application at doses ≥0.4 and 0.2 g/kg, respectively (NTP 1982, 1983b). Mice exposed dermally to nitrobenzene did not show this change. However, mice exposed to 0.8 g/kg (a dose that caused premature deaths in 9/10 males and 8/10 females) for 90 days by skin application had thymic atrophy with marked depletion of lymphocytes (NTP 1983b).

2.15 NEUROLOGICAL

Nitrobenzene has shown to induce neurological effects in humans and animals. Neurological effects were noted in the case of a woman who was occupationally exposed to nitrobenzene for 17 months at an unknown level. These effects included headache, nausea, vertigo, confusion, and paresthesia (Ikeda and Kita 1964). Similar effects were reported in case studies of acute oral ingestion of nitrobenzene (Carter 1936; Leader 1932; Myslak et al. 1971). Lesions in the brain (corpus callosum, centrum semiovale, dentate nuclei, and/or substantia nigra) have been observed in humans after accidental or intentional nitrobenzene ingestion (Dsouza et al. 2022; Kumar et al. 2017; Boukobza et al. 2015). In a case study of a woman who died by suicide after consumption of nitrobenzene, petechial hemorrhages were found in both cerebral hemispheres (Gupta et al. 2012). Levels of nitrobenzene associated with these effects were not reported in these studies. No studies on the effects on the neurological system in humans after dermal exposure were located.

In an acute lethality study, 4-hour exposure of male CRL:CD rats (Dupont 1981) to 439 ppm nitrobenzene resulted in hyperactive and aggressive behavior several days after exposure; at 555 ppm, rats exhibited tremors 1–2 days after exposure, and 6/10 rats died during this time frame. When CD rats and B6C3Fl mice were exposed to nitrobenzene at 124.5 ppm daily for 2 weeks, damage to the hindbrain (cerebellar peduncle), including bilateral cerebellar perivascular hemorrhage and malacia (cell breakdown), was observed in 8/19 mice (both sexes) and in 14/19 rats (both sexes) (Medinsky and Irons 1985). No brain lesions were found in F344 rats exposed to the same levels. The reason for these strain differences under similar conditions is not apparent. In the 90-day inhalation study, no neurologic signs of toxicity, alterations in brain weight, or brain histopathology changes were observed in B6C3F1 mice or F344 or CD rats exposed to concentrations up to 48.7 ppm nitrobenzene in air (Hamm et al. 1984).

Likewise, there were no effects on these parameters in rats and mice exposed to concentrations up to 24.8 and 49.1 ppm, respectively, for 2 years (Cattley et al. 1994, 1995; CIIT 1993).

A single gavage dose of 550 mg/kg nitrobenzene induced moderate to severe ataxia, loss of righting reflex, and lack of responsiveness to stimuli in male F344 rats (Morgan et al. 1985). At necropsy, these animals exhibited hemorrhages in the brain stem and cerebellum, and bilateral symmetric degeneration in the cerebellum and cerebellar peduncles (Morgan et al. 1985). Oladele et al. (2020b) evaluated neurobehavioral effects and brain histopathology in Wistar rats given 100 mg/kg/day nitrobenzene by daily gavage for 14 days. The exposed rats exhibited decreased exploratory behavior and increased defecation, and the study authors reported degenerative lesions in the cerebellum, cerebrum, and hippocampus. In addition, the study authors observed increased acetylcholinesterase activity and decreased dopamine levels in the brain (Oladele et al. 2020a). In a 2-week study of immune system effects, mice exposed to 300 mg/kg/day exhibited clinical signs of neurotoxicity including ataxia, circling behavior, and lethargy; at the same dose, there were premature mortalities related to treatment (Burns et al. 1994).

With oral exposure to 60 mg/kg/day in a repeat-dose and reproductive/developmental toxicity screening study, one female Sprague-Dawley rat experienced torticollis (a condition in which the neck muscles are contracted causing the head to tilt to one side) and abnormal gait during lactation (after about 50 days of exposure) (Mitsumori et al. 1994). Additionally, in this same study at 60 and 100 mg/kg/day, neuronal necrosis/gliosis in certain nuclei in the cerebellar medulla and pons were observed in 3/10 and 10/10 males, respectively (Mitsumori et al. 1994). Histopathology was not examined in the female rats in this study (Mitsumori et al. 1994).

Oral exposure to nitrobenzene for 90 days also resulted in clinical signs and neuropathology in rats and mice. Ataxia was observed in all female F344 rats exposed to 75 mg/kg/day (NTP 1983a). Male rats exposed to the highest dose of 150 mg/kg/day exhibited ataxia, lethargy, trembling, circling, and head tilt; at this dose, 9/10 males (along with 3/10 females) died before the end of the exposure period (NTP 1983a). It should be noted that all rats receiving doses of 75 and 150 mg/kg/day were cyanotic, which may have contributed to, and/or account for, the ataxia and other clinical signs. Brain weights were not affected by nitrobenzene exposure in rats, but histopathology changes consisting of brain stem hemorrhage, degeneration, and malacia were noted to occur at higher incidence (NTP 1983a). In males, hemorrhages were observed in the brain stem in 1/10, 4/10, 4/10, 5/10, and 2/10 animals exposed to 9.375, 18.75, 37.5, 75, and 150 mg/kg/day (respectively), but not in any control males. In female rats, a

higher incidence of brain stem hemorrhage was observed only in the 150 mg/kg/day group (7/10 versus 4/10 controls). At 150 mg/kg/day, both male and female rats exhibited degeneration and malacia in the brain stem (4/10 males and 3/10 or 4/10 females, compared with 0/10 controls of each sex). Other neuropathology findings in rats exposed to 150 mg/kg included degenerative changes in the pons, dentate nucleus, and cerebellar peduncle/olivary nucleus. These changes were described as spongy degeneration, necrosis, karyorrhexis, neutrophils, lymphocytes, plasma cells, and fusiform cells (NTP 1983a).

Mice were somewhat less sensitive to the neurological effects of nitrobenzene in the 90-day gavage study (NTP 1983a). Ataxia was observed at 300 mg/kg/day dose in 9/10 male and 1/10 female mice; three male mice (and no females) at this dose died prematurely (NTP 1983a). Females at this dose were characterized as irritable, and one female showed other neurological signs including hyperactivity and head bobbing. At 75 mg/kg/day, one male mouse was lethargic. Mice did not show cyanosis. There were no changes in brain weights and no increases in the incidences of histopathology findings in the brains of mice (NTP 1983a).

In the 12-day study of dermal exposure, all rats and mice died or were sacrificed moribund prior to study termination at doses ≥1.6 g/kg; ataxia, prostration, and dyspnea were seen in these animals prior to death (NTP 1982). Cyanosis was observed in all male and female rats by day 2 of treatment with ≥0.8 g/kg and in 2/5 male rats by day 8 of treatment with 0.4 g/kg (NTP 1982). At 0.8 g/kg, male Fischer F344 rats also displayed inactivity, possibly a consequence of hypoxia. No cyanosis or clinical signs of toxicity were noted in male rats at the lowest dose of 0.2 g/kg or in female rats treated with 0.2 or 0.4 g/kg (NTP 1982). The only histopathology findings in rats were small foci of hemorrhage in the cerebral and cerebellar cortex in 4/5 male and 5/5 female rats at the 1.6 g/kg dose (NTP 1982). Mice in this study did not show cyanosis at any dose, or clinical signs of neurotoxicity at doses that were not lethal (≤0.8 g/kg) (NTP 1982). As with the rats, 2/5 male mice and 1/5 female mice displayed small foci of hemorrhage in the cerebral and cerebellar cortex after dermal exposure to 1.6 g/kg nitrobenzene (NTP 1982).

In the follow-up 90-day dermal exposure study, all rats and 17/20 mice died prematurely with exposure to 0.8 g/kg nitrobenzene (NTP 1983b). Male and female rats displayed cyanosis, lethargy, and ataxia with exposure at this dose (NTP 1983b). Brain and brain stem hemorrhages were observed at low incidence in all groups of males including controls. At the highest dose of 0.8 g/kg, male and female rats exhibited effects in the cerebral and/or cerebellar white matter and the pons, including necrosis, vacuolization, and/or degeneration. Malacia was observed in the brain stem and pons in males and females at this dose. Control female rats showed no microscopic lesions in the brain. While there were no clear dose-related

trends for individual brain lesions in the treated female rats, when the reported lesions (brain and brain stem hemorrhages and vacuolization in the brain stem or cerebral or cerebellar white matter) were considered together, effects in the female rats occurred at doses as low as 0.1 g/kg (NTP 1983b). As was seen in the acute-duration study (NTP 1982), mice were less sensitive than rats to neurological effects of 90-day dermal exposure to nitrobenzene. Mice were not cyanotic at any dose, but did exhibit clinical signs including hypoactivity, circling, and head tilting or leaning with exposure to 0.8 g/kg nitrobenzene (NTP 1983b). There were no effects of treatment on brain weight or histopathology in mice (NTP 1983b).

Mechanisms. Some, but not all neurological effects may be mediated by hypoxia resulting from methemoglobinemia and/or erythrocyte hemolysis. As noted above, rats exhibiting neurological signs of toxicity were often cyanotic, while mice were not. There are few other data pertaining to neurotoxicity mechanisms of nitrobenzene. In the 14-day gavage study in Wistar rats described above, Oladele et al. (2020a) also showed that nitrobenzene exposure (100 mg/kg/day) increased oxidative stress in the cerebrum, mid brain, and cerebellum, as shown by statistically significant increases in malondialdehyde and hydrogen peroxide levels and decreases in levels of superoxide dismutase, reduced glutathione, and catalase. There is inconsistent information on the interaction between nitrobenzene and acetylcholinesterase activity. In cell-free experiments, nitrobenzene exhibited weak inhibition of acetylcholinesterase activity (Chen et al. 2019); however, in rats, oral exposure to 100 mg/kg/day nitrobenzene resulted in a statistically significant increase in brain acetylcholinesterase activity (Oladele et al. 2020a).

2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans following inhalation, oral, or dermal exposure to nitrobenzene. In experimental animals, nitrobenzene is a known testicular toxicant and has been used as a positive control in many studies aiming to evaluate toxic effects on spermatogenesis (Allenby et al. 1990, 1991; Linder et al. 1992). However, the evidence that nitrobenzene affects female reproduction in experimental animals is limited.

Testicular Effects. Evidence of testicular toxicity is seen in acute-, intermediate-, and chronic-duration studies of nitrobenzene exposure of rats and mice via inhalation, oral, and dermal routes. For example, in an acute-duration study in Fischer 344 rats, decreases in testicular weight and size were observed in rats exposed to 124.5 ppm for 2 weeks via inhalation (Medinsky and Irons 1985). Further, testicular lesions

were also observed in these rats, including an increase in multinucleated giant cells, Sertoli cell hyperplasia, interstitial edema, and severe dysfunctional spermiogenesis. Few sperm, with arrested maturation at primary and secondary spermatocyte stages, were present in seminiferous tubules, and the lumen of the ductus epididymis contained a reduced number of sperm. After a 2-week recovery period, the lesions were still present, although the Sertoli cell hyperplasia and the increased numbers of multinucleated giant cells were less severe (Medinsky and Irons 1985). At the same exposure concentration, male CD rats and B6C3F1 mice exhibited similar effects. In addition, male mice showed testicular degeneration and increased multinucleated giant cells at 35.8 ppm (Medinsky and Irons 1985).

Hamm et al. (1984) reported that both F344 and Sprague-Dawley rats exposed to nitrobenzene at 50 ppm for 90 days had testicular atrophy, bilateral degeneration of the seminiferous tubules, and a reduction in, or absence of, mature sperm in the epididymis. The lesions were more severe in Sprague-Dawley rats, which exhibited complete degeneration of the epithelium in seminiferous tubules. No testicular lesions were observed in B6C3Fl mice under the same exposure conditions (Hamm et al. 1984). In chronic-duration inhalation experiments conducted by Cattley et al. (1994, 1995; CIIT 1993), testicular lesions (bilateral atrophy of the testes and epididymal hypospermia) were observed in male CD rats at the highest exposure concentration (24.8 ppm) and epididymal hypospermia was observed in male mice at the highest concentration tested in that species (49.1 ppm) (Cattley et al. 1994, 1995; CIIT 1993).

Acute- and intermediate-duration oral studies in experimental animals provide additional evidence for testicular effects of nitrobenzene. In rats, single gavage doses of 250–300 mg/kg nitrobenzene resulted in decreased testicular weights, degeneration of seminiferous epithelium, sloughing of cells into the tubular lumen, loss of mature spermatids, increased numbers of multinucleated giant cells, decreased sperm counts, and abnormal sperm morphology (Levin et al. 1988; Linder et al. 1992; McLaren et al. 1993a). When nitrobenzene doses of 60 mg/kg/day were administered by gavage for 3 days, no testicular effects were seen (Kawaguchi et al. 2004), but with 14 days of exposure at this dose, testes and epididymal weights were decreased, as were sperm counts and motility (Iida et al. 1997; Kawashima et al. 1995). Two weeks of exposure by gavage to doses of 100 mg/kg/day reduced testicular and epididymal weights and also resulted in decreases in serum hormone levels (testosterone, prolactin, luteinizing hormone, and follicle stimulating hormone) in Wistar rats (Oladele et al. 2020c).

With intermediate-duration (21–90 days) oral exposures to ≥40 mg/kg/day, similar but more severe testicular effects were observed in rats (Iida et al. 1997; Kato et al. 2002; Kawaguchi et al. 2004; Kawashima et al. 1995; Mitsumori et al. 1994; NTP 1983a). As part of a study examining a new method

to evaluate mitochondrial function in sperm, mature male Sprague-Dawley rats were given nitrobenzene by daily gavage for 49 days before sperm were collected for analysis of motility (Kato et al. 2002). In the groups exposed to 40 and 60 mg/kg/day, there were no motile sperm; exposure to 20 mg/kg/day did not alter sperm motility (Kato et al. 2002). In the two rat studies that examined more than one dose level, isolated occurrences of seminiferous tubule atrophy were seen at doses of 20–37.5 mg/kg/day (Mitsumori et al. 1994; NTP 1983a). Only one intermediate-duration oral study in mice included examination of the testes. NTP (1983a) observed testicular atrophy in mice at 150 mg/kg/day, but not 75 mg/kg/day, after 90 days of treatment. At the highest dose in this study (300 mg/kg/day), half of the male mice died or were sacrificed moribund prior to termination (NTP 1983a).

In the two studies that evaluated nitrobenzene toxicity after dermal exposure, effects on the testes similar to those seen in the inhalation and oral studies were observed. After 12 days of dermal exposure to 0.8 g/kg, rats exhibited atrophy of the seminiferous tubules, absence of spermatids and spermatozoa, increased multinucleated giant cells, and decreased testes weight, while these effects were not seen in mice at the same doses (NTP1982). After 90 days of dermal exposure, similar but more severe testicular effects were seen at 0.4 g/kg in rats, while mice exhibited slightly less severe changes, including decreased testes weight, testicular atrophy, and hypospermatogenesis, at the same dose (NTP 1983b). Sloughing of spermatocytes, spermatids, and spermatozoa into the tubular lamina was reported in a few mice (incidence not reported) in the group dosed with 0.2 g/kg (NTP 1983b).

Other Reproductive Effects. Intermediate-duration (90 days) oral exposure to nitrobenzene resulted in uterine atrophy in two female rats given doses of 150 mg/kg/day (NTP 1983a). In addition, after 90 days of exposure via dermal application at 0.8 g/kg, 6/10 female rats and 5/10 female mice displayed atrophy of the uterus (NTP 1983b). It should be noted that all female rats and 8/10 female mice died before the end of the exposure period at this dose (0.8 g/kg). The study authors also reported that the ovaries of a few rats were congested (NTP 1983b); however, the exact number of rats experiencing this effect was not stated.

In a 2-generation inhalation study in Sprague-Dawley rats, 10 weeks of nitrobenzene exposure resulted in a decrease in fertility indices at 40 ppm for F0 and F1 generations, while other reproductive parameters were unaltered (Dodd et al. 1987). The study data suggested that the decrease in fertility was caused by males. Dodd et al. (1987) observed atrophy of seminiferous tubules, spermatocyte degeneration, and reduced testicular and epididymal weights in the F0 and F1 generations with 40 ppm exposure. When pregnant rats were exposed by inhalation to nitrobenzene concentrations up to 39.4 ppm on GDs 6–15 and New Zealand white rabbits were exposed to concentrations up to 104 ppm on GDs 7–19, there were no

significant adverse effects on numbers of corpora lutea, implantations, or resorptions (Biodynamics 1983, 1984; Tyl et al. 1987).

Despite the effects on testicular weight, sperm count, and sperm motility, oral exposure of male rats to 60 mg/kg/day nitrobenzene by gavage for 14 days did not affect copulation or fertility rates (Kawashima et al. 1995). However, when the same dose was administered for 21 or 28 days, the fertility index decreased to <20 and 0%, respectively (Kawashima et al. 1995). In the repeat-dose and reproductive/ developmental toxicity screening study reported by Mitsumori et al. (1994), male and female Sprague-Dawley rats were exposed from pre-mating through lactation day 4 (for a total of 54 days). Mitsumori et al. (1994) did not observe any differences in fertility or copulation indices at any dose (up to 100 mg/kg/day), although significant testicular toxicity was demonstrated at ≥60 mg/kg/day. Because the rats were only exposed for 14 days before mating, the lack of effect on fertility is not unexpected based on the findings of Kawashima et al. (1995); in fact, the study authors recommended a pre-mating treatment period longer than 14 days to detect effects on fertility. Testicular histopathology was not evaluated until the animals had been exposed for 54 days in the study by Mitsumori et al. (1994).

Mechanisms. The mechanism(s) by which nitrobenzene induces testicular toxicity and effects on spermatogenesis has not been fully elucidated, but may involve oxidative stress, induction of apoptosis in germ cells, and/or effects on Sertoli cell secretion of hormones or growth factors needed for germ cell differentiation and survival. Oladele et al. (2020c) observed increased lipid peroxidation and decreased levels of reduced glutathione in the testes of rats given 100 mg/kg/day nitrobenzene by daily gavage for 14 days. With this exposure regimen, the rats also exhibited decreased testicular and epididymal weights, as well as atrophic and degenerated seminiferous tubules (Oladele et al. 2020c). Using a cell-free system, Ohkuma and Kawanishi (1999) showed that nitrosobenzene, a metabolite of nitrobenzene, may cause oxidative DNA damage when this metabolite is reduced by reduced nicotinamide adenine dinucleotide (NADH) in the presence of Cu(II). The study authors suggested that the germ cell epithelium of the testis is exquisitely sensitive to oxidative DNA damage due to the continuous proliferation occurring in that tissue, and that oxidative DNA damage may be involved in the mechanism of nitrobenzene's effects on sperm production (Ohkuma and Kawanishi 1999).

Shinoda et al. (1998) exposed adult male Sprague-Dawley rats to single oral doses of nitrobenzene (250 mg/kg) and assessed DNA fragmentation in the testes at various times after dosing. The study authors observed DNA fragmentation using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) in late pachytene spermatocytes of exposed animals beginning 24 hours after dosing,

and these were in the same location where degenerating spermatocytes were observed upon microscopic examination. DNA laddering was observed (using gel electrophoresis), and electron micrographs of the degenerating spermatocytes showed nuclear chromatin condensation with crowding of cytoplasmic constituents; these findings are indicative of apoptosis. Shinoda et al. (1998) suggested that nitrobenzene could alter the secretion of Sertoli cell factors, leading to deficiencies in growth factors or hormones, and thus trigger apoptosis in germ cells. Using genetically modified mice that express a dysfunctional FasL protein, Richburg and Nañez (2003) showed that Fas-mediated signaling was not responsible for germ cell apoptosis induced by nitrobenzene, but that a dysfunctional Fas-signaling system increases the sensitivity of mice to germ cell apoptosis following nitrobenzene exposure.

McLaren et al. (1993b) observed decreased incorporation of 35S-methionine into newly synthesized proteins secreted by cultured seminiferous tubules obtained at different stages of spermatogenesis from rats exposed by gavage to 300 mg/kg nitrobenzene. The decrease was seen in stages VI–VIII and IX–XII, but not stages II–V, corresponding to the observed histological evidence of degeneration of pachytene spermatocytes at stages VI–XII at the same time after dosing. In experiments using seminiferous tubules from immature, late pubertal, and young adult rats cultured *in vitro* with nitrobenzene, McLaren et al. (1993b) observed age-dependent differences in the effect of nitrobenzene on protein secretions. Total 35S-methionine incorporation was decreased by nitrobenzene only in seminiferous tubules from young adult (70-day-old) rats, and not in 28- or 45-day-old rats. Secretion of individual proteins (not further identified) was also reduced by nitrobenzene, and the relationship to age varied by protein (McLaren et al. 1993b). These data suggest that nitrobenzene may affect spermatogenesis in part by reducing the secretion of hormones and growth factors needed for germ cell development and survival.

2.17 DEVELOPMENTAL

No studies were located regarding developmental effects in humans after inhalation, oral, or dermal exposure to nitrobenzene. The effects of nitrobenzene on the developing organism have been evaluated in rats and rabbits exposed by inhalation (Biodynamics 1983, 1984; Dodd et al. 1987; Tyl et al. 1987) and in rats exposed orally (Mitsumori et al. 1994).

When pregnant Sprague-Dawley rats were exposed to nitrobenzene via inhalation on GDs 6–15, there was no treatment-related effect on malformation incidence at concentrations up to 39.4 ppm (Tyl et al. 1987). The incidences of litters with variations consisting of ecchymosis (discoloration of the skin due to bleeding underneath) on the trunk and hole in the parietal bone were significantly elevated at the highest

concentration (39.4 ppm). The latter finding (hole in the parietal bone) may reflect delayed ossification (DeSesso and Scialli 2018; Fritz and Hess 1970). No increased malformations or variations were observed in offspring of New Zealand rabbits exposed to nitrobenzene concentrations up to 100 ppm on GDs 7–19 (Biodynamics 1984). In rabbits, the only effects noted on the offspring were significant decreases in the mean number of viable male fetuses in the groups exposed to 40 and 100 ppm (3.1 and 3.2 males per group, respectively, compared with 4.9 males in the control group) (Biodynamics 1984). The study authors (Biodynamics 1984) attributed the difference to an unusually high mean number of male fetuses in the control group, which consisted of 4.9 males and 3.2 females.

In a 2-generation inhalation exposure study in CD rats, Dodd et al. (1987) observed a 12% decrease in mean body weight of the F1 offspring on postnatal day (PND) 21 in the group exposed to 40 ppm nitrobenzene. The only oral study reporting assessment of developmental effects was a repeat-dose and reproductive/ developmental toxicity screening study in rats (Mitsumori et al. 1994). In this study, offspring body weights measured on PND 4 were significantly decreased in males at 20 mg/kg/day, while body weights of both male and female pups were reduced by more than 20% at 60 mg/kg/day. At 60 mg/kg/day, pup viability on PND 4 was also significantly reduced (66.9% compared with 99.1% in controls) (Mitsumori et al. 1994).

2.18 OTHER NONCANCER

Metabolic acidosis is a consequence of nitrobenzene-induced methemoglobinemia and subsequent tissue hypoxia, which triggers a switch to anaerobic respiration and accumulation of lactic acid. Metabolic acidosis was observed in the case of a 45-year-old woman who ingested about 50 mL of a solution containing 20% nitrobenzene in a suicide attempt (Shrestha et al. 2020). She was treated with hemodialysis and oral methylene blue administration, and was discharged 6 days later (Shrestha et al. 2020). No other studies were located regarding other noncancer effects of nitrobenzene exposure.

2.19 CANCER

U.S. Federal agencies and international scientific organizations have thoroughly reviewed the literature on nitrobenzene's carcinogenicity. Using a weight-of-evidence evaluation approach, EPA (2009a) has deemed nitrobenzene "likely to be carcinogenic to humans" by any route of exposure. The HHS NTP has determined that nitrobenzene is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in studies of animals (NTP 2021). IARC concluded nitrobenzene is possibly

carcinogenic in humans (Group 2B) based on inadequate evidence in humans and sufficient evidence in animals (IARC 1996, 2019).

Carreón et al. (2014) evaluated the association between bladder cancer and occupational exposure to o-toluidine, aniline, and nitrobenzene in a cohort of 1,786 workers at a rubber chemical manufacturing plant in New York. A total of 37 bladder cases were identified. Exposure was assessed using each employee's work history and assigned to exposure probability categories reflecting total exposure to the three compounds; exposure to nitrobenzene alone or as the primary contaminant was not evaluated. The standardized incidence ratios for bladder cancer were increased compared with a state-specific referent population for the highest probability of exposure, and analyses by duration and cumulative exposure indices yielded similar results. The study authors noted that measurements of exposure concentrations indicated that o-toluidine was present at the facility at higher concentrations than aniline or nitrobenzene, and that o-toluidine had been shown to be associated with increased risk of bladder cancer in other epidemiological studies (Carreón et al. 2014). For these reasons, it is not possible to ascertain the contribution of nitrobenzene exposure to the increased risk of bladder cancer in this study.

A 2-year, chronic-duration bioassay evaluated the carcinogenic effect of nitrobenzene in experimental animals (Cattley et al. 1994, 1995; CIIT 1993). Male and female B6C3F1 mice and F344 rats, and male CD rats were exposed 5 days/week, 6 hours/day to nitrobenzene via inhalation for 2 years. Mice were exposed to 0, 5, 24.8, or 49.1 ppm nitrobenzene, while rats were exposed to 0, 1, 5, or 24.8 ppm nitrobenzene. Increased incidences of liver and kidney tumors were observed in rats, and increased incidences of lung and mammary gland tumors were observed in mice. In male F344 and CD rats, significant increases in the incidences of hepatocellular adenomas and combined adenomas or carcinomas were observed at the highest exposure level (24.8 ppm). The incidences of these effects also showed statistically significant dose-related trends. In F344 rats, but not CD rats, males also exhibited significant increases in renal tubular adenomas and combined adenomas or carcinomas at 24.8 ppm, with significant dose-related trends. No increase in liver or kidney tumors was observed in female F344 rats (female CD rats were not included in this study). Female F344 rats exposed to 24.8 ppm nitrobenzene did experience a significant increase in endometrial stromal polyps, with a significant dose-related trend. Endometrial stromal polyps are noncancerous, and the relevance to humans of endometrial polyps in rats has been questioned based on differences in etiology and hormone sensitivity (Davis 2012).

Neither male nor female mice exhibited significant increases in liver tumor incidences when evaluated using pairwise comparison; however, a significant dose-related trend for increased hepatocellular

adenomas was observed in females (Cattley et al. 1994, 1995; CIIT 1993). In male B6C3F1 mice, a significant increase in the incidence of lung alveolar/bronchiolar adenoma or carcinoma was detected at all exposure concentrations (≥5 ppm), with a significant increase in adenoma incidence at ≥24.8 ppm. Female mice did not have increased incidences of lung tumors. Male (but not female) mice exposed to the highest concentration (49.1 ppm) also exhibited a significant increase in the incidence of thyroid follicular cell adenoma, with a significant dose-response by trend test. In female mice, the only statistically significant tumor finding was an increase in the incidence of mammary gland adenocarcinomas at 49.1 ppm; this tissue was not examined for tumors at lower exposure concentrations.

Apart from studies of genotoxicity (see Section 2.20), no studies exploring potential carcinogenic modes of action for nitrobenzene were located.

2.20 GENOTOXICITY

No studies were located regarding genotoxic effects in humans after exposure to nitrobenzene. Numerous studies have been published evaluating nitrobenzene's genotoxicity potential *in vitro*, summarized in Table 2-4, and *in vivo* using experimental animals, summarized in Table 2-5. The current evidence indicates that nitrobenzene is not mutagenic but does result in chromosomal aberrations and DNA damage.

Table 2-4. Genotoxicity of Nitrobenzene *In Vitro*

		Results		
Species (test system)	Endpoint	With activation	Without activation	Reference
Salmonella typhimurium	Gene mutation	–	–	Anderson and Styles 1978; Assmann et al. 1997; Chiu et al. 1978; Dellarco and Prival 1989; EPA 1984a; Garner and Nutman 1977; Haworth et al. 1983; Ho et al. 1981; Mestankova et al. 2016; Shimizu et al. 1983; Vance and Levin 1984
S. typhimurium UmuC	Gene mutation	–	–	Bonnefoy et al. 2012
Human peripheral blood lymphocytes	Chromosomal aberrations	ND	+	Huang et al. 1995, 1996
Human sperm	Chromosomal aberrations	ND	-	Kamiguchi and Tateno 2002
V79 hamster lung fibroblasts	Micronuclei	ND	+	Bonacker et al. 2004

Table 2-4. Genotoxicity of Nitrobenzene *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Human peripheral blood lymphocytes	Micronuclei	ND	+	Bonnefoy et al. 2012
Human kidney cells	Micronuclei	ND	+	Robbiano et al. 2004
Rat primary kidney cells	Micronuclei	ND	+	Robbiano et al. 2004
Human thyroid cells	DNA damage	ND	+	Mattioli et al. 2006
Human kidney cells	DNA damage	ND	+	Robbiano et al. 2004
Rat primary kidney cells	DNA damage	ND	+	Robbiano et al. 2004
Human hepatocytes	Unscheduled DNA synthesis	ND	–	Butterworth et al. 1989
Rat hepatocytes	Unscheduled DNA synthesis	ND	–	Butterworth et al. 1989
Human thyroid cells	Unscheduled DNA synthesis	ND	–	Mattioli et al. 2006

+ = positive result; – = negative result; DNA = deoxyribonucleic acid; ND = no data

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

Species (test system)	Endpoint	Result	Reference
F344 rat peripheral blood lymphocyte and isolated spleen lymphocyte	Sister chromatid exchange	–	Kligerman et al. 1983
Mouse bone marrow and spermatocyte	Chromosomal aberrations	+	Aly et al. 2014
Male Sprague-Dawley rats (liver, thyroid, and kidney cells)	DNA damage	+	Mattioli et al. 2006
Sprague-Dawley rats (kidney cells)	DNA damage	+	Robbiano et al. 2004
Male F344 rat hepatocytes	Unscheduled DNA synthesis	–	Mirsalis et al. 1982
Kunmig mice	DNA binding	+	Li et al. 2003a, 2003b

+ = positive result; – = negative result; DNA = deoxyribonucleic acid

Bacterial Mutagenicity. In all *Salmonella typhimurium* strains, nitrobenzene was negative for mutagenicity, regardless of metabolic activation (Anderson and Styles 1978; Assmann et al. 1997; Bonnefoy et al. 2012; Chiu et al. 1978; Dellarco and Prival 1989; EPA 1984a; Garner and Nutman 1977; Haworth et al. 1983; Ho et al. 1981; Suzuki et al. 1983, 1987; Vance and Levin 1984). None of these studies showed a positive result for nitrobenzene (Table 2-4). Two studies (Suzuki et al. 1983, 1987) looked at nitrobenzene in combination with the co-mutagen, norharman (9H-pyrido[3,4-b] indole). Neither nitrobenzene nor norharman was mutagenic in *S. typhimurium* strains TA98 or TA100 without

metabolic activation; however, together with activation, reverse mutations were induced in the TA98 strain, but not in the TA100 strain. Further studies indicated that a nitroreductase-deficient TA98NR was negative for reverse mutations with activation and norharman, indicating that the presence of nitroreductase was required for mutagenicity (Suzuki et al. 1987).

Clastogenicity and Aneugenicity. Data on clastogenicity are mixed, with the majority of the evidence suggesting a genotoxic effect on the chromosome. Huang et al. (1995, 1996) observed increases in the percent of chromosomal aberrations in human peripheral blood lymphocytes from 12.4 to 33.2% as doses increased from 0.05 to 0.80 mmol/L. However, nitrobenzene did not induce structural chromosomal aberrations *in vitro* in human spermatozoa incubated with 500 µg/mL for 120 minutes without activation (Kamiguchi and Tateno 2002). Increased chromosomal aberrations were observed in mouse bone marrow cells and spermatocytes following *in vivo* gavage administration of 300 mg/kg nitrobenzene (Aly et al. 2014). Micronuclei were induced *in vitro* in hamster lung fibroblasts, primary human and rat kidney cells, and human peripheral lymphocytes. Bonacker et al. (2004) demonstrated the induction of micronuclei from nitrobenzene exposure using V79 hamster lung fibroblasts possibly by affecting tubulin assembly and spindle apparatus. A method to detect aneugens using antibody staining, indicated the micronucleus effects were aneugenic, as the staining was mostly kinetochore-positive. A statistically significant increase in micronuclei was observed in primary human and rat kidney cells exposed to 0.250–0.50 mM nitrobenzene (Robbiano et al. 2004). Bonnefoy et al. (2012) found 13–14% of human blood cells had micronuclei when exposed *in vitro* to 0.01–10 µg/mL concentrations of nitrobenzene. A cytogenetic analysis of lymphocytes in the peripheral blood or in splenic blood of rats exposed *in vivo* to nitrobenzene at concentrations of 5–50 ppm for 6 hours/day, 5 days/week for 21 days did not reveal an increase in sister chromatid exchange or chromosome aberrations in bone marrow (Kligerman et al. 1983). Robbiano et al. (2004) observed a dose-dependent increase in micronucleated rat kidney cells due to broken and detached chromosomes separated from the spindle apparatus in rats treated *in vivo* with 300 mg/kg nitrobenzene via gavage.

DNA Damage, Synthesis, and Binding. There is mixed evidence as to whether or not nitrobenzene may cause DNA damage, with consistent positive evidence of DNA damage and consistent negative results for unscheduled DNA synthesis. DNA damage was positive *in vitro* in human thyroid cells, human kidney cells, and rat kidney cells. Robbiano et al. (2004) observed a dose-dependent increase in the DNA fragmentation, as measured by comet assay, in primary rat and human kidney cells exposed to concentrations of 0.125–0.50 mM nitrobenzene for 48 hours. Mattioli et al. (2006) evaluated DNA fragmentation on human thyroid cells and found a dose-dependent increase in tail length and tail moment

at concentrations ranging from 1.25 to 5 mM. In an *in vivo* analysis presented in Mattioli et al. (2006), nitrobenzene induced a significant increase in DNA fragmentation in thyroid, liver, and kidney cells in Sprague-Dawley rats administered a single dose of 620 mg/kg. However, in two studies that evaluated unscheduled DNA synthesis both *in vivo* in rat hepatocytes (Mirsalis et al. 1982) and *in vitro* with human and rat cells (Butterworth et al. 1989), DNA repair response was not induced. Lastly, Li et al. (2003a, 2003b) observed that nitrobenzene can form adducts with hepatic DNA in male mice in a dose-response and time-course study. Mice were administered nitrobenzene intraperitoneally in corn oil at doses of 0.1–100 μg/kg and 10 mg/kg animals, and a dose-related increase in hepatic DNA adducts was observed at all dose levels within 2 hours of exposure (Li et al. 2003b). In the time course study, mice were administered a 4.1 μg/kg dose of nitrobenzene and animals were sacrificed between 4 hours and 21 days after exposure (Li et al. 2003b). Adducts were initially increased, followed by reduction over time.

Genotoxicity of nitrobenzene indicates that it is not mutagenic and does not induce sister chromatid exchange or unscheduled DNA synthesis but does have consistent positive results for induction of micronuclei and DNA damage. Mixed results for chromosomal aberrations and a single study on DNA binding were inconclusive.

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

3.1 TOXICOKINETICS

Case reports and experimental exposure studies in humans provide limited information on the absorption, metabolism, and excretion of nitrobenzene. Studies in rats, mice, and rabbits exposed primarily by oral administration provide further details on the toxicokinetics. An overview of the information is provided below.

- Nitrobenzene is systemically absorbed in humans and animals after inhalation, oral, and dermal exposure, as evidenced by measurements of this compound in blood and by measurements of radioactivity in animals exposed to radiolabelled nitrobenzene. The rate of absorption has not been quantified. The extent of oral absorption has been estimated to be at least 35-65% based on urinary excretion of radioactivity, which may underestimate total absorption.

- Nitrobenzene is preferentially distributed to adipose tissue, from where it may be redistributed into systemic circulation. Based on measurement of radioactivity in animals exposed orally, nitrobenzene or its metabolites may also be distributed to liver, kidneys, and lungs.

- Nitrobenzene may be metabolized by oxidative or reductive pathways, but the reductive pathways appear to yield the ultimate toxicants. Reduction of nitrobenzene occurs in the gastrointestinal tract via oxygen-insensitive nitroreductase enzymes produced by gut microflora. Nitrobenzene reduction may also occur in hepatic microsomes and in erythrocytes via oxygen-sensitive nitroreductases.

- Redox cycling of nitrobenzene metabolites, especially nitrosobenzene and phenylhydroxylamine, is believed to be responsible for conversion of hemoglobin to methemoglobin. In addition, metabolism of nitrobenzene yields free radicals at several steps in the reductive pathways.

- Species differences in metabolism of nitrobenzene have been observed and may correlate to differences in susceptibility to nitrobenzene hematotoxicity.

- Urinary metabolites of nitrobenzene in humans and mammals include nitrophenols and aminophenols, and animal studies have also detected p-hydroxyacetanilide. The urinary metabolites are often conjugated with sulfate or glucuronide.

- The primary route of elimination of nitrobenzene is via urinary excretion of metabolites.

3.1.1 Absorption

Nitrobenzene is absorbed across the respiratory tract, as demonstrated indirectly in case reports of symptoms after inhalation exposure (Ikeda and Kita 1964; Lee et al. 2013) and directly in studies of experimental human exposures (Piotrowski 1967; Salmowa et al. 1963). The absorption of inhaled

nitrobenzene has been demonstrated qualitatively in a case report of elevated methemoglobin in a 37-year-old man who was exposed to nitrobenzene via inhalation while cleaning a nitrobenzene pump (Lee et al. 2013). Similarly, Ikeda and Kita (1964) reported methemoglobinemia in a 47-year-old woman whose job for the prior 1.5 years involved painting pan lids with a product that contained nitrobenzene as a solvent. The study authors detected metabolites of nitrobenzene (p-nitrophenol and p-aminophenol) in the subject's urine. The levels of these metabolites in urine declined over time in parallel with decreases in the patient's methemoglobin levels (Ikeda and Kita 1964).

Salmowa et al. (1963) estimated the lung retention of nitrobenzene in a study of seven male volunteers who were exposed for 6 hours to concentrations of 1–6 ppm. The subjects were exposed through a gas mask to prevent dermal absorption. Expired air was collected and analyzed for nitrobenzene, and absorption was estimated as the difference between the mass of nitrobenzene inhaled and the mass exhaled. Using this method, the study authors estimated that ~80% of the nitrobenzene vapor was retained in the lungs. Piotrowski (1967) conducted an experiment with male volunteers exposed to the same concentration range for repeated exposures (6 hours/day for ≥4 days). The detection of the nitrobenzene metabolite, p-nitrophenol, in the urine of the exposed subjects demonstrated absorption of inhaled nitrobenzene.

Oral absorption of nitrobenzene in humans has been shown indirectly in case reports of human poisoning after ingestion of this compound. Only one case report (Martínez et al. 2003) reported measurement of nitrobenzene in blood or tissues. Martínez et al. (2003) reported that the concentration of nitrobenzene in the blood was 3.2 μg/mL in an 82-year-old man 48 hours after he ingested about 250 mL of nitrobenzene. Myslak et al. (1971) described a case in which a 19-year-old female consumed about 50 mL of nitrobenzene; high levels of the nitrobenzene metabolites, p- aminophenol and p-nitrophenol, were detected in the patient's urine. Several other fatal human poisonings have been reported where the ingested substance is known to be nitrobenzene (Gupta et al. 2000, 2012; Kumar et al. 2017). In addition, numerous cases of methemoglobinemia in humans have been reported due to oral exposure to nitrobenzene (Agrawal et al. 2011; Balwani et al. 2017; Boukobza et al. 2015; Chongtham et al. 1997; D'sa et al. 2014; Kumar et al. 1990; Perera et al. 2009; Saxena and Prakash Saxena 2010). These case reports demonstrate absorption of nitrobenzene from the gastrointestinal tract. No quantitative estimates of oral absorption in humans were located.

Studies of rats, mice, and rabbits exposed to radiolabelled [^{14}C]-nitrobenzene suggest oral absorption estimates of 35–65% based on urinary excretion of radioactivity over ≥3 days after dosing (Albrecht and

Neumann 1985; Levin and Dent 1982; Parke 1956; Rickert et al. 1983; Robinson et al. 1951). Estimates of oral absorption based only on urinary excretion may underestimate the total uptake of nitrobenzene. Rabbits were shown to excrete small amounts of radioactivity (~1% of administered dose) as CO_2 and aniline in expired air (Parke 1956), and rats excreted between 2 and 4% of the administered dose in the bile (Rickert et al. 1983). In addition, Rickert et al. (1983) observed comparable amounts of radioactivity in the feces of rats given nitrobenzene via oral and intraperitoneal administration, suggesting that fecal excretion may not reflect unabsorbed nitrobenzene.

Rickert et al. (1983) investigated species differences and dose dependence of the oral absorption of nitrobenzene. In this study, mice exhibited lower total urinary excretion of radioactivity than rats given the same dose (35 versus ~60% in rats over 3 days after a dose of 225 mg/kg). However, the total recovery of radioactivity was also lower in mice (54 versus ~75–80% in rats). Dose did not markedly alter oral absorption estimates. In both F344 and CD rats, a 10-fold difference in oral dose (22.5 versus 225 mg/kg) did not change the percentage of dose excreted in urine (Rickert et al. 1983), suggesting little effect of dose on absorption in this dose range.

A portion of the radioactivity absorbed via the gastrointestinal tract is likely already in the form of nitrobenzene metabolites, as there is evidence for metabolism of nitrobenzene by gut microflora. This was demonstrated in a study showing that antibiotic-treated rats did not develop methemoglobinemia after oral exposure to nitrobenzene (Levin and Dent 1982). After a gavage dose of 225 mg/kg ^{14}C-nitrobenzene, antibiotic-treated rats excreted similar levels of most urinary metabolites, but significantly less p-hydroxy-acetanilide than control rats (0.9% of administered dose versus 16.2% in controls). In agreement with this finding, the antibiotic-treated rats had normal methemoglobin concentrations (2–3%), while the control rats still showed elevated methemoglobin (20%) 96 hours after exposure (Levin and Dent 1982).

A case report of poisoning in an infant exposed to nitrobenzene via dermal contact provides qualitative evidence of dermal absorption. Mallouh and Sarette (1993) reported that a 2-month-old infant whose mother had treated his skin with hair oil containing 1% nitrobenzene developed methemoglobinemia (31.5% methemoglobin).

Feldmann and Maibach (1970) evaluated dermal uptake of liquid nitrobenzene in volunteers. The subjects received a dermal application of ^{14}C-labelled nitrobenzene in acetone on the forearm (4 μg/cm^2 applied to a 13-cm^2 area, unoccluded) and were instructed not to wash the site for 24 hours. Based on the

amount of radioactivity in urine collected over the 5 days following application, the study authors estimated dermal absorption of about 1.53% of the applied dose. The absorption rate, estimated for each hour after application, was highest in the first 24 hours (0.022% per hour) and declined to 0.006% per hour in the final 24 hours of urine collection (Feldmann and Maibach 1970). The study authors suggested, however, that the continued excretion of radiolabel may have reflected redistribution from adipose tissue instead of absorption. Dermal absorption of liquid ^{14}C-nitrobenzene was measured in monkeys exposed on shaved abdominal skin at a dose of 4 μg/cm^2 (Bronaugh and Maibach 1985). Based on urinary excretion over 5 days (corrected for excretion via other routes), the study authors estimated that 4.2% of the applied dose was absorbed (Bronaugh and Maibach 1985).

Dermal absorption of nitrobenzene vapor was evaluated in a study of male volunteers (Piotrowski 1967). The volunteers were exposed to concentrations of 5–30 μg/L nitrobenzene (6 hours/day for ≥4 days) in an exposure chamber but were supplied clean air to breathe. The subjects were exposed either clothed or naked to assess the degree of protection afforded by clothes. The rate of absorption of nitrobenzene vapor across the skin was estimated from urinary excretion of p-nitrophenol. The rates of dermal absorption estimated were between 0.23 and 0.3 mg/hour/μg/L for the range of exposure concentrations. Based on these data, the study author estimated that normal clothes reduced absorption by a small amount (20–30%) (Piotrowski 1967).

The percutaneous uptake of ^{14}C-labelled nitrobenzene and related compounds across human and monkey skin *in vitro* was measured using flow-through diffusion cells with the tops covered with parafilm to prevent volatilization. Bronaugh and Maibach (1985) estimated that 7.8% of the applied dose of 4 μg/cm^2 nitrobenzene permeated across dermatomed human skin over 24 hours, and a similar absorption fraction was reported for monkey skin (6.2%) under the same conditions. These absorption estimates are higher than the *in vivo* estimates (1.5% for humans and 4.2% in monkeys). The study authors indicated that the use of occlusion in the *in vitro* experiments likely increased skin penetration by limiting evaporation of nitrobenzene.

3.1.2 Distribution

There are few data on the distribution of nitrobenzene to tissues in humans or animals. As noted in Section 3.1, nitrobenzene was detected in the blood of an elderly man who had ingested nitrobenzene (Martínez et al. 2003). Nitrobenzene was found in the stomach, liver, brain, and blood during autopsies of five patients who died from nitrobenzene poisoning. The highest concentrations were in the liver

(0.124 mg/kg tissue) and brain (0.164 mg/kg tissue) (Wirtschafter and Wolpaw 1944). In female rats exposed to 25 mg/kg ^{14}C-nitrobenzene by gavage, radioactivity was widely distributed in the tissues sampled, with the highest levels in the blood and kidneys (see Table 3-1). When rabbits were given ^{14}C-nitrobenzene (250 mg/kg), the highest levels of radioactivity in tissues sampled 8 days after dosing were in the intestinal and renal adipose tissue (Parke 1956). No information was located on tissue distribution after inhalation or dermal exposure in humans or animals.

Table 3-1. Distribution of Nitrobenzene-Derived Radioactivity in Tissues of Female Wistar Rats after Oral Exposure

	Radioactivity in tissue (pmol/mg)/dose (μmol/kg)	
Tissue	1 day after dosing	7 days after dosing
Blood	229±48[a]	134±19
Kidney	204±27	48±2.4
Liver	129±9.5	26.5±3.5
Lung	62±14	29±4.1

[a]Mean±standard deviation of three animals given 0.2 mmol/kg nitrobenzene.

Source: Albrecht and Neumann 1985

Nitrobenzene may be redistributed from adipose or other tissue into the bloodstream. Patel et al. (2008) reported the case of a 20-year-old male who had ingested about 75 mL of nitrobenzene. The patient exhibited very high methemoglobin (66.7%) and was treated with methylene blue, which decreased methemoglobin to 5.4% within an hour. However, 1 day later, his methemoglobin level rose again to 15.8% before declining again. The study authors suggested that the secondary rise in methemoglobin may have resulted from redistribution of nitrobenzene from tissues.

In blood, nitrobenzene metabolites are bound to hemoglobin and, to a lesser extent, plasma proteins. Albrecht and Neumann (1985) compared the binding of nitrobenzene and acetanilide to hemoglobin in blood from female Wistar rats exposed by gavage. Results showed that nitrobenzene had higher affinity for hemoglobin (~10-fold higher binding index and specific binding) compared with acetanilide. At 1 and 7 days after a gavage dose of 25 mg/kg ^{14}C-nitrobenzene in female Wistar rats, the hemoglobin binding indices were 72.8 and 70 mmol/mol Hb/dose (mmol/kg), and specific binding values were 1,030 and 1,024 pmol/mg/dose (mmol/kg). In contrast, hemoglobin binding indices and specific binding of acetanilide were in the range of 7–12 and 102–177, respectively. Specific binding of nitrobenzene to

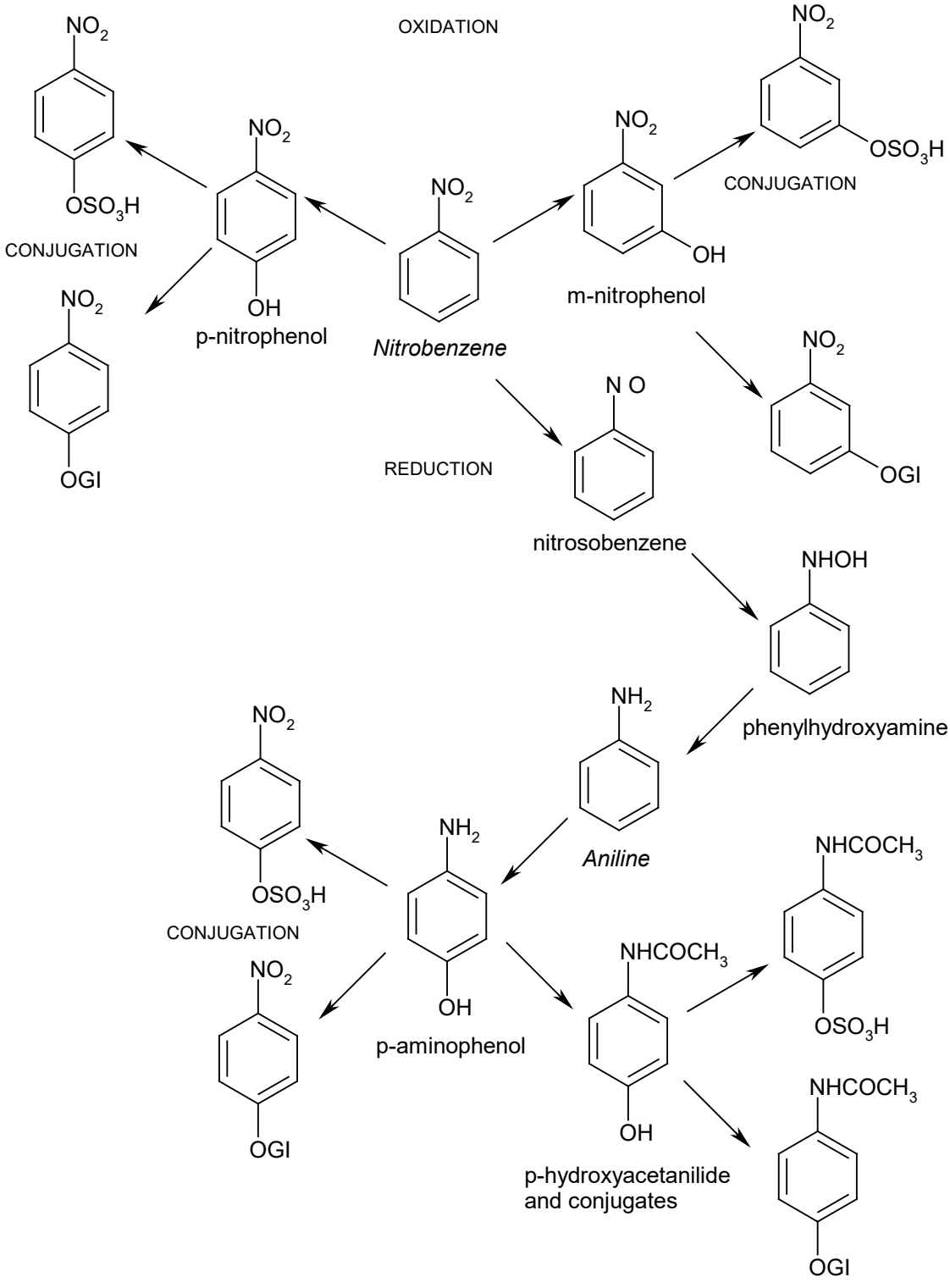
plasma proteins was comparable to that of acetanilide 1 day after dosing, and about 10-fold higher 7 days after dosing.

Goldstein and Rickert (1984) observed marked differences between rats and mice in the distribution of nitrobenzene-derived radiolabel to red blood cells and spleen. After single oral doses of 75–200 mg/kg ^{14}C-nitrobenzene, total and bound radiolabels in erythrocytes were 6–13-fold higher in rats than in mice. In the spleen, total and bound radiolabel levels were also higher in rats than in mice. In animals receiving the highest dose, peak binding in rats occurred 24 hours after dosing, while the peak occurred 10 hours after dosing in mice. At the peak, bound radiolabel in the erythrocytes and spleen were 429 and 74 nmol equivalents ^{14}C-nitrobenzene/g tissue in rats; in mice, bound radiolabel in erythrocytes peaked at 76 nmol equivalents ^{14}C-nitrobenzene/g tissue and no significant increase in bound radiolabel was detected in spleen. Goldstein and Rickert (1984) used sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) to separate the compounds in blood and spleen that were covalently bound to the nitrobenzene-derived radiolabel. The study authors showed that most of the radiolabel in erythrocytes was bound to hemoglobin, and most of the radiolabel in the spleen was bound to methemoglobin and another, unidentified component.

3.1.3 Metabolism

Overview. Nitrobenzene metabolism may proceed through oxidative or reductive pathways. The reductive metabolism of nitrobenzene is the toxicologically more important pathway in that the intermediates produced are believed to be responsible for the hematopoietic and carcinogenic action of this compound. Cytochrome P450 enzymes in hepatic microsomes can catalyze oxidation of nitrobenzene, forming p- and m-nitrophenols and derivative aminophenols, which are subsequently conjugated with sulfate or glucuronide prior to excretion. Nitrobenzene reduction may proceed via oxygen-insensitive (Type I) or oxygen-sensitive (Type II) pathways, and yields p-aminophenol through a series of intermediates including nitrosobenzene, phenylhydroxylamine, and aniline. As with the oxidative metabolites, p-aminophenol may be conjugated with sulfate or glucuronide or may be acetylated at the nitrogen to p-hydroxyacetanilide and subsequently conjugated with sulfate or glucuronide. Figure 3-1 displays a schematic of the metabolism of nitrobenzene.

Figure 3-1. Metabolism of Nitrobenzene



Source: EPA 2009a

Importance of Gut Microflora. Reduction of nitrobenzene has been shown to be catalyzed by Type I (oxygen-insensitive) nitroreductase and/or by Type II nitroreductase (oxygen sensitive). In Sprague-Dawley rats, both nitroreductase enzymes are found in gut microflora, but also in microsomes from the liver, brain, lung, heart, and kidney (Ask et al. 2004). The importance of nitrobenzene metabolism by microbes in the gut was demonstrated most clearly by Levin and Dent (1982), who showed that, unlike control rats, antibiotic-treated rats did not develop methemoglobinemia after oral nitrobenzene exposure. The urinary metabolites of nitrobenzene were comparable between the control and antibiotic-treated rats, except that the antibiotic-treated rats produced much less p-hydroxyacetanilide (0.9% of total dose excreted, compared with 16.2% in controls) and an unidentified metabolite (0.5 versus 3.7% in controls).

Experiments by Reddy et al. (1976) provide support for the importance of microflora. The study authors measured formation of aniline from nitrobenzene in homogenized liver, kidney, gut wall, and gut contents obtained from control rats, germ-free rats, or germ-free rats acclimatized to room air for 7 days prior to sacrifice. The formation of aniline was higher in the gut contents of germ-free and control rats (15.2 and 11.1 nmol/mg protein/hour) than the other tissues (0.7–3.3 nmol/mg/protein/hour). In the germ-free rats, very little aniline was formed in the gut contents (0.2 nmol/mg protein/hour) or other tissues (0.5–2 nmol/mg protein/hour), demonstrating the importance of metabolism by microbes in the gastrointestinal tract.

Urinary Metabolites. In humans exposed to nitrobenzene by inhalation, urinary metabolites include p-nitrophenol and p-aminophenol (Ikeda and Kita 1964; Salmowa et al. 1963). Rickert et al. (1983) compared the urinary metabolites produced by male F344 rats, CD rats, and B6C3F1 mice given an oral dose of 225 mg/kg nitrobenzene. Table 3-2 summarizes the results. As the table shows, the urinary metabolites differed between rats and mice, and even between the two strains of rat. Only mice excreted p-aminophenol. In addition, the excretions (as a percent of administered dose) of p- and m-nitrophenol and p-hydroxyacetanilide were much higher in rats than in mice. The reduced levels of p-hydroxyacetanilide excreted in mice may partly explain why this species is less sensitive to methemoglobinemia induced by nitrobenzene; as noted above, Levin and Dent (1982) showed the importance of this metabolite in the mechanism for methemoglobinemia.

Table 3-2. Metabolites Excreted in Urine by Male Rats and Mice within 72 Hours after an Oral Dose of 225 mg/kg Nitrobenzene

	Percent of dose excreted		
Metabolite	F344 rat[a]	CD rat[b]	B6C3F1 mouse[b]
p-Nitrophenol	19.9–22.4	13.0	7.2
m-Nitrophenol	10.2–11.4	7.9	6.2
p-Aminophenol	ND	ND	9.7
p-Hydroxyacetanilide	16.2–19.0	8.8	3.9
Unidentified (I)	4.5–9.8	25.3	4.8
Unidentified (II)	ND–3.7	5.7	2.6

[a]Levin and Dent (1982) and Rickert et al. (1983).
[b]Rickert et al. (1983).

ND = not detected

Rickert et al. (1983) also measured the metabolite levels in urine before and after enzyme hydrolysis to assess species and strain differences in conjugation. In F344 rats, nitrobenzene metabolites were excreted exclusively as sulfates. In CD rats, the largest fractions of each metabolite were excreted as sulfates, with smaller fractions excreted as glucuronides or free (unconjugated) metabolites. Finally, in B6C3F1 mice, p-hydroxyacetanilide was excreted primarily as the glucuronide conjugate, while the sulfate conjugate was most important for p-aminophenol, p-nitrophenol, and m-nitrophenol (i.e., there was little to no glucuronidation). Small amounts of free metabolites were also detected in the urine of mice (Rickert et al. 1983).

In rabbits and guinea pigs exposed to nitrobenzene by gavage, the primary urinary metabolite was reported to be p-aminophenol, followed by p- and m-nitrophenol and o- and m-aminophenol (Parke 1956). However, Rickert (1987) noted that Parke (1956) used an acid hydrolysis procedure to cleave conjugates in urine, which would have resulted in conversion of p-hydroxyacetanilide (if present) to p-aminophenol. Therefore, the primary metabolite in rabbits and guinea pigs is not known but is probably either p-hydroxyacetanilide or p-aminophenol.

Metabolic Mechanisms. The mechanism by which nitrobenzene induces methemoglobinemia and related effects has been reviewed extensively (EPA 2009a; Holder 1999; Rickert 1987). The mechanism begins with its reductive metabolism, which can occur via oxygen-insensitive or oxygen-sensitive pathways. As discussed above, microflora in the gut reduce nitrobenzene to aniline via a three-step process that includes nitrosobenzene and phenylhydroxylamine intermediates. The reaction is catalyzed by an oxygen-insensitive nitroreductase (nicotine adenine dinucleotide phosphate [NADPH] dehydrogenase) that is

present at highest levels in the microflora of the gut but is also present at lower levels in tissues including the liver, heart, brain, lung, and kidney (Ask et al. 2004). Once absorbed, the products of microbial metabolism (nitrophenols, aminophenols, and aniline) may be further metabolized to form reactive intermediates including nitrosobenzene, phenylhydroxylamine, and the benzene nitronium ion (EPA 2009a).

Hepatic microsomes and erythrocytes can also reduce nitrobenzene, but these reactions are catalyzed by oxygen-sensitive nitroreductases (NADH dehydrogenase and the mitochondrial form, ubiquinone). Reduction of nitrobenzene through this pathway yields a number of toxic and reactive intermediates, including nitrosobenzene, phenylhydroxylamine, and several free radicals (superoxide, nitroanion, and hydronitroxide) (EPA 2009a; Holder 1999).

Reduction of nitrobenzene by intestinal microflora appears to be the most important metabolic pathway for induction of methemoglobin, indicating that the metabolites from this pathway are responsible for this effect. Available data indicate that the oxygen-insensitive reduction of nitrobenzene by gut bacteria proceeds much more rapidly than the oxygen-sensitive pathway occurring in liver microsomes (EPA 2009a). In addition, *in vitro* incubation of erythrocytes with nitrobenzene does not trigger conversion of hemoglobin to methemoglobin (Facchini and Griffiths 1981). Finally, as discussed above, the importance of the bacterial reduction of nitrobenzene to its induction of methemoglobinemia was demonstrated when germ-free animals were shown to be resistant to this effect (Levin and Dent 1982).

Holder (1999) postulated that metabolic cycling between the nitrosobenzene and phenylhydroxylamine products of nitrobenzene reduction is responsible for the conversion of hemoglobin to methemoglobin. As proposed by Holder (1999), nitrosobenzene is converted to phenylhydroxylamine via oxidation of NADPH, and the latter converts hemoglobin to methemoglobin (via a phenylhydronitroxide intermediate) during its reversion to nitrosobenzene. Nitrosobenzene may also bind to cysteine groups on intact hemoglobin, resulting in denaturation of the globin moiety and dissociation of the heme group (Holder 1999; Smith and McHale 2018). Once dissociated from the globin chain, free heme can injure erythrocytes and tissues via induction of oxidative stress. In addition, ferric iron from heme may complex with chloride to form the hydrophobic compound hemin, which may accumulate in the membrane of erythrocytes and cause their lysis (Smith and McHale 2018). Erythrocyte damage may also result from lipid peroxidation via free radicals formed during the metabolism of nitrobenzene and its metabolites.

Damaged erythrocytes are removed from circulation by splenic macrophages. Splenic effects seen in animals exposed to nitrobenzene, including increases in spleen weight, sinusoidal congestion, and hemosiderosis, result from accumulation of the products of erythrocyte destruction. Further, breakdown of heme in the liver induces hemosiderosis there. In addition, release of hemoglobin from injured red blood cells can trigger renal effects via formation of nephrotoxic hemoglobin dimers (Smith and McHale 2018).

In summary, metabolites of nitrobenzene convert hemoglobin to methemoglobin and destroy erythrocytes through a variety of molecular events, leading to splenic, liver, and kidney effects such as congestion and hemosiderosis. In addition, several steps in the metabolic pathways for nitrobenzene release free radicals and/or reactive intermediates that can cause lipid peroxidation and injury to a variety of tissues.

3.1.4 Excretion

The major route of nitrobenzene elimination in humans and animals is urinary excretion of metabolites. After a 17-month occupational exposure to nitrobenzene (via inhalation), a 47-year-old woman was admitted to the hospital, where urinary analysis showed excretion of p-nitrophenol and p-aminophenol in the urine that gradually declined over 2 weeks (Ikeda and Kita 1964). In experimental studies of humans exposed to nitrobenzene by inhalation, excretion of p-nitrophenol in urine was initially rapid but reached a steady-state rate of excretion around the 4^{th} day (Piotrowski 1967; Salmowa et al. 1963). Piotrowski (1967) estimated that about 16% of the absorbed dose excreted in the urine as p-nitrophenol during the steady-state phase. Myslak et al. (1971) reported extensive excretion of those metabolites in the urine of a subject who ingested about 50 mL of nitrobenzene. The levels of nitrobenzene metabolites in urine reached their maximum peak on days 2 (198 mg/day for p-aminophenol) and 3 (512 mg/day for p-nitrophenol). Piotrowski (1967) examined the rates of urinary p-nitrophenol excretion in a volunteer after intake of a single oral dose of 5 mg p-nitrophenol or 30 mg nitrobenzene. The excretion of p-nitrophenol was very rapid; in contrast, excretion was slow after exposure to nitrobenzene, probably due to the slow rate of nitrobenzene metabolism to p-nitrophenol. The initial half-time of elimination of p-nitrophenol after exposure to nitrobenzene was around 5 hours, with a late-phase half-time of >20 hours (Piotrowski 1967). All p-nitrophenol was eliminated by 8 hours when subjects were exposed to nitrophenol directly (Piotrowski 1967). In humans exposed to nitrobenzene vapor through the skin, about 20% was excreted as p-nitrophenol in the urine the first day (Piotrowski 1967). Feldmann and Maibach (1970) applied [^{14}C]-labeled nitrobenzene dissolved in acetone to the forearm skin of six subjects. The cumulative urinary excretion of radiolabel over the five days following application was estimated to be

1.5±0.84% of the applied dose or about 58% of the absorbed dose. After intravenous administration of [^{14}C]-nitrobenzene, the study authors observed 60.5% of the radioactive label in the urine and estimated a half-life of 20 hours (Feldmann and Maibach 1970).

In laboratory animals, nitrobenzene is primarily excreted via the urine as metabolites, as shown by studies of animals exposed orally to radiolabelled nitrobenzene. Table 3-3 summarizes the distribution of radioactivity in excreta collected over 3–7 days from rats, mice, and rabbits given single gavage doses of 22.5–250 mg/kg.

Table 3-3. Excretion of Radioactivity in Animals Treated Orally with ^{14}C-Nitrobenzene

			Percent of administered dose excreted in				
Strain and species (sex)	Dose mg/kg	Duration of collection	Urine	Feces	Expired air	Total recovery	Reference
F344 rat (M)	225	3 days	58.4	16.4	2.3	77.1	Levin and Dent (1982)
F344 rat (M)	225	3 days	63.2	14.2	1.6	79	Rickert et al. (1983)
F344 rat (M)	22.5	3 days	65.8	21.4	1.0	88.2	Rickert et al. (1983)
CD rat (M)	225	3 days	60.8	11.8	2.5	75.1	Rickert et al. (1983)
CD rat (M)	22.5	3 days	64.5	11.5	0.8	76.8	Rickert et al. (1983)
Wistar rat (F)	25	7 days	65	15.5	NR	NR	Albrecht and Neumann (1985)
B6C3F1 mouse (M)	225	3 days	34.7	18.8	0.8	54.3	Rickert et al. (1983)
Rabbit (NS)	250	4–5 days	58	9	1.6[a]	70	Parke 1956
Giant chinchilla rabbit (F)	150–200	2 days	~55	NR	NR	NR	Robinson et al. 1951

[a]In first 30 hours after dosing; not measured subsequently.

M = male; F = female; NS = not specified; NR = not reported

The available studies did not suggest substantial dose-related differences in patterns of nitrobenzene excretion in rats over the dose range of 22.5–225 mg/kg (Rickert et al. 1983). In addition, experiments in F344 rats comparing oral and intraperitoneal exposure routes showed similar excretory patterns (Rickert et al. 1983). However, species differences were evident. As the table shows, mice excreted less radioactivity (in total) and lower amounts in urine than rats of either strain exposed to the same dose of nitrobenzene; higher fecal excretion was seen in the mice as well (Rickert et al. 1983). The excretory patterns of rabbits were more similar to those of rats than mice (Parke 1956; Rickert et al. 1983; Robinson et al. 1951) (see Table 3-3).

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

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

No PBPK models of nitrobenzene were located in the available literature.

3.1.6 Animal-to-Human Extrapolations

Nitrobenzene toxicity results from its metabolism (see Section 3.1.3 *Metabolic Mechanisms*), and there is evidence for species differences in metabolism that correspond to differences in toxicity. For example, mice appear to be somewhat less sensitive than rats to the induction of methemoglobin in several studies (Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; NTP 1982), and mice have been shown to excrete less of the p-hydroxyacetanilide urinary metabolite that is important to the mechanism of action (Rickert et al. 1983). In addition, there are species differences in the level and/or activity of methemoglobin reductases, the enzymes that convert methemoglobin back to hemoglobin. For example, when erythrocytes from several species were exposed to nitrite at the same concentration, methemoglobin was produced by all species, but declined more rapidly in erythrocytes from mice and rabbits than in erythrocytes from humans, cats, dogs (Stolk and Smith 1966), or rats (Smith et al. 1967). Lo and Agar (1986) measured the activity of NADH-dependent methemoglobin reductase in erythrocytes from a variety of species and observed that mice and rabbits had the highest activity, followed by rats, with the lowest activity in humans. In *in vitro* studies with monomethylhydrazine, methemoglobin levels in human blood were ~4 times higher than levels in rat blood at the same concentration of monomethylhydrazine (Clark and De La Garza 1967). These data suggest that sensitivity to methemoglobinemia increases (low to high) from mice ≈ rabbits < rats < humans (i.e., humans may be more sensitive than rodents).

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

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

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

Populations that are considered unusually susceptible to nitrobenzene toxicity are those groups that are susceptible to methemoglobinemia. For example, the first 6 months of postnatal life is a period of increased susceptibility to methemoglobinemia (termed infantile methemoglobinemia or blue baby syndrome) due to a number of factors (Goldstein et al. 1969; Greer and Shannon 2005; Von Oettingen 1941):

- Fetal hemoglobin, which remains in the blood for some time after birth, is more prone to conversion to methemoglobin than is adult hemoglobin.
- Levels of NADH-dependent methemoglobin reductase (the major enzyme responsible for reduction of methemoglobin to normal hemoglobin) in the newborn increase approximately 2-fold during the first 4 months of postnatal life to reach adult levels.
- Umbilical cord blood is deficient in the enzyme glucose-6-phosphate dehydrogenase and thus cannot readily convert the methemoglobin that is formed “naturally” back to hemoglobin as is readily done in adults.

Additionally, a condition described as “hereditary methemoglobinemia” may result from a genetic defect (Goldstein et al. 1969). The enzyme, methemoglobin reductase, is absent and persons are hypersensitive to any substances such as nitrite or aniline derivatives capable of producing methemoglobinemia. The trait is

inherited as an autosomal recessive allele. Thus, either sex may exhibit the trait, which is ordinarily detected by the presence of cyanosis at birth. Such individuals would be extremely sensitive to the effects of nitrobenzene.

A more common genetic defect was also described in which the enzyme, glucose-6-phosphate, dehydrogenase has decreased activity (Goldstein et al. 1969). The pattern of inheritance of this trait is linked to one of several alleles on the X chromosome. The phenotype is expressed as an incomplete dominant trait. Thus, female heterozygotes are not known to have severely depressed enzyme levels and males may have a wide range of activity. These phenotypes express a wide range of levels of glucose-6-phosphate dehydrogenase enzyme in the red blood cell. This defect is ordinarily without adverse effects. It is only when these individuals are challenged with compounds that oxidatively stress erythrocytes (such as primaquine) that there is a hemolytic response. Reactors to primaquine (and fava beans) are found predominantly among groups that live in or trace their ancestry to malaria-hyperendemic areas such as the Mediterranean region or Africa. The incidence of "primaquine sensitivity" among Kurds, a Middle Eastern population, is 53%. Among black Americans, the incidence is 13%. Thus, individuals already exhibiting primaquine sensitivity would be expected to be more vulnerable to the additional hemolytic crisis that often follows 5–6 days after nitrobenzene exposure (Gosselin et al. 1984; Von Oettingen 1941).

People who have preexisting or underlying diseases such as anemia, cardiovascular disease, lung disease, sepsis, or abnormal hemoglobin species (e.g., carboxyhemoglobin, sulfhemoglobin, or sickle cell hemoglobin), maybe at greater risk of developing the chemically induced methemoglobinemia at much lower levels of exposure to nitrobenzene (Goldfrank et al. 1998).

In addition, external factors such as medications and exposure to xenobiotics from the environment can also cause methemoglobinemia. Nitrite-based medications, which are widely used to treat angina and other cardiac-related problems, can cause methemoglobinemia and are reported as a complication of the therapeutic use of these drugs (Bojar et al. 1987; Marshall and Ecklund 1980). Self-administration of local anesthetic drugs like benzocaine have also been known to cause this condition (Nappe et al. 2015).

Dapsone, a commonly used anti-inflammatory for treating infections has severe side effects, including methemoglobinemia, and patients are often recommended to use a pulse oximeter (Ashurst et al. 2010; Mahmood et al. 2019) or co-oximeter (Toker et al. 2015) to monitor blood oxygen levels regularly, with

the latter providing more accuracy. Acquired methemoglobinemia can also be caused by malaria medication (Kudale et al. 2014).

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to nitrobenzene are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for nitrobenzene from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by nitrobenzene are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Levels of nitrobenzene in blood reflect recent exposure. Metabolism of nitrobenzene to p-nitrophenol and p-aminophenol in humans is well-known, and the presence of metabolites in the urine can be used to indicate exposure to nitrobenzene (Ikeda and Kita 1964; Piotrowski 1967). About 10–20% of a dose is eliminated in the urine as p-nitrophenol, which is used in biological monitoring of occupational exposures. A smaller fraction of a dose is eliminated in urine as p-aminophenol (Astier 1992; IARC 1996). Urinary levels of p-nitrophenol and aminophenol also reflect recent exposure to nitrobenzene. In addition, neither of these metabolites is specific to nitrobenzene exposure. p-Nitrophenol is also a metabolite of other insecticides such as methyl parathion, ethyl parathion, and O-ethyl-O-(4-nitrophenyl) phenylphosphonothioate (Barr et al. 2002; CDC 2021a; Kissel et al. 2005; McCann et al. 2002; Rubin et al. 2002). p-Aminophenol is a urinary metabolite of aniline (Kao et al. 1978; McCarthy et al. 1985; Parke 1956; Robinson et al. 1951) and N-acetyl p-aminophenol (Newton et al. 1982; Chang et al. 1993). Measurement of p-nitrophenol and p-aminophenol, therefore, should not be used to quantify nitrobenzene exposure.

The nitrobenzene metabolites, nitrosobenzene and phenylhydroxylamine, have been found to bind with hemoglobin in the blood of orally exposed mice and rats and could be used as biomarkers of exposure (Goldstein and Rickert 1984). The presence of these hemoglobin adducts in human blood may also serve as a potential biomarker of exposure to nitrobenzene.

3.3.2 Biomarkers of Effect

The presence of methemoglobinemia can indicate effects of nitrobenzene exposure, but is also not specific, as methemoglobinemia can result from genetic defects as well as exposure to some medicines and many other toxic substances.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Smyth et al. (1969) demonstrated synergism between orally administered nitrobenzene and six other common industrial compounds in rat studies using death (oral LD_{50}) as the endpoint. The combinations of chemicals showed increased lethality that varied from 20 to 47%. The compounds were: formalin, 20%; butyl ether, 28%; aniline, 32%; dioxane, 39%; acetone, 47%; and carbon tetrachloride, 47% (Smyth et al. 1969).

Alcohol also has the potential for enhancing the toxicity of nitrobenzene; however, the toxicokinetic mechanism is not known. It is clear, however, that alcohol does not simply enhance the absorption of nitrobenzene. When alcohol was given orally and nitrobenzene was given intravenously, there was increased toxicity in rabbits compared with nitrobenzene alone. Alcohol also enhanced the neural toxicity of nitrobenzene in rabbits when nitrobenzene was applied to the skin (Matsumara and Yoshida 1959).

In addition, there are several other chemicals that operate through a similar mechanism of action in causing increases in methemoglobin such as nitrates and nitrites. Exposure to multiple agents that induce methemoglobinemia would likely increase the risk of an adverse outcome.

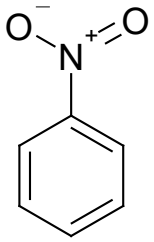
CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Nitrobenzene is a colorless to pale yellow oily liquid composed of a benzene ring with a single substituted nitro group. The compound is a synthetic chemical, and it does not occur naturally. It has an odor similar to bitter almonds or shoe polish. The chemical is primarily used in the synthesis of aniline and in producing the chemical intermediate to polyurethane. Nitrobenzene is also used as a solvent during petroleum refining and in the manufacture of cellulose ethers and acetates. It is a starting material for dinitrobenzenes, dichloroanilines, and other compounds including acetaminophen. Some of nitrobenzene's synonyms include mirbane oil and myrbane oil.

Table 4-1 lists common synonyms, trade names, and other pertinent identification information for nitrobenzene.

Table 4-1. Chemical Identity of Nitrobenzene

Characteristic	Information	Reference
Chemical name	Nitrobenzene	NLM 2021
Synonym(s) and registered trade name(s)	Nitrobenzol; essence of mirbane, essence of myrbane; oil of mirbane; mononitrobenzene; nitrobenzol; Caswell No.600	NLM 2021
Chemical formula	$C_6H_5NO_2$	Lide 2005
Chemical structure		Lide 2005
CAS registry number	98-95-3	Lei et al. 2008; Lide 2005

CAS = Chemical Abstracts Service

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Nitrobenzene is a liquid at room temperature. It is sparingly soluble in water and most organic solvents, and it represents a fire hazard. It is completely miscible in diethyl ether, benzene, and alcohol.

Nitrobenzene has a relatively high vapor pressure, which contributes to its flammability. Nitrobenzene has a relatively low K_{ow} value, suggesting that it is unlikely to bioaccumulate. Nitrobenzene's low K_{oc} indicates its high to moderate mobility in soil. The Henry's Law constant for nitrobenzene suggests that it will volatilize from moist soil and water surfaces. The high vapor pressure of nitrobenzene indicates that if released into the air, it will exist solely as a vapor in the atmosphere. Table 4-2 lists important physical and chemical properties of nitrobenzene.

Table 4-2. Physical and Chemical Properties of Nitrobenzene

Property	Information	Reference
Molecular weight	123.11 g/mol	Lei et al. 2008; Lide 2005
Color	Colorless to greenish-yellow or yellow	NLM 2021
Physical state	Crystals or oily liquid	Haynes 2015
Melting point(s)	5.7°C	Lide 2005
Boiling point(s)	210.8°C	Lei et al. 2008; Lide 2005
Critical temperature and pressure	720 K and 4.824 MN/M SQ	NLM 2021
Density	1.2037 g/cm^3 at 20°C	Lide 2005
Viscosity	1.863 mPas at 25°C	Lide 2005
Taste	Sweet (aqueous solutions)	NLM 2021
Odor	Volatile oil almond odor; pungent odor	NLM 2021
Odor threshold:		NLM 2021
Water	30–110 µg/L	
Air	4.7x10^{-3}–1.90 ppm	
Solubility:		Haynes 2015
Water	2.1 g/LH_2O at 25°C	
Organic solvent(s) at 20°C	Slightly soluble in carbon tetrachloride; very soluble in ethanol, diethyl ether, acetone, benzene	
Inorganic solvent(s)		
Partition coefficients:		
Log K_{ow}	1.85	Lei et al. 2008; Lide 2005
Log K_{oc}	1.94	NLM 2021
Relative Vapor Density	4.2 (air=1)	NLM 2021
Vapor pressure at 25°C	0.245 mmHg	NLM 2021
Henry's law constant	2.3 x10^{-5} atm m^3/mol at 25°C	Lei et al. 2008
Degradation half-life in air via reaction with OH radicals	44 days	EPA 2012
Heat of combustion	-10,420 Btu/pound	NLM 2021
Heat of vaporization	55.01 kJ/mol at 25°C	Haynes 2015
Autoignition temperature	900°F	NFPA 2002
Flashpoint	88°C	Haynes 2015

Table 4-2. Physical and Chemical Properties of Nitrobenzene

Property	Information	Reference
Flammability limits in air	1.8% by volume at 200°F	Lide 2005
Conversion factors:	1 mg/m^3=0.199 ppm[a] 1 ppm=5.04 mg/m^3	
Explosive limits	Moderate when exposed to heat or flame	NLM 2021
Incompatibilities and reactivity	Explosive reaction with solid or concentrated alkali and heat (e.g., sodium hydroxide or potassium hydroxide), aluminum chloride and phenol, aniline and glycerin, N_2O, and $AgClO_4$	NLM 2021

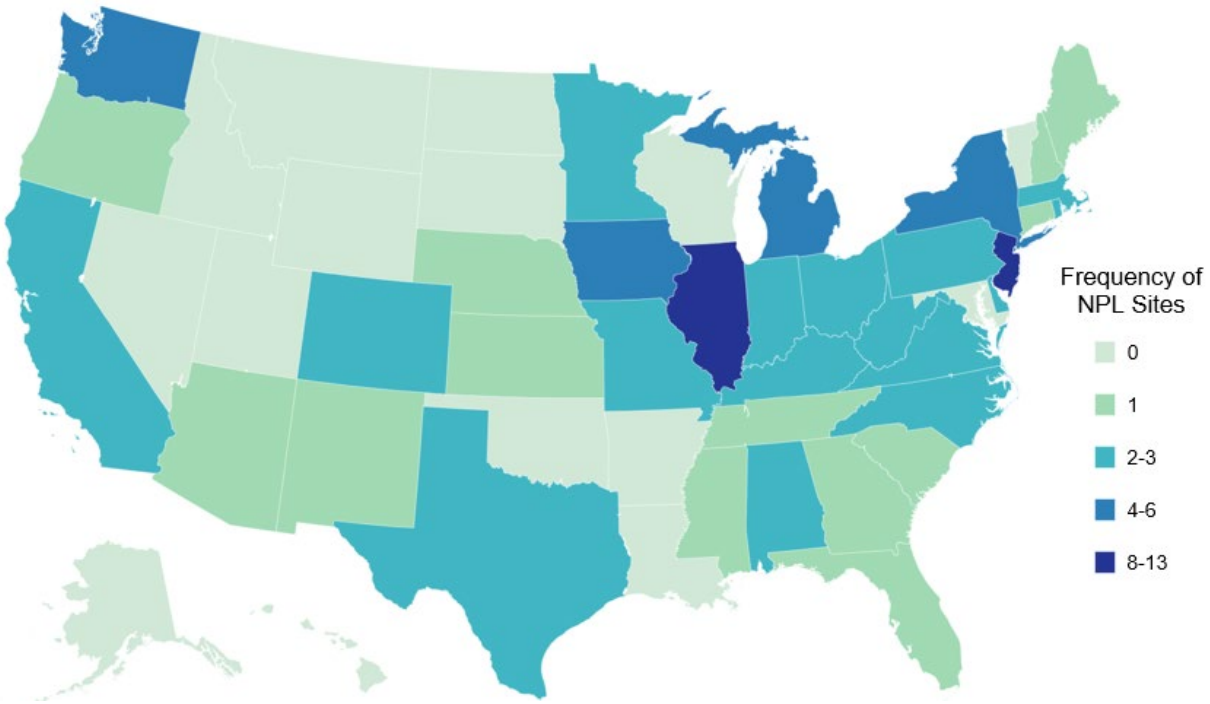
[a]Concentration (ppm) = 24.45 x concentration (mg/m^3)/molecular weight at standard temperature and pressure.

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Nitrobenzene has been identified in at least 92 of the 1,868 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2022a). However, the number of sites evaluated for nitrobenzene is not known. The number of sites in each state is shown in Figure 5-1.

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



Source: ATSDR 2022a

- The most likely routes of occupational exposure to nitrobenzene occurs through dermal exposure and inhalation of workplace air.
- Employees working in explosive, pharmaceutical, aniline, pesticide, and dye-stuff manufacturing are at a higher risk of exposure to nitrobenzene than the general population since nitrobenzene is used in these industries.
- The general population may be exposed to nitrobenzene via inhalation of air and possibly from drinking water.
- Nitrobenzene has the potential to volatilize if released to surface waters. Nitrobenzene is not expected to bioconcentrate or bioaccumulate in aquatic organisms. In water, nitrobenzene has

been shown to undergo photolysis and biodegradation is expected under both aerobic and anaerobic conditions. Nitrobenzene does not contain any functional groups that are susceptible to hydrolysis; therefore, this will not be an important environmental fate process in water.

- If released to soil, nitrobenzene is expected to possess moderate to high mobility and may leach to groundwater. It is expected to undergo biodegradation under both aerobic and anaerobic conditions.

- If released to air, nitrobenzene will be slowly degraded through reactions with hydroxyl radicals but may also undergo direct photolysis.

Human exposure to nitrobenzene results from releases to air and wastewater from industrial sources and from nitrobenzene as an air pollutant in ambient air, especially in urban areas. Its low volatility and weak sorption on soil suggests that surface waters and groundwater could be a route of exposure for the general population. Exposure is mitigated by environmental degradation, including photolysis and microbial biodegradation. Nitrobenzene is poorly bioaccumulated and is not biomagnified through the food chain. A number of fairly stable degradation products of nitrobenzene are formed during environmental degradation; some have similar effects, while others operate by different mechanisms. Moreover, whether or not nitrobenzene will be completely broken down (mineralized) at a particular site seems to be questionable. Nitrobenzene may be degraded in sewage treatment plants in aerobic conditions (WHO 2009), and when present at high concentrations, it also may inhibit the biodegradation of other wastes.

Monitoring studies reveal low and highly variable exposures through air and with a generally downward trend in exposure levels over two decades (Bozzelli and Kebbekus 1982; EPA 1985; Harkov et al. 1983; LaRegina et al. 1986). Occupational exposure is of concern due to the fact that nitrobenzene can be taken up very readily through the skin as well as by inhalation.

5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.2.1 Production

Nitrobenzene is produced commercially by the exothermic nitration of benzene with fuming nitric acid in the presence of a sulfuric acid catalyst at 50–65°C and then purified by washing and distilling with steam and redistilling (Booth 2012).

According to the Chemical Data Reporting (CDR) database, seven companies in the United States reported data for nitrobenzene: Malinckrodt, LLC; Huntsman Corporation; Tedia Company, Inc.; The

Chemours Company; The Dow Chemical Co.; Covestro, LLC; and BASF Corporation (EPA 2022). The total national aggregate production volumes estimated for 2016–2019 ranged from 1,000,000,000 to <5,000,000,000 pounds each year (these figures include imports); however, most of the individual company data for production volumes, imports, and exports were declared as confidential business information (CBI).

Table 5-1 lists the facilities in each state that manufacture or process nitrobenzene, the intended use, and the range of maximum amounts of nitrobenzene that are stored on site.

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

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AL	1	10,000	99,999	1, 13
AR	1	10,000	99,999	9, 12
CA	1	10,000	99,999	6
DE	1	1,000,000	9,999,999	6
IL	1	1,000	9,999	12
IN	1	100	999	12
KY	1	10,000	99,999	12
LA	2	1,000,000	9,999,999	1, 3, 6
NC	1	1,000,000	9,999,999	2, 3, 6
NE	1	1,000	9,999	9, 12
NY	1	1,000	9,999	12
OH	2	1,000	99,999	12
PA	2	1,000	99,999	10, 12
SC	1	1,000	9,999	6
TX	4	1,000	9,999,999	1, 3, 5, 6, 9, 12, 13

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

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

Source: TRI21 2022 (Data are from 2021)

5.2.2 Import/Export

According to the CDR, Mallinckrodt LLC and Tedia Company imported 28,287,333 and 33,331 pounds of nitrobenzene, respectively, into the United States in 2019 (EPA 2022). All of the other manufacturers reported no imports or declared this information as CBI. All manufacturers also reported no exports or declared export volumes as CBI in 2019.

5.2.3 Use

Approximately 90% of the worldwide production of nitrobenzene is used to produce aniline (Gatermann et al. 1995). Nitrobenzene is also widely used to produce other raw materials like quinolone antibiotics, azobenzene, and trinitrotoluene to make explosives, rubbers, pesticides, herbicides, insecticides, pharmaceuticals, and dyes (Dai et al. 2010b; Dong et al. 2010). Nitrobenzene is also used as a solvent in petroleum refining and as a solvent for coating materials and dye (Dai et al. 2010a; Dasgupta et al. 2018). It is also used to manufacture cellulose ethers and acetates, dinitrobenzene, dichloroaniline, and acetaminophen (Dasgupta et al. 2018). Nitrobenzene is used as a solvent for shoe dyes, and has been used in very small amounts as a flavoring agent and a perfume for soaps (Dunlap 1981; EPA 1981, 1985).

5.2.4 Disposal

Because nitrobenzene is listed as a hazardous substance, disposal of waste nitrobenzene is controlled by a number of federal regulations (see Chapter 7). Land disposal restrictions (treatment standards) apply to wastes containing nitrobenzene. These wastes may be chemically or biologically treated or incinerated by the liquid injection or fluidized bed methods (EPA 1988, 1989). In the past, EPA has not believed that releases of nitrobenzene to the environment are substantial (EPA 1984b).

5.3 RELEASES TO THE ENVIRONMENT

Most (97–98%) of the nitrobenzene produced is retained in closed systems for use in synthesizing aniline and other substituted nitrobenzenes and anilines (CMR 1987; EPA 1976). Most of these products go into the manufacture of various plastic monomers and polymers (50%) and rubber chemicals (27%); a smaller proportion goes into synthesis of hydroquinones (5%), dyes and intermediates (6%), drugs (3%), and pesticides and other specialty items (9%) (Dunlap 1981). A small fraction of the production is used directly in other processes or in consumer products (principally metal and shoe polishes).

The nitration of benzene in air leads to variable ambient levels in urban areas, making the assessment of releases to the air from waste sites difficult. Nevertheless, limited studies of municipal waste disposal facilities and the more complete evaluation of hazardous waste sites have found nitrobenzene infrequently present and, when present, concentrations have been generally low.

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ ≥10 full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under the Resource Conservation and Recovery Act (RCRA) Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005).

5.3.1 Air

Estimated releases of 15,993 pounds (~7.25metric tons) of nitrobenzene to the atmosphere from 21 domestic manufacturing and processing facilities in 2021, accounted for about 5% of the estimated total environmental releases from facilities required to report to the TRI (TRI21 2022). These releases are summarized in Table 5-2.

Table 5-2. Releases to the Environment from Facilities that Produce, Process, or Use Nitrobenzene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AL	1	1,798	0	0	0	0	1,798	0	1,798
AR	1	0	0	0	174	0	0	174	174
CA	1	0	0	0	0	0	0	0	0
DE	1	1	0	0	0	0	1	0	1
IL	1	1	0	0	5	0	1	5	7
IN	1	0	0	0	0	0	0	0	0
KY	1	221	0	0	0	0	221	0	221
LA	2	7,008	0	280,000	82	0	287,008	82	287,090
NE	1	27	0	0	0	0	27	0	27
NY	1	1	0	0	0	0	1	0	1
NC	1	4,959	0	0	0	0	4,959	0	4,959
OH	2	0	0	0	0	0	0	0	0
PA	2	4	0	0	0	250	4	250	254
SC	1	5	0	0	0	0	5	0	5
TX	4	1,969	0	3,215	0	0	5,184	0	5,184
Total	21	15,993	0	283,215	261	250	299,208	511	299,719

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

RF = reporting facilities; UI = underground injection

Source: TRI21 2022 (Data are from 2021)

Direct release of nitrobenzene to air during its manufacture is minimized by the passage of contaminated air through activated charcoal (EPA 1980a), and its subsequent use in closed systems as an intermediate similarly limits direct exposure during industrial processing. Nevertheless, as much as 8.3 million

pounds/year may be released from industrial processes (EPA 1976). The fraction of these manufacturing losses to air is not known.

Use of nitrobenzene as a chemical intermediate or in consumer products such as metal and shoe polishes could contribute to losses via fugitive emissions, wastewater, spills, and end-product usage. The extent to which these sources contribute to human exposure has not been evaluated quantitatively.

Nitrobenzene is a compound that has air emission data from the National Emissions Inventory (NEI), which is a comprehensive and detailed estimate of air emissions of criteria pollutants, criteria precursors, and hazardous air pollutants (HAPs) from air emissions sources (EPA 2017). Data from the 2017 NEI for nitrobenzene are presented in Table 5-3.

Table 5-3. Nitrobenzene Air Emissions as Reported to the National Emissions Inventory Database for 2017

Sector	Pollutant type	Emissions (pounds)
Industrial processes; chemical manufacturing	HAP	9,985
Industrial processes; not elsewhere classified	HAP	9,805
Industrial processes; storage and transfer	HAP	2,966
Fuel combustion; electric generation; coal	HAP	1,774
Industrial processes; petroleum refineries	HAP	667
Waste disposal	HAP	330
Fuel combustion; industrial boilers, industrial combustion engines; other	HAP	45
Industrial processes; pulp and paper	HAP	7
Industrial processes; ferrous metals	HAP	6
Industrial processes; cement manufacturing	HAP	5

HAP = hazardous air pollutant

Source: EPA 2017

The third principal source of nitrobenzene is the atmospheric photochemical reaction of nitrogen oxides with benzene, which presumably is derived from automobile fuels and, to a lesser extent, solvent uses of benzene (EPA 1976). As benzene releases decline, this source (not quantified) should diminish as well. The contribution of this source is difficult to estimate since most measurements of ambient atmospheric nitrobenzene have been made in urban areas near sites of nitrobenzene manufacture, use, and disposal

(see Section 5.5.1). Seasonal variations and those associated with air pollution episodes suggest that this source, although limited, may form a significant proportion of non-occupational human exposure.

5.3.2 Water

No nitrobenzene was released to surface water from domestic manufacturing or processing facilities required to report to the TRI (TRI21 2022).

The effluent discharge produced during nitrobenzene manufacture is the principal source of nitrobenzene release to water. Products from leather manufacturing, like nitrobenzene, are often released to streams or rivers despite regulations (Baby et al. 2000). Nitrobenzene can be found in wastewater from pesticide, explosive, colorant, and paper pulp production industries (Li et al. 2010). Lin et al. (2013) noted that nitrobenzene has been so widely used in the creation of chemicals like aniline, aniline dyes, drugs, explosives, paint, pesticides, shoe polishes, floor polishes, and metal polishes that wastewater may contaminate surface and groundwater. Losses to wastewater have been observed to be 0.09% of production in one plant and 2.0% in another (EPA 1976).

The nitrobenzene in wastewater may be lost to the air, degraded by sewage organisms or, rarely, carried through to finished water. The EPA has surveyed nitrobenzene levels reported in effluents from 4,000 publicly-owned treatment works (POTWs) and industrial sites. The highest value in effluent was >100 ppm in the organic chemicals and plastics industries (Shackelford et al. 1983). Nitrobenzene was detected in 1 of 33 industrial effluents at a concentration >100 µg/L (EPA 1979a). Reported nitrobenzene concentrations in raw and treated industrial wastewaters from several industries range from 1.4 to 91,000 µg/L (EPA 1983).

Nitrobenzene was reported at above detectable levels in 1.8% of the 1,245 reporting industrial stations (Staples et al. 1985) and in the finished effluent of only 3 of the POTWs and 1 oil refinery (Ellis et al. 1982). In analysis of runoff samples from 51 catchments in 19 cities, the National Urban Runoff Program found no nitrobenzene (Cole et al. 1984). These results suggest that commercial and industrial users of nitrobenzene are dispersed throughout the country, so that concern regarding sources must extend beyond those four states in which nitrobenzene is manufactured.

In 2005, there was an explosion at a petrochemical plant in Jilin, Jilin Province, China that resulted in approximately 100 tons of chemicals including benzene, aniline, and nitrobenzene being released into the Songhua River (Dai et al. 2010b).

Although nitrobenzene is sparingly soluble in water (2.1 g/L) (Haynes 2015), its pungent, characteristic odor ("bitter almonds" [Budavari 1983]; "shoe polish" [Ruth 1986]) is detectable at water concentrations as low as 30 ppb (EPA 1980a). Hence, human exposures to large releases or accumulations in the environment are likely noticeable.

5.3.3 Soil

Estimated releases of 261 pounds (~0.1 metric tons) of nitrobenzene to soil from 21 domestic manufacturing and processing facilities in 2021, accounted for <1% of the estimated total environmental releases from facilities required to report to the TRI (TRI21 2022). An additional 283,215 pounds (~128.46 metric tons), accounted for about 94.49% of the total environmental emissions, were released via underground injection (TRI21 2022). These releases are summarized in Table 5-2.

As a source of nitrobenzene exposure of humans, soil appears to rank a distant third in terms of its contribution. Nelson and Hites (1980) reported 8 ppm in the soil of a former dye manufacturing site along the Buffalo River, but failed to detect nitrobenzene in river sediments, as noted above. The presence of nitrobenzene in the soils of abandoned hazardous waste sites is inferred by its presence in the atmosphere above several sites (Harkov et al. 1985; LaRegina et al. 1986).

5.4 ENVIRONMENTAL FATE

5.4.1 Transport and Partitioning

Air. Nitrobenzene's measured vapor pressure of 0.245 torr at 25°C and measured Henry's Law constant of 2.4×10^{-5} atm- m^3/mol suggests it can volatilize to air from water and moist soils (Lyman et al. 1990). The vapor density reported for nitrobenzene relative to air is 4.1–4.25 (WHO 2003). Removal processes for nitrobenzene in air may involve settling of vapor due to its higher density relative to air (Bidleman 1988; EPA 1976). Washout by rainfall (either through solution in rain drops or by removal of nitrobenzene sorbed onto particulates) and dry fall of particulates are negligible, as estimated by EPA

(1980b) and expressly measured in field releases (EPA 1984c). Atmospheric residence time was estimated to be 190 days (EPA 1980b).

Water. Based on a study of benzene and other aromatic compounds, observations of nitrobenzene in water suggest that it does not bioaccumulate; it does not accumulate in soils and sediments, can be taken up by plants, has been reported in groundwater, and has not been associated with either direct or indirect effects in the atmosphere (Korte and Klein 1982). Korte and Klein (1982) noted that chemical concentration will affect biodegradability in water. Compared to benzene, which Korte and Klein (1982) concluded is readily biodegradable based on mineralization, nitrobenzene is not as readily biodegraded.

Piwoni et al. (1986) found that nitrobenzene was totally degraded before significant volatilization occurred in microcosms simulating land-application of wastewater. The EXAMS computer model (EPA 1982a) predicts volatilization half-lives of 12 days (river) to 68 days (eutrophic lake) and up to 2% sediment sorption for nitrobenzene.

In a study modeling a spill of nitrobenzene and benzene into the Songhua River in China on November 13, 2005 at 1:00 pm, Fu et al. (2008) stated that the pollution front was expected to meet a monitoring point approximately 500 km downstream at 5:00 am on November 24, 2005. They found that over time and distance, concentrations decreased due to dispersion and pollutant mass decreased due to volatilization from the river water to the air.

Activated carbon and activated carbon materials developed from woody biomass have been shown to be effective adsorbents to remove nitrobenzene from water (Dai et al. 2010a).

Sediment and Soil. Sediment sorption is not likely to be significant (EPA 1985). Nitrobenzene preferentially sorbs to soils with higher organic carbon content and soils containing weaker hydrated cations or negatively charged siloxane sites (Briggs 1981). Leaching through soil may occur.

In soil, nitrobenzene has a high to moderate mobility. In two Danish subsoils, the K_{oc} values were 170 and 370 (Løkke 1984). In another study, nitrobenzene, in conjunction with other pollutants, was added to a column of Lincoln fine sand over a 45-day period, which resulted in a retardation factor of 1.9. In river sediment and coal wastewater pond sediment, nitrobenzene K_{oc} reported values were 89 and 105.6, respectively. In snow, nitrobenzene has a logarithmic sorption coefficient (Log $K_{i\ snow\ surface/air}$) of -2.89 m^3/m^2 at -6.8°C (Roth et al. 2004).

Seip et al. (1986) explored the retardation factors of nitrobenzene in three typical Norwegian soils. One soil was sandy with a low organic content and two were organic soils. The K_{oc} and retardation factor for the sandy soil were 30.6 and 1.27, respectively, while the other two organic soils had K_{oc} values of 42.8 and 69.6. The retardation factors of the two organic soils were 3.36 and 5.52. Briggs (1981) compared the soil sorption coefficient (K_d) expressed in terms of organic matter (K_{om}), where K_{om}=100xK_d (percent organic matter), for a wide variety of chemicals and soils, to the K_{ow}.

Jury et al. (1984) also classified nitrobenzene as intermediately mobile but noted that its loss from soil would be enhanced by evaporation of water. Moreover, because nitrobenzene has relatively poor diffusive flux, the material would tend to move as a bolus within soil. Jury et al. (1984) hypothesized that a deposit 10 cm deep in soil would have a half-life for effective volatilization of about 19 days.

Other Media. The National Institute of Technology and Evaluation (NITE) organization of Japan conducts standardized bioconcentration studies on pollutants or other chemicals of concern. Bioconcentration factors (BCF) of 3.1–4.8 and 1.6–7.7 were measured for carp exposed to 0.125 and 0.0125 mg/L nitrobenzene, respectively, over a 6-week exposure period (NITE 2022). Veith et al. (1979) found that the 28-day flow-through test for fathead minnows (*Pimephales promelas*) yielded a BCF of 15. A 3-day static measurement gave a BCF of <10 for the golden orfe (Freitag et al. 1982). In the Metcalf model "farm pond" microcosm (Lu and Metcalf 1975), the Ecological Magnification Index (EM: ratio of concentration of parent material in organism to concentration of parent material in water) was about 8 in mosquitofish (*Gambusia affinis*) after a 24-hour exposure. Longer exposures of other species, however, did not increase the value; the EM values were 0.7 in snails (*Physa sp*.); 0.8 in mosquito (*Culex quinquifasciatus*) larvae; 0.15 in *Daphnia magna*; and 0.03 in alga (*Oedogonium*). Bioaccumulation is not expected to be significant in terrestrial animals (EPA 1985). These data suggest that the potential for bioconcentration and bioaccumulation in aquatic organisms is low.

Nitrobenzene may accumulate in terrestrial plants. The relatively rapid uptake of ^{14}C-labeled nitrobenzene into mature soybean (*Glycine max* (L.) Merr) plants was reported by McFarlane et al. (1987a, 1987b). Plant uptake is, therefore, a possible route of human exposure to nitrobenzene.

5.4.2 Transformation and Degradation

Air. Atmospheric photochemical decomposition is an important removal route for nitrobenzene in the environment (EPA 1985). Nitrobenzene irradiated in a pyrex glass reaction vessel with a xenon lamp with wavelengths >300 nm was degraded 38% after a 5-hour exposure period (EPA 1985). p-Nitrophenol and nitrosobenzene were reported to be the principal photodegradation products of nitrobenzene vapors exposed to ultraviolet (UV) light in air (EPA 1985; Hastings and Matsen 1948). In another direct photolysis study, both o- and p-nitrophenols were observed as photodegradation products (Nojima and Kanno 1977). If released into the air, nitrobenzene exists solely as a vapor in the ambient atmosphere, with an estimated half-life for reaction with photochemically-produced hydroxyl radicals of 44 days (EPA, 2012). This reaction results in the formation of dinitrobenzene, nitrophenols, and dicarbonyls as reaction products (Kao 1994).

Water. Nitrobenzene does not hydrolyze; however, photolysis and biodegradation are significant degradation pathways in water (Bao et al. 2012). Nitrobenzene absorbs sunlight in the UV and blue spectral region, so direct photolysis may degrade nitrobenzene in aqueous systems (Wang et al. 2008). In near-surface water, the measured average annual photolytic half-life is 133 days (Simmons and Zepp1986). Near the surface of water bodies or in shallow waters, nitrobenzene may degrade by direct photolysis, with a half-life of 2.5–>6 days (Zepp et al. 1987). Wang et al. (2008) studied the kinetics and mechanism of phototransformation of nitrobenzene in four samples taken from different sections of the Songhua River. The study found that nitrobenzene had a relatively short half-life in natural river water (17.2–21.5 hours) that indicated indirect photodegradation may have played an important role in the loss of nitrobenzene. Under both natural and simulated solar irradiations, the main organic products of photodegradation were observed to be o-, m-, and p-nitrophenols and phenol (Wang et al. 2008).

Photochemical oxidation of nitrobenzene by hydrogen peroxide also yields p-, o-, and m-nitrophenols (Draper and Crosby 1984), with an estimated half-life of 250 days (NBS 1973). Through the reaction of hydrated electrons in eutrophic lakes or through reactions with nitrate in sunlight, degradation can occur with half-lives of 22 days and 11 hours, respectively (Zepp et al. 1987). Wang et al. (2008) found that nitrate concentration and alkalinity were the main factors affecting the photochemical fate of nitrobenzene in natural river water, suggesting that decomposition of nitrobenzene mediated by hydroxyl radicals was predominant in water solution with high nitrate concentrations.

Nitrobenzene was not readily biodegradable in a standardized ready test; however, it is expected to biodegrade under environmental conditions. Nitrobenzene achieved 3.3% of its theoretical biological oxygen demand (BOD) at a concentration of 100 mg/L using an activated sludge inoculum at 30 mg/L over the course of a 2-week incubation period and the MITI test (Organisation for Economic Cooperation and Development [OECD] 301C) (NITE 2022). In a laboratory-scale waste treatment study, Davis et al. (1981) estimated that 25% of the nitrobenzene was degraded and 75% was lost through volatility in a system yielding a loss of about 80% of initial nitrobenzene in 6 days. In a stabilization pond study, the half-life for volatilization was about 20 hours, with approximately 3% adsorbed to sediments (Davis et al. 1983).

A study investigating the use of the solar thermal electrochemical photo (STEP) concept to degrade nitrobenzene in wastewater demonstrated the experimental ease of solar-driven thermo- and electrochemical degradation of nitrobenzene in wastewater (Gu et al. 2017). The electrode reaction yields CO_2, nitric acid, and hydrogen. A study examining the use of zero-valent iron (ZVI) to reduce nitrobenzene found ZVI to be another feasible way to reduce nitrobenzene in groundwater (Dong et al. 2010). Dong et al. (2010) found that ZVI could be used to reduce nitrobenzene to aniline, resulting in an observed nitrobenzene reduction rate constant of 0.0006 $minute^{-1}$ and a half-life of 115.5 minutes. The final removal efficiency using ZVI was 80.98% (Dong et al. 2010).

Under laboratory conditions, direct photolysis of nitrobenzene in solvents such as isopropanol yields phenylhydroxylamine, which can be oxidized to nitrobenzene by oxygen (Hurley and Testa 1966, 1967). Phenylhydroxylamine and nitrobenzene can then combine to form azoxybenzene. However, these reactions may not be important under natural conditions in the absence of hydrogen donors (i.e., under environmental conditions) (EPA 1982b). Zepp et al. (1987) reported that hydrated electrons from dissolved organic matter could significantly increase photoreduction of compounds such as nitrobenzene, and that photolysis of nitrate ions to hydroxyl radicals increased nitrobenzene photodegradation (Zepp et al. 1987). Algae do not enhance photolysis of nitrobenzene (Zepp and Schlotzhauer 1983). Photolysis may be an important pathway in natural waters (EPA 1985), but probably only under conditions where biodegradation is poor or absent and where both UV irradiance and appropriate facilitating molecules occur in relatively clear waters.

Sediment and Soil. Nitrobenzene biodegrades under both aerobic and anaerobic conditions in soil. When a solution of nitrobenzene and other pollutants was passed through a column packed with Lincoln fine sand in a 45-day soil column transport experiment, 20–40% of the nitrobenzene was degraded

(Wilson et al. 1981). More than 99.9% of nitrobenzene added to an aerobic oil microcosm was removed during passage; the experiment, which was set up to mimic the fate of nitrobenzene in municipal wastewater applied to soil, suggests that the compound is degraded in treatment facilities (Piwoni et al. 1986). Under aerobic conditions, nitrobenzene had a half-life of 13.4–56 days in two different waste sludges mixed with a Derby soil in batch reactor experiments (Kincannon and Lin 1985). Nitrobenzene was transformed or removed with a half-life of 56 minutes in sediments from ponds and streams that had a sediment:water ratio of 0.13 (Wolfe 1992). Nitrobenzene was reduced in soils containing sulfide minerals. Nitrobenzene was typically reduced to aniline, and the rate of reaction was determined by dissolution rate and mineral solubility (Yu and Bailey 1992).

Other Media. Nitrobenzene may be almost completely removed by activated sludge treatment (EPA 1983; Gomółka and Gomółka 1979). Gomółka and Gomółka (1979) experimented with a 45-day soil column transport containing a solution of nitrobenzene and other pollutants that were passed through a column packed with Lincoln fine stand; 20–40% of the chemical was degraded with a half-life of 56 days. Similarly, anaerobic soil microcosm containing reed canary glass was created to understand the effect of nitrobenzene in municipal wastewater applied to soil; 99.9% of the added nitrobenzene was removed demonstrating the compound was biodegraded (Wilson et al. 1981). Pitter (1976) obtained 98% removal of chemical oxygen demand (COD) at a rate of 14 mg COD/hour/g dry weight of activated sludge with nitrobenzene as the sole carbon source. Tabak et al. (1981) obtained 100% biodegradation in settled domestic wastewater in 7 days. Hallas and Alexander (1983) reported 100% degradation in 10 days after a 6-day lag under aerobic conditions with municipal sewage effluent. Similar results have been reported by a number of researchers (Davis et al. 1981, 1983; Kincannon et al. 1983; Patil and Shinde 1988; Stover and Kincannon 1983) using a variety of model sewage treatment reactors and wastewater sources, including adapted industrial sludges.

Nitrobenzene was either highly resistant to degradation or inhibited biodegradation of other components of the waste in several biodegradation studies (EPA 1979b; Davis et al. 1981; Korte and Klein 1982; Lutin et al. 1965; Marion and Malaney 1963). However, these effects were observed at concentrations (≥50 mg/L) of nitrobenzene much higher than those detected in ambient waters (see Section 5.5.2).

Nitrobenzene is also degradable by anaerobic processes, but more slowly than described above. Chou et al. (1978) reported that nitrobenzene was 81% removed in 110 days by acclimated domestic sludge in an anaerobic reactor, and Hallas and Alexander (1983) found that 50% was degraded in 12 days under similar conditions. Canton et al. (1985) measured an 8% decrease in nitrobenzene after 8 days in unadapted

media but reported a half-life of <2 weeks in adapted media. As soon as degradation began, aniline was detected (Hallas and Alexander 1983).

5.5 LEVELS IN THE ENVIRONMENT

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

Table 5-4 shows the lowest limit of detections that are achieved by analytical analysis in environmental media. An overview summary of the range of concentrations detected in environmental media is presented in Table 5-5.

Table 5-4. Lowest Limit of Detection for Nitrobenzene Based on Standards[a]

Media	Detection limit	Reference
Air at landfill sites	0.05 ppb	Harkov et al. 1985
Air	0.02 mg/sample	NIOSH 1984
Air	0.5 mg/m^3	NIOSH 1977
Air	130 pptv	Francis et al. 2009
Wastewater	3.6 µg/L (FID); 13.7 µg/L (ECD)	EPA 1982c
Wastewater	5.42 µg/L	Zhang et al. 2007
Water	1.9 µg/L	EPA 1982c
Surface water	0.1 ng/L	Gatermann et al. 1995
Groundwater	10 µg/L	EPA 1986a
Soil and solid waste	137 mg/kg[a]	EPA 1986b
Soil and solid waste	19 mg/kg[a]	EPA 1986c
Soil and solid waste	660 µg/kg[b]	EPA 1986a
Soil and solid waste	12.5 µg/L[d]	EPA 2014
Blood	0.32 ng/mL	CDC 2021a

Table 5-4. Lowest Limit of Detection for Nitrobenzene Based on Standards[a]

Media	Detection limit	Reference
Urine	0.8 mg/L	Dangwal and Jethani 1980
Honey	<2 µg/kg	Castle et al. 2004

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.
[b]Approximate detection limit in high-level soil and sludges.
[c]Approximate detection limit in low-level soil and sediments.
[d]Detection limit in water. Detection limit in solids and wastes is several orders of magnitude higher.

ECD = electron capture detector; FID = flame ionization detector

Table 5-5. Summary of Environmental Levels of Nitrobenzene

Media	Low	High	For more information
Outdoor air (ppbv)	0.020	5.7	Section 5.5.1
Surface water (ppb)	0.0005	115	Section 5.5.2
Groundwater (ppb)	0.05	139	Section 5.5.2
Drinking water (ppb)	0.7	100	Section 5.5.2
Soil and sediment (ppb)	2	8,000	Section 5.5.3

Detections of nitrobenzene in air, water, and soil at NPL sites are summarized in Table 5-6.

Table 5-6. Nitrobenzene Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
Water (ppb)	21.5	74.9	37.4	24	12
Soil (ppb)	5,550	13,900	30.0	14	10
Air (ppbv)	No data				

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

5.5.1 Air

Most of the information on nitrobenzene levels in air is derived from reports from New Jersey, in which ambient air in urban, rural, and waste disposal areas were monitored extensively. In 1978, nitrobenzene levels averaged 0.40 ppbv in industrial areas, and 0.02 and 0.09 ppbv in two residential areas, but in 1982,

levels in residential areas were approximately ≤0.3 ppbv, while levels in industrial areas were ≥0.9 ppbv (Bozzelli and Kebbekus 1982). Again, most of the samples were negative for nitrobenzene. The highest values were 3.5–5.7 ppbv.

Harkov et al. (1983) reported low levels of nitrobenzene (0.07–0.1 ppbv) in approximately 85% of air samples of nitrobenzene in their study of airborne toxic chemicals in summer. Nitrobenzene was not detected during the winter (Harkov et al. 1984; Lioy et al. 1983).

Studies of air over waste disposal sites (Harkov et al. 1985) are confounded by weather and timing. Air at one landfill showed a mean nitrobenzene concentration of 1.32 ppbv and another of 0.3 ppbv; but at two other sites (measured during snow and/or rain), nitrobenzene was not detected. LaRegina et al. (1986) summarized these studies by noting that the highest value for nitrobenzene was 14.48 ppbv at a hazardous waste site, whereas nitrobenzene was often undetectable elsewhere (especially in rural areas or at sanitary landfills) or anywhere in the air during the winter.

Very little information is available for other areas of the United States. EPA (1978) found only one positive value of 107 ng/m^3 (0.020 ppbv) at a plant site in Louisiana. EPA (1985) data showed <25% of United States air samples positive, with a median concentration of approximately 0.01 ppbv.

5.5.2 Water

Nitrobenzene concentrations in the effluent of a Los Angeles County municipal wastewater treatment plant was about 200 ppb in 1978 and <10 ppb in 1980 (Young et al. 1983). Nitrobenzene was not reported in runoff samples in 1982 in a nationwide project (Cole et al. 1984). Kopfler et al. (1977) list nitrobenzene as one of the chemicals found in finished tap water in the United States, but do not report its concentrations or locations. EPA (1979c) reported only one positive sample (total sample number not stated) in Hartford, Connecticut sewage treatment plant influents, and no nitrobenzene was detected in samples taken from three other major metropolitan areas. Nitrobenzene was detected in only 0.4% of the 836 ambient surface water stations involved in EPA's STORET database (Staples et al. 1985).

Data from EPA's Water Quality Portal from 2000 to 2022 is presented in Tables 5-7 and 5-8 for both surface water and groundwater.

Table 5-7. Annual mean Nitrobenzene Concentrations (ppb) Measured in Surface Water at Locations Across the United States[a]

Year	Number of samples	Average of the arithmetic mean at all locations	Maximum concentration	Percent detected
2000–2004	137	84.8	115	43.8%
2005–2009	98	0.095	0.47	38.8%
2010–2014	490	0.093	0.26	3.49%
2015–2019	298	2.37	3.2	3.02%
2022	109	0.046	0.07	11.9%

[a]As of October 20, 2022.

Source: EPA 2022

Table 5-8. Annual mean Nitrobenzene Concentrations (ppb) Measured in Groundwater at Locations Across the United States[a]

Year	Number of samples	Average of the arithmetic mean at all locations	Maximum concentration	Percent detected
2000–2004	139	20.8	139	3.59%
2005–2009	252	7.08	40	54.4%
2010–2014	75	4.89	5	49.3%
2015–2019	14	ND	ND	ND
2022	1	0.37	0.37	100%

[a]As of October 20, 2022.

ND = not detected

Source: EPA 2022

Many studies have examined the concentration and distribution of nitrobenzene in surface water in China after a spill containing nitrobenzene occurred following a plant explosion. Nitrobenzene has been detected in rivers, especially in North China, at a mean concentration of 18.1 ng/L (Li et al. 2010). Fu et al. (2008) found that the peak concentration measured at Harbin, the capital of Hoeilongjiang Province located approximately 500 km downstream of the spill, 10 days after the spill was 0.58 mg/L; the guideline for nitrobenzene in China for drinking water is 0.017 mg/L. Another study, conducted at the Songhua River years after the spill, resulted in detections of nitrobenzene in all water and ice samples at concentrations between 0 and 0.65 µg/L (Dai et al. 2010b). In a study of the Yellow river, concentrations of nitrobenzene ranged from 0.128 to 8.427 µg/L, and nitrobenzene was the predominant contaminant in all locations sampled (He et al. 2006).

Nitrobenzene was a contaminant that was monitored for during the EPA's first unregulated Contaminant Monitoring Rule (UCMR). UCMR 1 required monitoring for 26 contaminants including nitrobenzene between 2001 and 2003 using analytical methods developed by EPA, consensus organizations, or both. Nitrobenzene was detected in 2 of the 3,867 public water systems tested (33,937 total samples analyzed) at concentrations of 21.6 and 100 µg/L (EPA 2003). Data from other nations indicate that nitrobenzene is occasionally detected in drinking water at low levels. It was detected in 1 of 14 samples of treated water in the United Kingdom and was detected in potable water from the Netherlands at a maximum value of 0.7 µg/L (WHO 2009).

Nitrobenzene was found in all 33 water samples taken from the eastern part of the North Sea at concentrations from 0.5 to 2.5 ng/L (Gatermann et al. 1995). The highest concentrations were found in the open sea and concentrations increased in a north-west direction.

5.5.3 Sediment and Soil

With the exception of the data shown in Table 5-6 regarding levels of nitrobenzene at NPL sites, there a few data regarding nitrobenzene levels in soil. Nitrobenzene was detected at a level of 8 ppm (mg/kg) in soil at one of two sampling sites along the bank of the industrially polluted Buffalo River in New York (Nelson and Hites 1980). Nitrobenzene was not detected at any of three sediment sampling sites in this study. Nitrobenzene was detected in 1 of 10 soil samples collected from the Gas Works Park in Seattle, Washington, at a concentration of 0.79 mg/kg (Turney and Goerlitz 1990). Nitrobenzene was detected in the sediment of the River Havel, Gmund Germany in March of 1993 at 4 ng/g dry weight (Lopes and Furlong 2001), and in the Spree River at Dahme and at Spandau shipping canal at 2 and 10 ng/g dry weight, in March 1993 and July 1994, respectively (Ricking et al. 2003).

5.5.4 Other Media

Nitrobenzene has not been found in other environmental media. It has not been detected as a bioaccumulated material in fish samples (Staples et al. 1985). No monitoring of plant tissues has been reported, even though uptake of nitrobenzene by plants has been observed (McFarlane et al. 1987a, 198b).

Nitrobenzene is a component of Frow mixture occasionally used to control Acarine, a parasite infestation, in honeybees, and nitrobenzene residues may be found in honey as a result (Castle et al. 2004). However,

of 49 samples of honey tested at a detection limit of 2 µg/kg, none contained a detectable level of nitrobenzene residue (Castle et al. 2004).

5.6 GENERAL POPULATION EXPOSURE

General exposure of the population to nitrobenzene is limited to variable concentrations in air and possibly drinking water (WHO 2009). Its occurrence in public water systems was low in the Unregulated Contaminant Monitoring Rule (UCMR) 1 testing results but has been detected in groundwater (see Section 5.5.2). Air levels can be high in the vicinity of manufacturing or production facilities (especially petroleum refining, leather finishing and some chemical manufacturers). Urban areas have much higher levels in the summer than winter due to both the formation of nitrobenzene by nitration of benzene (from motor vehicle fuels) and the higher volatility of nitrobenzene during the warmer months. Table 5-9 summarizes the geometric mean and percentiles of nitrobenzene in the blood of the U.S. general population from the National Health and Nutrition Examination Survey (NHANES) survey years 2013–2016. In each age and demographic group, the number of detections were too low to calculate the geometric means and representative percentiles.

Table 5-9. Geometric Mean and Selected Percentiles of Blood Nitrobenzene (in ng/mL) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES)

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)[a]				Sample size
			50th	75th	90th	95th	
Total	2013–2014	Not calculated	<LOD	<LOD	<LOD	<LOD	3,180
	2015–2016	Not calculated	<LOD	<LOD	<LOD	<LOD	3,037
Age group							
12–19 years	2013–2014	Not calculated	<LOD	<LOD	<LOD	<LOD	593
	2015–2016	Not calculated	<LOD	<LOD	<LOD	<LOD	537
20 years and older	2013–2014	Not calculated	<LOD	<LOD	<LOD	<LOD	2,587
	2015–2016	Not calculated	<LOD	<LOD	<LOD	<LOD	2,500
Sex							
Females	2013–2014	Not calculated	<LOD	<LOD	<LOD	<LOD	1,649
	2015–2016	Not calculated	<LOD	<LOD	<LOD	<LOD	1,534
Males	2013–2014	Not calculated	<LOD	<LOD	<LOD	<LOD	1,531
	2015–2016	Not calculated	<LOD	<LOD	<LOD	<LOD	1,503
Race/ethnicity							
Mexican American	2013–2014	Not calculated	<LOD	<LOD	<LOD	<LOD	505
	2015–2016	Not calculated	<LOD	<LOD	<LOD	<LOD	542

Table 5-9. Geometric Mean and Selected Percentiles of Blood Nitrobenzene (in ng/mL) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES)

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)[a]				Sample size
			50th	75th	90th	95th	
All Hispanics	2013–2014	Not calculated	<LOD	<LOD	<LOD	<LOD	810
	2015–2016	Not calculated	<LOD	<LOD	<LOD	<LOD	947
Non-Hispanic White	2013–2014	Not calculated	<LOD	<LOD	<LOD	<LOD	1,283
	2015–2016	Not calculated	<LOD	<LOD	<LOD	<LOD	986
Non-Hispanic Black	2013–2014	Not calculated	<LOD	<LOD	<LOD	<LOD	615
	2015–2016	Not calculated	<LOD	<LOD	<LOD	<LOD	634
Asians	2013–2014	Not calculated	<LOD	<LOD	<LOD	<LOD	363
	2015–2016	Not calculated	<LOD	<LOD	<LOD	<LOD	345

[a]The lowest limit of detection (LLOD) for nitrobenzene in blood in both NHANES 2013–2014 and 2015–2016 is 0.3200 ng/mL. All values that fell below the LLOD were recorded as the LLOD divided by the square root of 2. All of the measured values for nitrobenzene in blood were below the LLOD and were assigned the same value below the LLOD, resulting in a standard deviation of 1 for all groups.

CI = confidence interval; LOD = limit of detection

Source: CDC 2021b

Nitrobenzene is absorbed after dermal, inhalational, or oral exposure and then metabolized to various intermediates as discussed in Chapter 3. About 10–20% of a dose is eliminated in the urine as p-nitrophenol, which is used in biological monitoring of occupational exposures. Table 5-10 summarizes urinary levels of p-nitrophenol in the U.S. general population for NHANES survey years 2011–2014. As mentioned in Chapter 3, this metabolite is not specific to nitrobenzene; it is also a metabolite of organophosphate insecticides like parathion.

Table 5-10. Geometric Mean and Selected Percentiles of Urinary Nitrobenzene (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES)

	Survey years	Geometric mean (95% CI)	Selected percentiles				Sample size
			50th	75th	90th	95th	
Total	2011–2012	0.64 (0.57–0.71)	0.63	1.17	2.17	3.31	2,350
	2013–2014	0.64 (0.60–0.69)	0.61	1.18	2.17	3.21	2,584
Age group							
6-11 years	2011–2012	0.61 (0.50–0.75)	0.60	1.21	2.08	2.78	394
	2013–2014	0.84 (0.72-0.98)	0.68	1.66	3.11	4.09	411

Table 5-10. Geometric Mean and Selected Percentiles of Urinary Nitrobenzene (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES)

	Survey years	Geometric mean (95% CI)	Selected percentiles				Sample size
			50th	75th	90th	95th	
12–19 years	2011-2012	0.62 (0.51-0.74)	0.64	1.15	1.85	2.51	376
	2013–2014	0.66 (0.57–0.77)	0.68	1.23	1.82	2.71	415
20+ years	2011–2012	0.64 (0.57–0.72)	0.63	1.18	2.23	3.48	1,580
	2013–2014	0.62 (0.58–0.67)	0.57	1.14	2.07	3.21	1,758
Sex							
Male	2011–2012	0.67 (0.61–0.74)	0.70	1.24	2.21	3.19	1,190
	2013–2014	0.67 (0.62–0.73)	0.65	1.17	2.02	3.03	1,306
Female	2011–2012	0.60 (0.52–0.70)	0.58	1.13	2.14	3.48	1,160
	2013–2014	0.62 (0.56–0.68)	0.58	1.19	2.32	3.40	1,278
Race/ethnicity							
Mexican American	2011–2012	0.62 (0.52–0.73)	0.68	1.18	1.84	2.51	285
	2013–2014	0.68 (0.58–0.80)	0.67	1.30	2.07	2.64	403
Non-Hispanic Black	2011–2012	0.70 (0.55–0.87)	0.72	1.37	2.34	3.47	642
	2013–2014	0.76 (0.69–0.84)	0.78	1.37	2.49	3.59	576
Non-Hispanic White	2011–2012	0.62 (0.54–0.71)	0.60	1.15	2.13	3.31	752
	2013–2014	0.60 (0.56–0.64)	0.57	1.10	2.03	3.16	986
All Hispanic	2011–2012	0.64 (0.58–0.72)	0.68	1.16	1.98	2.98	546
	2013–2014	0.69 (0.60–0.79)	0.67	1.29	2.17	2.82	637
Asians	2011–2012	0.72 (0.63–0.81)	0.62	1.54	2.84	3.87	325
	2013–2014	0.74 (0.62–0.89)	0.63	1.39	3.16	4.90	284

CI = confidence interval

Source: CDC 2021b

Nitrobenzene in water is expected to be semi-volatile; thus, there is potential for inhalation exposure during showering and bathing. ATSDR's three-compartment Shower and Household-Use Exposure (SHOWER) model predicts air concentrations in the shower stall, bathroom, and main house throughout the day by estimating the contribution from showering or bathing and the contribution from other water sources in the house, such as the dishwasher, clothes washer, and faucets (ATSDR 2022b). This information, along with human activity patterns, is used to calculate a daily time-weighted average (TWA) exposure concentration via inhalation exposure and from dermal uptake from skin contact. ATSDR's SHOWER model is available by sending a request to showermodel@cdc.gov. Using maximum potable water levels from Section 5.5.2 and representative outdoor air levels discussed in

Section 5.5.1, Reasonable Maximum Exposure (RME) levels are estimated. Table 5-11 presents estimated intakes of nitrobenzene from such activities for various demographic groups.

Table 5-11. Reasonable Maximum Exposure for Daily Inhalation Dose and Administered Dermal Dose in µg/kg/day for the Target Person

Exposure group	Inhalation	Dermal
Birth–<1 year	5.4	0.36
1–<2 years	5.8	0.33
2–<6 years	3.9	0.29
6–<11 years	2.3	0.23
11–<16 years	1.5	0.19
16–<21 years	1.2	0.18
Adult	1.1	0.17
Pregnant and breastfeeding women	1.6	0.17

Source: ATSDR 2022b

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Occupational exposure can be significantly higher than the exposure of the general population. NIOSH (1988) identified about 10,600 workers (mainly chemists, equipment servicers, and janitorial staff) as potentially exposed workers in facilities where nitrobenzene is used. Additionally, Hanley et al. (2012) found that workers at a rubber chemical manufacturing plant in New York were occupationally exposed to nitrobenzene, ortho toluidine, and aniline. At an industrial exposure level of 5 mg/m^3 (1 ppmv), which is the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL), a worker would receive about 25 mg of nitrobenzene during an 8-hour day (Dunlap 1981). Additional information on regulations regarding occupational exposure is found in Chapter 7. Nitrobenzene is readily absorbed through the skin, as well as taken up by inhalation and ingestion; the need for worker protection is dependent on the scenario and may include the use of respirators and gloves, among other protections.

Based on the New Jersey air studies and on estimates of releases during manufacture, only populations in the vicinity of manufacturing activities (i.e., producers and industrial consumers of nitrobenzene for subsequent synthesis) and petroleum refining plants are likely to have any significant exposure to anthropogenic nitrobenzene. However, consideration of possible groundwater and soil contamination and uptake of nitrobenzene by plants expands the potentially high exposure group to include people living in

and around abandoned hazardous waste sites. Children may be exposed to nitrobenzene if they play in dirt that has been contaminated with nitrobenzene.

Residents of cities getting drinking water from the Songhua River, which was contaminated by a spill of nitrobenzene after an explosion at a petrochemical plant in Jilin, Jilin Province, China, were at high risk of exposure after the spill.

CHAPTER 6. ADEQUACY OF THE DATABASE

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

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

6.1 INFORMATION ON HEALTH EFFECTS

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

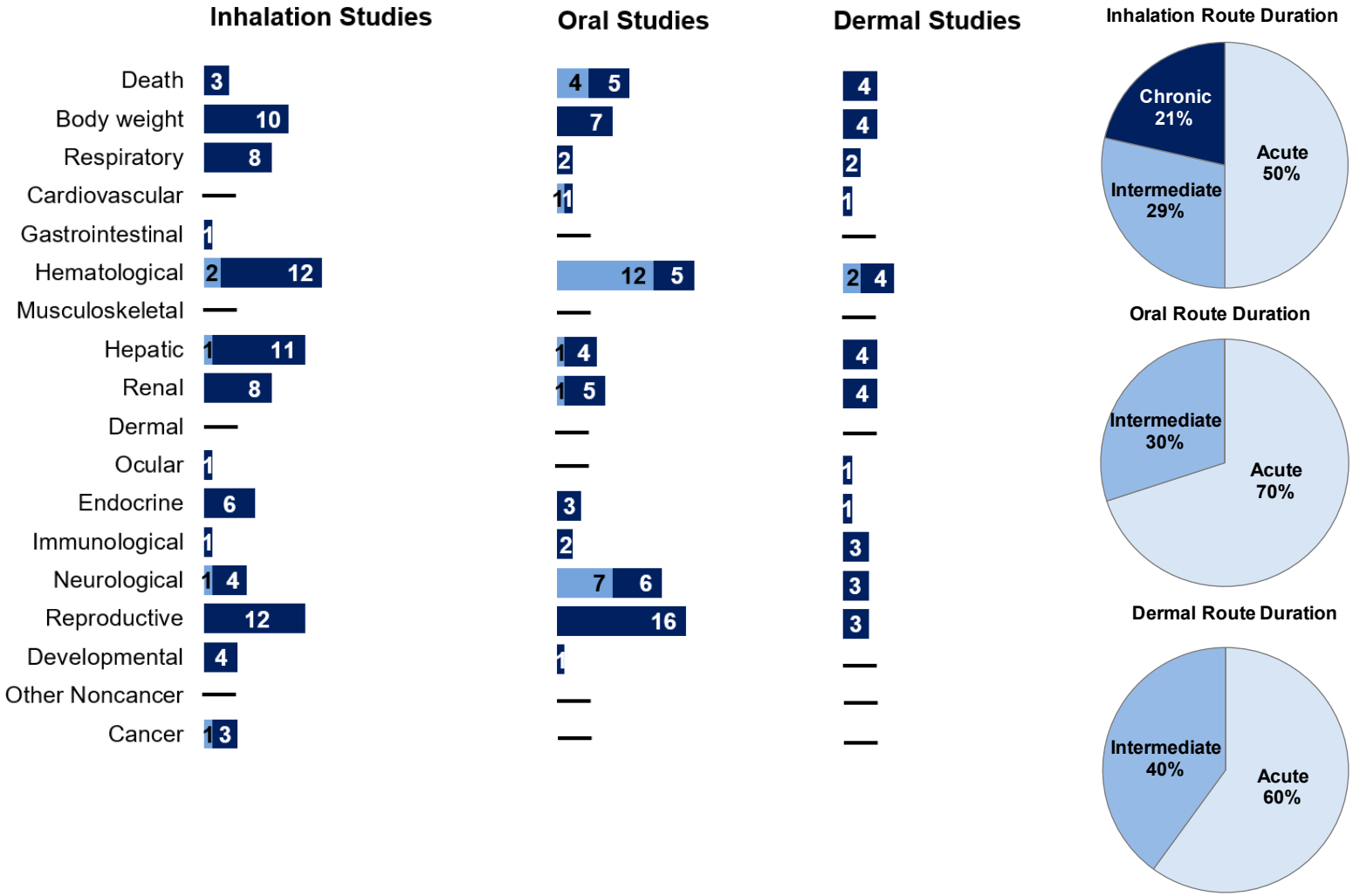
6.2 IDENTIFICATION OF DATA NEEDS

Acute-Duration MRLs. The inhalation database was adequate to derive an inhalation MRL. Medinsky and Irons (1985) provided full toxicological evaluations for nitrobenzene's toxicity with acute-duration via inhalation exposure. The available data were adequate for deriving an oral MRL. Burns et al. (1994) provided full toxicological evaluations for nitrobenzene's toxicity with acute-duration exposure via oral exposure.

Intermediate-Duration MRLs. The data were adequate to derive an intermediate MRL for both inhalation and oral routes of exposure. Hamm et al. (1984) and NTP (1983a) provided comprehensive toxicological evaluations of nitrobenzene's toxicity with intermediate-duration exposure via inhalation and oral exposure, respectively.

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

Potential hematological, reproductive, and neurological effects were the most studied endpoints.
The majority of studies examined oral exposure in **animals** (versus **humans**)



*Includes studies discussed in Chapter 2; the number of studies include those finding no effect. Most studies examined multiple endpoints.

Chronic-Duration MRLs. The data were adequate to derive an MRL for inhalation exposure based on the data provided in Cattley et al. (1994, 1995; CIIT 1993). However, no studies were located that evaluated the chronic effects of nitrobenzene exposure after oral exposure. Therefore, a chronic-duration oral exposure study is needed to have an adequate database to derive an MRL.

Health Effects. Overall, the database for nitrobenzene is relatively complete, with comprehensive toxicological evaluations in rats and mice exposed for acute and intermediate durations via inhalation, oral, and dermal studies. However, there are no chronic-duration oral or dermal studies, which could improve the completeness of the database. In addition, studies designed to evaluate the health effects of acute-duration inhalation at low exposure concentrations would improve the database.

Hematology. Available animal studies on nitrobenzene provide abundant evidence for hematological toxicity through all exposure routes. However, none of the available studies identified a NOAEL for this endpoint. Additional testing at lower doses would be useful for determining no-effect levels for hematologic effects.

Respiratory. The available inhalation studies on nitrobenzene demonstrate the potential for nitrobenzene to cause toxicity in the nasal passages and lungs. As with the hematology changes, the available studies did not identify NOAELs, as effects were seen at all exposure concentrations. Otherwise, the available data on nitrobenzene toxicity to the respiratory system appear to be adequate.

Endocrine. Several studies noted effects on the adrenal and thyroid glands after nitrobenzene exposure. Both Hamm et al. (1984) and NTP (1983a) observed cellular vacuolization of the zona reticularis in the adrenal gland. Additionally, chronic-duration nitrobenzene inhalation resulted in thyroid follicular cell hyperplasia. Additional studies that explore the endocrine effects of nitrobenzene exposure would be helpful to better understand the potential implications of an altered endocrine system.

Developmental. Two studies were located that evaluated developmental outcomes as a result of nitrobenzene exposure via inhalation. The potential developmental effects of nitrobenzene would be better understood if oral and/or dermal developmental toxicity studies were available.

Cancer. A chronic-duration inhalation study of nitrobenzene identified carcinogenic effects in rats and mice. No chronic-duration oral studies assessing nitrobenzene's carcinogenic potential were located; such studies would improve the database for this endpoint. Further, epidemiological studies on cancer in humans exposed to nitrobenzene would better delineate the carcinogenic potential of nitrobenzene in humans.

Genotoxicity. No studies were located regarding genotoxic effects in humans after inhalation, dermal, or oral exposure to nitrobenzene. However, many studies have been published evaluating nitrobenzene's mutagenicity/genotoxicity potential *in vitro* (see Table 2-4), some using human cells, and *in vivo* using experimental animals (see Table 2-5). The evidence is fairly conclusive that nitrobenzene does not induce mutations in bacteria. However, there is some evidence that nitrobenzene may causes chromosomal aberrations, micronuclei, and DNA damage and/or adducts. Additional research on these endpoints, particularly in relevant tissues (lung, liver, kidney, and mammary glands) from animals exposed *in vivo*, would serve to better characterize the potential genotoxic effects of nitrobenzene.

Epidemiology and Human Dosimetry Studies. Only one relevant epidemiology study was located. Additional epidemiological studies on the toxicity of nitrobenzene would improve the database on this chemical regarding understanding the relevance of the effects seen in experimental animals for humans.

Biomarkers of Exposure and Effect. Urinary levels of p-nitrophenol and p-aminophenol reflect recent exposure to nitrobenzene. The limitation of using these metabolites as biomarkers of exposure, however, is that they are nonspecific. p-Nitrophenol is a metabolite of not only nitrobenzene, but also of insecticides such as methyl parathion, ethyl parathion, and O-ethyl-O-(4-nitrophenyl) phenylphosphonothioate. A measurement of p-nitrophenol and p-aminophenol, therefore, should not be used to confirm or quantify nitrobenzene exposure. Nitrobenzene can be biomonitored in urine, but it is only reflective of recent exposure.

The presence of increased levels of methemoglobin may indicate exposure to nitrobenzene. However, it is an effect that is common to several other toxic substances. Therefore, methemoglobinemia by itself would not serve as a satisfactory biomarker of effect for nitrobenzene. Further study in this area does not appear to be potentially useful.

Absorption, Distribution, Metabolism, and Excretion. Nitrobenzene is readily absorbed following exposure by any route. Absorption data for humans exposed to nitrobenzene via inhalation and the dermal route indicate that it is efficiently absorbed by these routes. Although absorption studies using the oral route have not been located for humans, the available case studies suggest that it can also be absorbed via ingestion. The lipophilicity of nitrobenzene suggests a high degree of absorption. In animals, absorption studies using oral and dermal routes suggest extensive absorption. No quantitative absorption studies using inhalation exposure are available, but the available toxicity data suggest that absorption does take place. This does not appear to be a priority area for further research.

Following accidental ingestion of nitrobenzene in humans, the highest concentrations were found in the liver, brain, blood, and stomach. Delayed rise in the methemoglobin levels in severe methemoglobinemia after antidote administration may be attributed to the release of nitrobenzene stores from the adipose tissue. However, there is a lack of supportive evidence of significant accumulation of nitrobenzene or its metabolites in the body. Data in animals are limited to oral studies in rats and mice that indicate that there is some distribution to the blood, liver, brain, kidney, and lung. Not all tissues have been analyzed in these studies. No data on distribution of nitrobenzene are available for humans or animals after inhalation and dermal exposure. Additionally, some of the crucial studies that examine absorption of nitrobenzene are older, and newer studies that leverage current technology to quantify the absorption of nitrobenzene in humans and/or animals would be relevant to better understand the toxicokinetics of nitrobenzene. Comprehensive distribution studies for nitrobenzene administered to mice and rats via all three routes would be very helpful in predicting the organ systems at potential risk in exposed humans. PBPK models help quantitatively predict the internal dosimetry of nitrobenzene and its metabolites in a target tissue and their delayed retention.

Metabolism data available for nitrobenzene suggest that species and/or strain differences in toxicity may be related to the metabolic activities of intestinal bacteria that convert it to its toxic metabolite, aniline. This is an area in which further study may be helpful in making comparisons of human sensitivity with that of other animals, and thus may aid in the interpretation of the currently available animal studies and their relevance to humans.

Excretion data are available for humans exposed to nitrobenzene via the inhalation, oral, and dermal routes. The available animal studies have used the oral route. Urine appears to be the major route of excretion, although this has not been clearly established, especially after inhalation and dermal exposure. There is no apparent need for further studies in this area.

Comparative Toxicokinetics. Species and strain differences in response to nitrobenzene exposure have been noted in studies using mice and rats. The reasons for these differences and the toxicokinetics involved are not understood. Additional toxicokinetic studies comparing metabolism of nitrobenzene in rats, mice, and humans would strengthen the available understanding. In addition, the development of a PBPK/PD model for nitrobenzene would also be useful, in order to reduce the uncertainty in extrapolating dose and effect information from animals to humans.

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

Physical and Chemical Properties. No specific data needs are identified for these properties. Available values are generally accepted and can be used to estimate nitrobenzene's environmental fate.

Production, Import/Export, Use, Release, and Disposal. Production methods for nitrobenzene are well-described in the literature, and there does not appear to be a need for further information. Available data indicate that most nitrobenzene produced in the United States is consumed in the production of aniline. Nitrobenzene is widely used in the workplace to produce raw materials (especially aniline) and as a solvent. Information on the uses of nitrobenzene is available in the literature and more information is not needed. Because nitrobenzene is listed as a hazardous substance, disposal of waste nitrobenzene is controlled by a number of federal regulations. Land disposal restrictions (treatment standards) apply to wastes containing nitrobenzene. Data on the amounts of nitrobenzene disposed was not located in the literature. Nitrobenzene is regulated under EPCRA, the Clean Air Act, the Clean Water Act, and RCRA. Several regulations govern disposal of nitrobenzene. Additional regulatory information is not needed.

Environmental Fate. The environmental fate of nitrobenzene is fairly well understood within the context of recognition of the importance of conditions in estimating or modelling environmental concentrations. The most critical condition is the presence/absence of a viable, competent, and functioning population of microorganisms for biodegradation. The next most critical factor is the amount of sunlight. For exposure assessment modelling accuracy, more data are needed on fate in soil, both in the root zone where plants are exposed and in the saturated and unsaturated zones where groundwater may become contaminated. Metabolism in plants is poorly characterized to date, so information on the

nature and quantity of plant metabolites would assist in the assessment of exposure via ingestion of plants.

Bioavailability from Environmental Media. The available information indicates that nitrobenzene is well absorbed following inhalation, oral, or dermal exposure. It is expected to be well-absorbed by persons breathing or having dermal contact with contaminated air or ingesting water, soil, plants, or any environmental materials that contain it. It would be useful to have information on its absorption after dermal contact with contaminated soil or plant material.

Food Chain Bioaccumulation. Uptake and accumulation of nitrobenzene through food chains are well-understood regarding animal tissues, especially fish. However, more information about plant tissues would be helpful.

Exposure Levels in Environmental Media. Data are available on nitrobenzene occurrence in air, surface waters, soil, sediments, and aquatic animals. However, much of these data are from studies performed decades ago and more current monitoring data is needed to identify current exposure risks.

Exposure Levels in Humans. There is very little information on human exposure to nitrobenzene outside of the workplace. More detailed exposure analyses that take transformation pathways into account can be performed for local sites and the potentially affected populations. Further, it would be useful to know more about the relationship of the organoleptic properties of nitrobenzene with respect to tolerable exposures. For example, it would be useful to know whether its taste and aroma are deterrents to high levels of human exposure.

6.3 ONGOING STUDIES

No relevant ongoing studies were identified in the National Institute of Health (NIH) RePORTER (2022) database.

CHAPTER 7. REGULATIONS AND GUIDELINES

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

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

Table 7-1. Regulations and Guidelines Applicable to Nitrobenzene

Agency	Description	Information	Reference
	Air		
EPA	RfC	$9x10^{-3}$ mg/m^3 ($2x10^{-3}$ ppm)	IRIS 2009
WHO	Air quality guidelines	No data	WHO 2010
	Water & Food		
EPA	Drinking water standards and health advisories	Not listed	EPA 2018a
	National primary drinking water regulations	Not listed	EPA 2009b
	RfD	$2x10^{-3}$ mg/kg/day	IRIS 2009
WHO	Drinking water quality guidelines	Guidelines not established[a]	WHO 2022, WHO 2009
FDA	Substances added to food (formerly EAFUS)	Not listed	FDA 2022
	Cancer		
HHS	Carcinogenicity classification	Reasonably anticipated to be a human carcinogen	NTP 2021
EPA	Carcinogenicity classification	Likely to be carcinogenic to humans	IRIS 2009
	Inhalation unit risk	$4x10^{-5}$ per µg/m^3	
IARC	Carcinogenicity classification	Group 2B[b]	IARC 1996
	Occupational		
OSHA	PEL (8-hour TWA) for general industry, shipyards, and construction	1 ppm (5 mg/m^3)[c]	OSHA 2021a, 2021b, 2021c
NIOSH	REL (up to 10-hour TWA)	1 ppm (5 mg/m^3)[c]	NIOSH 2019
	IDLH	200 ppm	

Table 7-1. Regulations and Guidelines Applicable to Nitrobenzene

Agency	Description	Information	Reference
	Emergency Criteria		
EPA	AEGLs-air	No data	EPA 2018b
DOE	PACs-air		DOE 2018a
	PAC-1[d]	3 ppm	
	PAC-2[d]	20 ppm	
	PAC-3[d]	200 ppm	

[a]Reason: nitrobenzene is rarely found in drinking water at concentrations of health concern.
[b]Group 2B: possibly carcinogenic to humans.
[c]Skin notation.
[d]Definitions of PAC terminology are available from U.S. Department of Energy (DOE 2018b).

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

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APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

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

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

APPENDIX A

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(s):	Nitrobenzene
CAS number(s):	98-95-3
Date:	January 2024
Profile Status:	Final
Route:	Inhalation
Duration:	Acute
MRL	0.1 ppm (0.5 mg/m^3)
Critical Effect:	Increased methemoglobin
Reference:	Medinsky and Irons 1985
Point of Departure:	$BMCL_{1SD}$ = 16.3 ppm ($BMCL_{HEC}$ = 2.91 ppm)
Uncertainty Factor:	30
LSE Graph Key:	2
Species:	Rat

MRL Summary: An acute-duration inhalation MRL of 0.1 ppm was derived for nitrobenzene based on a LOAEL of 9.1 ppm ($BMCL_{1SD}$ of 16.3 ppm) for increased methemoglobin in a 14-day study in female CD rats (Medinsky and Irons 1985). The $BMCL_{1SD}$ of 16.3 ppm was adjusted to continuous-duration exposure and converted to a human equivalent concentration ($BMCL_{HEC}$) of 2.91 ppm. The $BMCL_{HEC}$ was divided by a total uncertainty factor of 30 (10 for human variability and 3 for animal to human extrapolation after dosimetric adjustment).

Selection of the Critical Effect: The database of acute-duration inhalation toxicity studies of nitrobenzene consists of two developmental toxicity studies in rabbits (Biodynamics 1983, 1984), a developmental toxicity study in rats (Tyl et al. 1987), and 14-day studies in CD and F344 rats and B6C3F1 mice that included comprehensive toxicological evaluations (Medinsky and Irons 1985). Table A-1 shows the lowest effect levels from the acute-duration studies. The studies by Tyl et al. (1987) and Biodynamics (1983, 1984) identified developmental effects in rats and rabbits at exposure concentrations of 39.4 and 41 ppm, respectively. Maternal effects seen in the rat dams included decreased maternal weight gain at 39.4 ppm and increased spleen weight at 9.8 ppm. Increased methemoglobin levels were observed in maternal rabbits exposed to 41 ppm in the developmental toxicity study (Biodynamics 1984). In the 14-day studies (Medinsky and Irons 1985), hematological effects occurred at all exposure levels in all species; the LOAEL was 9.1 ppm. The hematologic effects seen in rats and mice at the lowest exposure level consisted of increased spleen weight (F344 and CD rats), decreased erythrocyte count (CD rats), and splenic lesions (all animals). In F344 rats, increased liver and kidney weight were also seen at the LOAEL of 9.1 ppm and mild hepatocyte necrosis was observed in CD rats at 35.8 ppm. The data presented in Table A-1 indicate that hematological effects occur at the lowest exposure concentrations and appear to represent a critical effect of nitrobenzene.

Table A-1. Summary of Acute-Duration Inhalation NOAEL and LOAEL Values for Nitrobenzene

Species	Duration	NOAEL (ppm)	LOAEL (ppm)	$NOAEL_{ADJ}$[a] (ppm)	$LOAEL_{ADJ}$[a] (ppm)	Effect	Reference
Hematological effects							
F344 rat	14 days, 5 days/week, 6 hours/day		9.1		1.6	Splenic lesions (not specified); increased relative spleen weight (33%)	Medinsky and Irons (1985)
CD rat	14 days, 5 days/week, 6 hours/day		9.1		1.6	Decreased RBC count; increased relative spleen weight (44% in females); splenic lesions (not specified); increased methemoglobin in females (6.3 versus 4.8% in controls)	Medinsky and Irons (1985)
B6C3F1 mouse	14 days, 5 days/week, 6 hours/day		9.1		1.6	Splenic lesions (not specified)	Medinsky and Irons (1985)
CD rat	GDs 6–15, 6 hours/day	1.06	9.8	0.3	2.5	Increased absolute and relative maternal spleen weights (13–15%)	Tyl et al. (1987)
New Zealand white rabbit	GDs 7–19, 6 hours/day	9.9	41	2.5	10	Increased maternal methemoglobin (1.4 versus 1.0% in controls)	Biodynamics (1984)
Renal effects							
F344 rat	14 days, 5 days/week, 6 hours/day		9.1 M		1.6 M	Increased relative kidney weight (15%) in males	Medinsky and Irons (1985)
Hepatic effects							
F344 rat	14 days, 5 days/week, 6 hours/day		9.1 M		1.6 M	Increased relative liver weight (13%) in males	Medinsky and Irons (1985)
CD rat	14 days, 5 days/week, 6 hours/day	9.1	35.8	2.3	8.7	Mild hepatocyte necrosis	Medinsky and Irons (1985)

Table A-1. Summary of Acute-Duration Inhalation NOAEL and LOAEL Values for Nitrobenzene

Species	Duration	NOAEL (ppm)	LOAEL (ppm)	$NOAEL_{ADJ}$[a] (ppm)	$LOAEL_{ADJ}$[a] (ppm)	Effect	Reference
Developmental effects							
CD rat	GDs 6–15, 6 hours/day	9.8	39.4	2.5	9.9	Increased incidences of litters with variations (hole in parietal bone and ecchymosis on trunk)	Tyl et al. (1987)
New Zealand white rabbit	GDs 7–19, 6 hours/day	9.9	41	2.5	10	Decreased mean number viable male fetuses	Biodynamics (1984)

[a]Acute-duration inhalation studies were adjusted from intermittent daily exposures to the equivalent of 24-hour continuous exposure. The duration adjusted values were calculated as:

$$Adjusted\ Daily\ Dose = Intermittent\ dose \times \frac{hours\ per\ day\ exposed}{24\ hours} \times \frac{days\ per\ week\ exposed}{7\ days}$$

F = female(s); GD = gestation day; LOAEL = lowest-observed-adverse-effect level; $LOAEL_{ADJ}$ = LOAEL adjusted to continuous exposure; M = male(s); NOAEL = no-observed-adverse-effect level; $NOAEL_{ADJ}$ = NOAEL adjusted to continuous exposure

***Selection of the Principal Study*:** Medinsky and Irons (1985) conducted comprehensive toxicological evaluations, while the gestational exposure studies were limited to developmental endpoints. In addition, the lowest LOAELs were identified for hematological, renal, and liver effects in rats and mice in the study by Medinsky and Irons (1985). Therefore, the study by Medinsky and Irons (1985) was selected as the principal study.

Summary of the Principal Study:

Medinsky MA, Irons RD. 1985. Sex, strain, and species differences in the response. In: Ricker D, ed. Toxicity of nitroaromatic compounds. New York: Hemisphere Publishing Corporation, 35-51.

Medinsky and Irons (1985) compared effects of nitrobenzene in F344 rats, CD rats, and B6C3F1 mice. Groups of 10 male and 10 female animals per species and strain were exposed whole body to nitrobenzene vapor at concentrations of 0 (control), 10, 35, or 125 ppm (nominal) or 0, 9.1 or 35.8 ppm (analytical) for 6 hours/day, 5 days/week for 14 days. Animals were observed for general health status prior to and at the end of each exposure. Half of the animals (5/sex/species/strain/concentration) were sacrificed 3 days after the last exposure and the other half were sacrificed 14 days after the end of exposure. At necropsy, animals were weighed, and blood was collected for hematologic (erythrocyte and leukocyte counts; hematocrit, hemoglobin concentration, and mean cell volume) and serum chemistry analysis. Selected organs were weighed (liver, spleen, kidney, testes, and brain) and the following tissues were examined for histopathology (specifically the adrenal glands, bone marrow, brain, sternum, colon, duodenum, testes with epididymis, heart, ileum, left kidney, liver, mesenteric lymph nodes, lungs, nose and turbinates, ovaries, pancreas, spleen, stomach, thyroid glands, thymus, trachea, urinary bladder, uterus, and gross lesions).

There were no deaths or clinical signs of toxicity in F344 rats. In contrast, CD rats and mice exposed to 124.5 ppm nitrobenzene exhibited premature mortality and morbidity. After the 4^{th} exposure day, five male and three female CD rats were found dead; the rest of the group showed hyperpnea and wheezing and were sacrificed at the end of the first week. Mice at this exposure level were prostrate and exhibited dyspnea and were sacrificed between exposure days 2 and 4. Histopathology evaluation showed perivascular hemorrhage in the cerebellar peduncle in both rats and mice sacrificed early. No deaths occurred at lower exposure levels, and no brain lesions were observed in F344 rats at any exposure level or in CD rats or mice exposed to lower concentrations.

The study authors reported methemoglobin levels and relative organ weights in rats (but not mice) at each of the two sacrifice times (3 and 14 days after end of exposure). Table A-2 shows methemoglobin levels and significant organ weight changes in the groups sacrificed 3 days after exposure ended. As Table A-2 shows, a significant increase in methemoglobin level was reported in female CD rats exposed to 124.5 ppm. These animals were sacrificed early, so it is not clear when the methemoglobin levels were measured. No other statistically significant alterations in methemoglobin level were observed in rats at either sacrifice time, although there were concentration-dependent increases in methemoglobin in F344 rats sacrificed 3 days after treatment ended. For mice, the study authors reported only that the methemoglobin level in the group exposed to 124.5 ppm and sacrificed early was between 13 and 31%.

Table A-2. Methemoglobin Levels (%)[a] in 14-Day Inhalation Study of Rats and Mice

Effect	0	9.1 ppm	35.8 ppm	124.5 ppm
F344 rats, male	0	1.9±0.7	6.6±0.2	11.7±1.2
F344 rats, female	3.6±2.2	4.8±0.8	6.6±0.8	13.4±2.1
CD rats, male	6.9±1.3	6.1±0.5	8.7±1.0	14.0±1.3[b]
CD rats, female	4.8±0.7	6.3±0.6	7.3±1.4	31.3±2.5*[b]

[a]Methemoglobin levels are reported as mean±standard error for five animals/group. Results marked with an asterisk (*) are significantly different from control ($p<0.05$) as reported by the study authors. Methemoglobin levels were not reported for mice.
[b]Five male and three female CD rats died after the 4th day of exposure and the rest were sacrificed at the end of week 1 (5 days of exposure). It is not clear when the methemoglobin levels were measured in these animals.

Source: Medinsky and Irons 1985

Other hematology changes were primarily described qualitatively. F344 rats showed dose-related decreases in erythrocyte counts and decreased hemoglobin and hematocrit at 124.5 ppm. CD rats at 124.5 ppm exhibited decreased erythrocyte counts (~40% lower than controls), and the study authors noted that "less marked suppression was observed in animals exposed to 35 and 10 ppm nitrobenzene sacrificed on day 3 after exposure." Hemoglobin and hematocrit values were decreased, and MCV increased only at the highest concentration in CD rats. In mice, the only hematology change was an increase in MCV at the highest concentration.

No information was reported in the publication on organ weights in mice. At sacrifice 3 days after exposure ended (see Table A-3), relative liver weights were increased at ≥9.1 ppm in male F344 rats (≥13% relative to controls) and at concentrations ≥35.8 ppm in female F344 rats (≥ 27%). Both male and female F344 rats showed increased relative kidney weights at ≥9.1 ppm (≥15% in males and ≥6% in females). In the groups sacrificed 2 weeks after exposure ended, liver and kidney weights did not differ from controls. No effects on liver or kidney weights were observed in CD rats at either sacrifice time.

Rats of both strains showed markedly increased relative spleen weights. At sacrifice 3 days after exposure ended (see Table A-3), relative spleen weights were significantly increased at ≥9.1 ppm in female CD rats (≥44%) and at ≥35.8 ppm in male CD rats (74% relative to controls), male F344 rats (≥89%), and female F344 rats (≥111%). In the groups sacrificed 2 weeks after exposure ended, spleen weights did not differ from controls in CD rats, while spleen weights remained elevated relative to controls at ≥35.8 ppm in female F344 rats and at 124.5 ppm in male F344 rats.

A marked (44% relative to controls) decrease in testes weight was observed in male F344 rats exposed to 124.5 ppm and sacrificed 3 days after the end of treatment (see Table A-3). A similar magnitude of decrease was seen in the group exposed to this concentration and sacrificed after 2 weeks of recovery. No effect on testes weight was reported in male CD rats exposed to concentrations up to 35.8 ppm at either sacrifice time.

Table A-3. Significant Organ Weight Changes[a] in 14-Day Inhalation Study of Rats and Mice (Groups Sacrificed 3 Days after Exposure Ended)

Effect	0	9.1 ppm	35.8 ppm	124.5 ppm
F344 rats, male				
Relative liver weight (% body weight)	3.78±0.21	4.28±0.15* (13%)	5.02±0.08* (33%)	5.52±0.30* (46%)
Relative kidney weight (% body weight)	0.34±0.02	0.39±0.02* (15%)	0.39±0.01* (15%)	0.42±0.02* (24%)
Relative spleen weight (% body weight)	0.18±0.03	0.22±0.02 (22%)	0.34±0.02* (89%)	0.58±0.05* (222%)
Relative testes weight (% body weight)	1.31±0.05	1.33±0.03 (2%)	1.41±0.08 (8%)	0.73±0.08* (-44%)
F344 rats, female				
Relative liver weight (% body weight)	3.32±0.29	3.42±0.22 (3%)	4.22±0.34* (27%)	4.88±0.25* (47%)
Relative kidney weight (% body weight)	0.35±0.05	0.37±0.02* (6%)	0.43±0.08* (23%)	0.43±0.03* (23%)
Relative spleen weight (% body weight)	0.18±0.04	0.24±0.01 (33%)	0.38± 0.05* (111%)	0.68±0.06* (278%)
CD rats, male				
Relative spleen weight (% body weight)	0.19±0.04	0.20±0.03 (5%)	0.33±0.05* (74%)	NR[b]
CD rats, female				
Relative spleen weight (% body weight)	0.18±0.04	0.26±0.02* (44%)	0.35±0.06* (94%)	NR[b]

[a]Organ weights are reported as mean±standard error (percent change from control) for five animals/group. Results marked with an asterisk (*) are significantly different from control ($p<0.05$) as reported by the study authors. Organ weights were not reported for mice.
[b]Five male and three female CD rats died after the 4th day of exposure and the rest were sacrificed at the end of week 1 (5 days of exposure); organ weights were not reported for these animals.

NR = not reported

Source: Medinsky and Irons 1985

Histopathology changes related to treatment were observed in the spleen, kidneys, liver, and testes of rats (both strains) and mice; and in the lungs of CD rats and B6C3F1 mice. The study authors reported the histopathology incidences for the 35.8 and 124.5 ppm groups combined across the two sacrifice times. Histopathology findings were not reported quantitatively for the control or 9.1 ppm groups, and only selected endpoints were reported for the 35.8 ppm group.

The study authors stated that "splenic lesions were evident in all animals and dose groups exposed to nitrobenzene." Table A-4 shows the incidences of splenic lesions in the 35.8 and 124.5 ppm groups; the specific nature and incidences of splenic lesions in the 9.1 ppm group were not reported.

Table A-4. Splenic Noncancer Histopathology Changes[a] in 14-Day Inhalation Study of Rats and Mice (Combined Across Groups Sacrificed 3 and 14 Days after Exposure Ended)

Effect	0	9.1 ppm[b]	35.8 ppm	124.5 ppm
F344 rats, male				
Spleen, hemosiderosis	NR	Lesions present, nature and incidence not specified	10/10	10/10
Spleen, sinusoidal congestion			10/10	10/10
Spleen, EMH			10/10	10/10
Spleen, capsular hyperplasia			5/10	7/10
F344 rats, female				
Spleen, hemosiderosis	NR	Lesions present, nature and incidence not specified	10/10	10/10
Spleen, congestion			10/10	10/10
Spleen, EMH			10/10	10/10
Spleen, capsular hyperplasia			1/10	0/10
CD rats, male				
Spleen, hemosiderosis	NR	Lesions present, nature and incidence not specified	10/10	9/10
Spleen, congestion			5/10	10/10
Spleen, EMH			9/10	10/10
Spleen, lymphoid hypoplasia			9/10	0/10
Spleen, stromal hyperplasia			4/10	0/10
CD rats, female				
Spleen, hemosiderosis	NR	Lesions present, nature and incidence not specified	10/10	9/10
Spleen, congestion			4/10	10/10
Spleen, EMH			10/10	10/10
B6C3F1 mice, male				
Spleen, hemosiderosis	NR	Lesions present, nature and incidence not specified	2/9	0/10
Spleen, congestion			0/9	10/10
Spleen, EMH			9/10	10/10
Spleen, lymphoid hypoplasia			5/9	0/10
Spleen, stromal hyperplasia			3/9	0/10

Table A-4. Splenic Noncancer Histopathology Changes[a] in 14-Day Inhalation Study of Rats and Mice (Combined Across Groups Sacrificed 3 and 14 Days after Exposure Ended)

Effect	0	9.1 ppm[b]	35.8 ppm	124.5 ppm
B6C3F1 mice, female				
Spleen, hemosiderosis	NR	Lesions present, nature and incidence not specified	5/10	0/10
Spleen, congestion			5/10	10/10
Spleen, EMH			10/10	10/10
Spleen, lymphoid hypoplasia			1/10	0/10
Spleen, stromal hyperplasia			5/10	0/10

[a]Histopathology findings are reported as number affected/number examined. Results marked with an asterisk (*) are significantly different from control ($p<0.05$) as reported by the study authors.
[b]The study authors reported that "splenic lesions were evident in all animals and dose groups exposed to nitrobenzene" but did not specify the nature or the incidence(s) of the lesions in the 9.1 ppm group.

EMH = extramedullary hematopoiesis; NR = not reported

Source: Medinsky and Irons 1985

Rats of both sexes and strains showed renal changes. At the highest concentration, hydropic degeneration of cortical tubular cells, focal hyalinosis, and basophilic degeneration of tubular epithelial cells were reported in CD rats, and severe hyaline nephrosis was reported in F344 rats. The study authors noted that "degenerative tubular epithelial changes occurred in a small number of mice," and that minimal to moderate multifocal degenerative changes were noted in renal tubular epithelium of male mice exposed to 35 ppm." Incidences of renal lesions in mice were not given.

Hepatic effects in CD rats and in mice included necrosis and hydropic degeneration, with most rats and most male mice (and a few female mice) affected at 124.5 ppm (both CD rats and mice exposed to this concentration were sacrificed early). The study authors also reported (qualitatively) that all male CD rats sacrificed 3 days after the end of exposure to 35 ppm showed mild individual hepatocyte necrosis, while mice of both sexes exposed to 35 ppm exhibited nonnecrotic hepatocyte degenerative changes.

In the testes, multinucleated giant cells were observed in both species and strains at 124.5 ppm. In addition, F344 and CD rats in this exposure group exhibited dyspermiogenesis, while F344 rats also showed interstitial edema and interstitial Sertoli cell hyperplasia. Most mice at the highest exposure level had testicular degeneration. The study authors noted also that 1 of 10 mice exposed to 35 ppm showed acute testicular degeneration.

Lung histopathology changes in CD rats were limited to the highest exposure group (124.5 ppm, at which animals died or were sacrificed early) and consisted of perivascular edema and vascular congestion. In mice, moderate bronchiolar hyperplasia was observed in most animals of both sexes at the highest exposure level, and mild bronchial hyperplasia was seen in mice sacrificed 3 days after exposure to 35 ppm (incidence not reported).

Selection of the Point of Departure for the MRL: As shown in Table A-1, the lowest LOAELs for acute inhalation exposure to nitrobenzene were identified for hematological, renal, and liver effects in rats and mice in the 14-day study by Medinsky and Irons (1985). The effects of nitrobenzene on the blood, spleen, liver, and kidneys are all believed to originate with the formation of methemoglobin from

nitrobenzene metabolites, as detailed in *Metabolic Mechanisms* in Section 3.1.3. Thus, the point of departure (POD) for the acute-duration inhalation MRL was based on benchmark dose (BMD) modeling of methemoglobin levels. Medinsky and Irons (1985) provided quantitative data on methemoglobin levels in rats, but not in mice (see Table A-2). The highest methemoglobin level was measured in female CD rats exposed to 124.5 ppm, despite the fact that these animals were exposed for only 5 days. Thus, the data for female CD rats were selected for BMD modeling.

BMD modeling was conducted to identify a POD using the data for changes in methemoglobin levels in female CD rats administered nitrobenzene via inhalation (Table A-2). The highest exposure group (124.5 ppm) was omitted from modeling due to the mortality and early sacrifice of these animals. BMD modeling of continuous data was conducted with the EPA's Benchmark Dose Software (BMDS) (version 3.2). For these data, the Exponential, Hill, Linear, Polynomial, and Power continuous models available within the software were fit employing a benchmark response (BMR) of 1 standard deviation (SD). An adequate fit was judged based on the $\chi 2$ goodness-of-fit p value ($p>0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was constant. If a constant variance model was deemed appropriate based on the statistical test provided in BMDS (i.e., Test 2; p-value >0.1), the final BMD results were estimated from a constant variance model. If the test for homogeneity of variance was rejected (p-value <0.1), the model was run again while modeling the variance as a power function of the mean to account for this nonconstant variance. If this nonconstant variance model did not adequately fit the data (i.e., Test 3; p-value <0.1), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMCL was selected if the BMCLs estimated from different models varied >3-fold; otherwise, the BMCL from the model with the lowest Akaike information criterion (AIC) was selected.

Results of the modeling are shown in Table A-5. For female CD rat data, there was a marginal difference in the response and variance among concentration levels (test 1 p-value=0.088). The constant variance model did provide an adequate fit to the data (Test 2; p-value >0.1). With the constant variance model applied, the Exponential 2, Exponential 3, Polynomial, Power, and Linear models provided adequate fit to the means; the Exponential 4, Exponential 5 and Hill models did not. Of the fit models, the BMCLs were sufficiently close (<3-fold). The 2-degree Polynomial and Power models converged on the Linear model and had the lowest AIC; therefore, the Linear model was selected. Predicted BMC_{1SD} and $BMCL_{1SD}$ values are 31 and 16 ppm, respectively. Figure A-1 shows the fit of the linear model to the data.

Table A-5. Results from Benchmark Dose (BMD) Analysis (Constant Variance) of Increased Methemoglobin Levels in Female CD Rats Following Inhalation Exposure to Nitrobenzene (Medinsky and Irons 1985)

					Scaled residuals[c]	
Model	BMC_{1SD}[a] (ppm)	$BMCL_{1SD}$[a] (ppm)	p-Value[b]	AIC	Dose below BMC	Dose above BMC
Exponential (model 2)[d]	33.17	19.57	0.40	68.89	0.64	-0.13
Exponential (model 3)[d]	33.18	19.57	0.40	68.89	0.64	-0.13
Exponential (model 4)[d]			NA	70.18	-1.2×10^{-6}	-6.1×10^{-7}
Exponential (model 5)[d]			65,535	72.18	-0.0004	0.0006
Hill[d]			<0.0001	72.18	-1.5×10^{-7}	1.0×10^{-7}
Polynomial (2-degree)[d]	31.39	16.27	0.43	68.79	0.61	-0.15

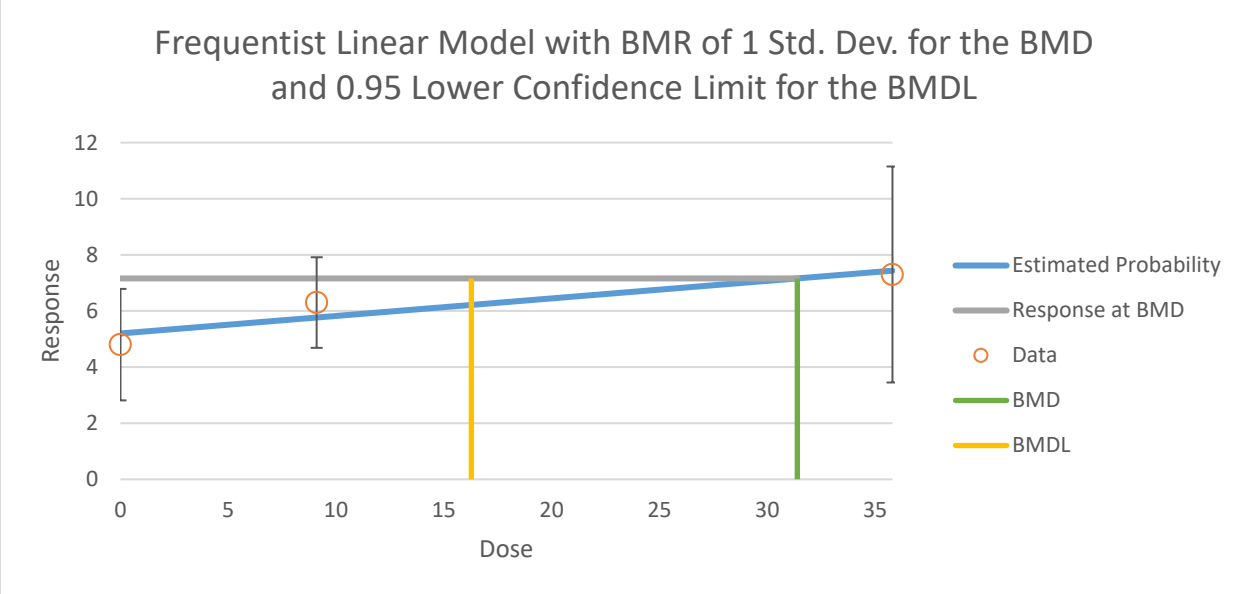
Table A-5. Results from Benchmark Dose (BMD) Analysis (Constant Variance) of Increased Methemoglobin Levels in Female CD Rats Following Inhalation Exposure to Nitrobenzene (Medinsky and Irons 1985)

Model	BMC_{1SD}[a] (ppm)	$BMCL_{1SD}$[a] (ppm)	p-Value[b]	AIC	Scaled residuals[c]	
					Dose below BMC	Dose above BMC
Power[d]	31.39	16.27	0.43	68.79	0.61	-0.15
Linear[e]	**31.39**	**16.27**	**0.43**	**68.79**	**0.61**	**-0.15**

[a]BMC and BMCL values for models that do not provide adequate fit are not included in this table.
[b]Values <0.1 fail to meet conventional χ^2 goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMC.
[d]Restricted model.
[e]Recommended model (lowest AIC). The constant variance model provided an adequate fit to the data (Test 2; p-value >0.1). With the constant variance model applied, the Exponential 2, Exponential 3, Polynomial, Power, and Linear models provided adequate fit to the means; the Exponential 4, Exponential 5, and Hill models did not. Of the fit models, the BMCLs were sufficiently close (<3-fold). The 2-degree Polynomial and Power models converged on the Linear model and had the lowest AIC; therefore, the Linear model was selected.

AIC = Akaike Information Criterion; BMC = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMCL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., $_{1SD}$ = exposure dose associated with a change of 1 standard deviation from the control)

Figure A-1. Fit of the Linear Model (Constant Variance) to Data for Nitrobenzene, Methemoglobin Levels in Female CD Rats (Medinsky and Irons 1985)



Adjustment for Intermittent Exposure: The animals in the study by Medinsky and Irons (1985) were exposed 6 hours/day, 5 days/week. Therefore, the $BMCL_{1SD}$ of 5 ppm was adjusted for intermittent exposure as follows:

$$BMCL_{ADJ} = BMCL1SD \times \frac{6\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 16.3\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 2.91\ ppm$$

Human Equivalent Concentration: The hematological effects of nitrobenzene are systemic, so the $BMCL_{ADJ}$ was converted to a human equivalent concentration ($BMCL_{HEC}$) using guidance from EPA (1994) on dosimetric adjustments for extrarespiratory (systemic) effects. Blood:gas partition coefficients were not identified in the available literature for nitrobenzene. In the absence of a chemical-specific blood:gas partition coefficient, EPA (1994) recommends using a default value of 1. Therefore, the $BMCL_{HEC}$ was calculated by the following equation:

$$BMCL_{HEC} = BMCL_{ADJ} \times \frac{(HB/g)_A}{(HB/g)_H} = 2.91 * 1 = 2.91\ ppm$$

where:

$\frac{(HB/g)_A}{(HB/g)_H}$ = is the blood: air partition coefficient for animals (a) to humans (h)

Uncertainty Factor: The $BMCL_{HEC}$ is divided by a total uncertainty factor of 30:

- 10 for human variability
- 3 for animal to human extrapolation after dosimetric adjustment

MRL = $BMCL_{HEC}$ ÷ uncertainty factors
MRL = 2.91 ppm ÷ (3x10) = 0.097 ppm ≈ 0.1 ppm (rounded to one significant figure)

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APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s):	Nitrobenzene
CAS number(s):	98-95-3
Date:	January 2024
Profile Status:	Final
Route:	Inhalation
Duration:	Intermediate
MRL	0.003 ppm (0.02 mg/m^3)
Critical Effect:	Hematological, renal, hepatic, and endocrine effects
Reference:	Hamm et al. 1984
Point of Departure:	LOAEL = 5 ppm ($LOAEL_{HEC}$ = 0.89 ppm)
Uncertainty Factor:	300
LSE Graph Key:	8
Species:	Rat

MRL Summary: An intermediate-duration inhalation MRL of 0.003 ppm was derived for nitrobenzene based on a LOAEL of 5 ppm for hematological, renal, hepatic, and endocrine effects (anemia, hemosiderin deposits, and histopathological changes in the kidneys, liver, and adrenal glands) in a 90-day study in rats and mice (Hamm et al. 1984). The LOAEL of 5 ppm was adjusted to continuous-duration exposure and converted to a human equivalent concentration ($LOAEL_{HEC}$) of 0.89 ppm. The $LOAEL_{HEC}$ was divided by a total uncertainty factor of 300 (10 for human variability, 10 for use of a LOAEL, and 3 for animal to human extrapolation after dosimetric adjustment).

Selection of the Critical Effect: There are two studies that evaluated toxicity in animals exposed by inhalation for intermediate durations (Dodd et al. 1987; Hamm et al. 1984). Table A-6 shows the lowest effect levels from these two studies. Dodd et al. (1987) was a 2-generation reproduction study with Sprague-Dawley rats in which a serious LOAEL of 40 ppm was identified for effects that included decreased fertility rates, atrophy of seminiferous tubules, spermatocyte degeneration, and reduced testicular and epididymal weights. The NOAEL was 10 ppm. Hamm et al. (1984) exposed F344 and Sprague-Dawley rats and B6C3F1 mice to nitrobenzene for 90 days and evaluated a comprehensive list of endpoints. Effects seen at the lowest exposure level (5 ppm) in the 90-day studies included hematological, renal, hepatic, and endocrine changes in rats and mice. All of these effects were considered for use in deriving the intermediate-duration inhalation MRL.

Selection of the Principal Study: The LOAEL of 5 ppm in the study by Hamm et al. (1984) was lower than the NOAEL of 10 ppm in the reproductive toxicity study by Dodd et al. (1987). Therefore, Hamm et al. (1984) was selected as the principal study for derivation of the intermediate-duration inhalation MRL.

Table A-6. Summary of Relevant NOAEL and LOAEL Values Considered for Derivation of an Intermediate-Duration Inhalation MRL for Nitrobenzene

Species	Duration	NOAEL (ppm)	LOAEL (ppm)	$NOAEL_{ADJ}$[a] (ppm)	$LOAEL_{ADJ}$[a] (ppm)	Effect	Reference
Hematological effects							
F344 rat	90 days, 5 days/week, 6 hours/day		5		0.89	Increased methemoglobin in males (3.0 versus 1.2% in controls); hematology changes indicative of hemolytic anemia in females; minimal to slight acute sinusoidal congestion and moderate to marked hemosiderin deposition in spleen in both sexes	Hamm et al. 1984
CD rat	90 days, 5 days/week, 6 hours/day		5		0.89	Splenic lesions: moderate hemosiderin pigmentation (males); slight to moderate sinusoidal congestion (both sexes)	Hamm et al. 1984
B6C3F1 mouse	90 days, 5 days/week, 6 hours/day		5		0.89	Splenic lesions: minimal to slight hemosiderin deposition (both sexes); slight to moderate sinusoidal congestion (females)	Hamm et al. 1984
Renal effects							
F344 rat	90 days, 5 days/week, 6 hours/day		5 M		0.89	Minimal nephrosis in males	Hamm et al. 1984
Hepatic effects							
CD rat	90 days, 5 days/week, 6 hours/day		5 M		0.89 M	Hepatic microgranulomas in males	Hamm et al. 1984
Endocrine effects							
B6C3F1 mouse	90 days, 5 days/week, 6 hours/day		5 F		0.89 F	Minimal to slight cortical cell vacuolization in zona reticularis of adrenal glands in females	Hamm et al. 1984

Table A-6. Summary of Relevant NOAEL and LOAEL Values Considered for Derivation of an Intermediate-Duration Inhalation MRL for Nitrobenzene

Species	Duration	NOAEL (ppm)	LOAEL (ppm)	$NOAEL_{ADJ}$[a] (ppm)	$LOAEL_{ADJ}$[a] (ppm)	Effect	Reference
Reproductive and developmental effects							
CD rat	10 weeks (2 generations), 5 days/week, 6 hours/day	10	40 (SLOAEL)	1.8	7.1	Decreased fertility rate, atrophy of seminiferous tubules, spermatocyte degeneration, and reduced testicular and epididymal weights; decreased F1 offspring body weights (12%) also occurred at this exposure level	Dodd et al. 1987

[a]Intermediate-duration inhalation studies were adjusted from intermittent daily exposures to the equivalent of 24-hour continuous exposure. The duration adjusted values were calculated as:

$$Adjusted\ Daily\ Dose = Intermittent\ dose \times \frac{hours\ per\ day\ exposed}{24\ hours} \times \frac{days\ per\ week\ exposed}{7\ days}$$

LOAEL = lowest-observed-adverse-effect level; $LOAEL_{ADJ}$ = LOAEL adjusted to continuous exposure; NOAEL = no-observed-adverse-effect level; $NOAEL_{ADJ}$ = NOAEL adjusted to continuous exposure

Summary of the Principal Study:

Hamm TE, Gibson JE, Irons RD, et al. 1984. Ninety-day inhalation toxicity study of nitrobenzene in F-344 rats, and CD rats and B6C3Fl mice with cover letter. Chemical Industry Institute of Technology. Submitted to the U.S. Environmental Protection Agency under section 8D. OTS0206507. 878214291.

Groups of male and female F-344 rats, CD rats, and B6C3F1 mice were exposed by inhalation (whole body) to nitrobenzene vapor at concentrations of 0 (control), 5, 16, or 50 ppm (nominal) or 0, 5, 15.8, or 48.7 ppm (analytical) for 6 hours/day, 5 days/week for 90 days. Experimental groups consisted of 10 male and 10 female animals of each strain and species. Animals were examined for clinical abnormalities twice daily and weighed once per week. Urine was collected for urinalysis. Animals were sacrificed at the end of the exposure period. Blood was drawn from the heart for hematology and serum chemistry analysis. Animals underwent gross necropsy and select tissues (spleen, brain, ovaries, kidney, testes, and brain) were weighed. Other tissues were prepared for histological analysis (cerebrum, pituitary gland, larynx, jejunum, pancreas, thymus, kidney, prostate, oviducts, lacrimal gland, sternum, nose/turbinates, thalamus, thyroid glands, esophagus, ileum, salivary gland, spleen, adrenal glands, testes, uterus, mammary glands, bone marrow, gross lesion, cerebellum, parathyroid, stomach, cecum, lymph nodes, heart, lungs, epididymis, urinary bladder, skeletal muscle, rib bone, medulla, trachea, duodenum, colon, liver, seminal vesicles, ovaries, eyes and optic nerve, peripheral nerve, and zymbal's gland).

There was no difference in survival between control groups and the nitrobenzene exposure groups. No biologically significant (≥10% less than controls) effects on body weight were observed. The only clinical sign of toxicity that was noted was diarrhea in female F344 rats at all exposure levels. Treatment-related hematology changes were observed in both rats and mice. Increased serum methemoglobin was observed at all exposure levels in male F344 rats; at ≥15.8 ppm in female F344 and male CD rats; and at 48.7 ppm in female CD rats and mice of both sexes. Hematology changes indicative of hemolytic anemia were seen in rats but not mice. Decreased erythrocyte counts, hematocrit, and/or hemoglobin, and increased erythrocyte width were evident at all exposure concentrations in female F344 rats, at ≥15.8 ppm in male and female CD rats, and at 48.7 ppm in male F344 rats. Clinical chemistry and urinalysis changes were not considered to be biologically significant, with the exception of a 2-fold increase in serum ALT in male mice.

Organ weight changes in male and female F344 rats included increased absolute and relative liver weights and increased absolute spleen weights at ≥15.8 ppm. Males also exhibited reduced testes weight at 48.7 ppm. Male and female CD rats had increased absolute and relative liver and spleen weights (≥15.8 ppm in females and at 48.7 ppm in males). Increased absolute and relative kidney weights and decreased absolute and relative testes weights were also noted in male CD rats exposed to 48.7 ppm. Spleen and liver weights were also increased in male and female mice exposed to 48.7 ppm. Male mice at that concentration showed increases in absolute and relative-to-brain-weight kidney weight, but not relative-to-body-weight kidney weight.

Enlarged spleen was observed at gross necropsy in animals of both species, strains, and sexes at the highest exposure level. Histopathology findings related to nitrobenzene exposure were observed in the respiratory tract, spleen, bone marrow, liver, and adrenal glands of both species, strains, and sexes. In addition, treatment-related increased incidences of lesions were observed in the kidneys and male reproductive organs of both strains of rat and in the thyroid glands of male CD rats.

Table A-7 summarizes the statistically significant treatment-related effects that occurred at all exposure levels in rats or mice. These included hematological, hepatic, renal, and endocrine effects.

Table A-7. Significant Noncancer Effects[a] Occurring at All Concentrations in a 90-Day Inhalation Study of Rats and Mice

Effect	0	5 ppm	15.8 ppm	48.7 ppm
F344 rats, male				
Methemoglobin (%)	1.2 ± 0.4	3.0 ±1.0*	4.4±1.3*	10.1±1.2*
Spleen, sinusoidal congestion	0/10	10/10* (2.0)	10/10* (1.8)	10/10* (2.8)
Spleen, hemosiderin deposition	10/10 (2.0)	10/10 (3.4)*	10/10 (3.7)*	10/10 (3.6)*
Kidney, nephrosis	0/10	6/10* (1.0)	9/10* (1.6)	10/10* (2.2)
F344 rats, female				
Erythrocyte count (10x6/mm^3)	9.07±0.18	8.7±0.18*	8.09±0.21*	7.36±0.23*
Hemoglobin (g/dL)	16.24±0.42	15.67±0.41*	14.81±0.28*	14.34±0.33*
Hematocrit (%)	48.58±1.68	47.12±1.04*	44.83±0.74*	42.25±1.06*
Spleen, sinusoidal congestion	0/10	10/10* (1.4)	10/10* (1.6)	10/10* (2.0)
Spleen, hemosiderin deposition	10/10 (2.5)	10/10 (3.8)*	10/10 (3.9)*	10/10 (4.4)*
CD rats, male				
Spleen, sinusoidal congestion	0/10	8/9* (1.6)	10/10* (2.4)	10/10* (2.4)
Spleen, hemosiderin deposition	10/10 (2.1)	9/9 (3.2)*	10/10 (4.9)*	10/10 (3.8)*
Liver, microgranulomas	0/10	7/9*	9/10*	7/10* (1.0)
CD rats, female				
Spleen, sinusoidal congestion	0/10	7/9* (1.6)	10/10* (2.6)	10/10* (3.9)
B6C3F1 mice, male				
Spleen, hemosiderin deposition	0/10	7/9* (1.0)	7/9* (2.3)	9/9* (2.6)
B6C3F1 mice, female				
Spleen, acute sinusoidal congestion	0/10	10/10* (2.9)	7/7* (4.0)	5/6* (3.8)
Spleen, hemosiderin deposition	1/7 (2.0)	10/10* (1.9)	7/7* (3/1)	6/6* (3.7)
Adrenal gland, cortical cell vacuolization	0/10	10/10* (1.8)	7/7* (3.4)	6/6* (4.3)

[a]Hematology changes are reported as mean±standard deviation. Histopathology findings are reported as number affected/number examined (mean severity score). Severity was scored as follows: 1=minimal; 2=slight; 3=moderate; 4=marked; and 5=very severe. Results marked with an asterisk (*) are significantly different from control ($p<0.05$) as reported by the study authors.

Source: Hamm et al. 1984

In F344 rats, additional treatment-related effects seen at the highest concentration (48.7 ppm) included: hyperplasia of the bronchial epithelium in males; proliferation of mesenchymal cells in the spleen, fibroblastic hyperplasia of the splenic capsule, accumulation of lymphocytes and macrophages in the spleen, and extramedullary hematopoiesis in the spleen; bone marrow hyperplasia; disorganized hepatic cords, vascular ectasia, centrilobular hepatocyte degeneration, periportal hepatocyte basophilia, and focal hepatocyte necrosis; nephrosis in females; increased basophilia of medullary cells in adrenal glands; and male reproductive tract changes consisting of moderate to severe degeneration of tubular epithelial cells, absence of mature sperm in epididymis, slight to moderate interstitial edema, and minimal to slight interstitial cell hyperplasia.

In CD rats, additional treatment-related effects seen at ≥15.8 ppm included centrilobular hepatocyte hypertrophy in females and toxic nephrosis in males and a slight reduction in mature sperm in two males. At the highest concentration (48.7 ppm), additional effects were seen in males including epithelial hyperplasia/metaplasia and goblet cell hyperplasia in the nasal turbinates; basophilia of adrenal medullary cells and thyroid follicular cell hypertrophy; and reproductive organ effects (bilateral testicular atrophy, complete loss of seminiferous epithelium, and absence of mature sperm in lumen of epididymis; increased interstitial cell hyperplasia, interstitial testicular atrophy, and multinucleate giant cells).

At the highest exposure level in B6C3F1 mice, treatment-related effects included hyperplasia of the bronchial mucosa; bone marrow hyperplasia; and centrilobular hepatocyte hyperplasia with some cord disorganization (females) and basophilic hepatocytes (males).

Selection of the Point of Departure for the MRL: As shown in Table A-7, effects seen at the lowest exposure level in the study by Hamm et al. (1984) included spleen congestion and/or hemosiderin deposition (significantly increased in severity but not incidence) in all species, strains, and sexes; increased methemoglobin and nephrosis in male F344 rats; hematology changes in female F344 rats; liver effects in male CD rats; and adrenal lesions in female B6C3F1 mice.

BMD modeling was conducted on the methemoglobin data for male F344 rats. In the absence of a biologically based benchmark for the methemoglobin level that is associated with adverse effects in rodents, a BMR of 1 SD was used. The data were fit to all continuous models in EPA's BMDS (version 3.2). Adequate model fit was judged by four criteria: chi-square goodness-of-fit p-value ($p \geq 0.1$), visual inspection of the dose-response curve, lower confidence limit on the benchmark dose (BMDL) <10 times the lowest non-zero dose, and scaled residual (>-2 and <+2) at the data point (except the control) closest to the predefined BMR. No adequate model fits were obtained.

The incidences of spleen congestion in nearly every species, strain, and sex (as well as adrenal cortical cell vacuolization in female mice) increased from 0 to ~100% at the lowest exposure level, precluding BMD modeling. Modeling was not considered for other histopathology changes (nephrosis, liver microgranulomas) that were seen at the LOAEL because the modeling results might not be adequately protective for the splenic and adrenal lesions.

In the absence of a suitable POD from modeling of the data, the LOAEL of 5 ppm was selected as the POD. Although the effects seen at the LOAEL were numerous and many occurred at high incidence, the lesions are likely all related to the effects of nitrobenzene on the blood, and severity scores indicated that the lesions were of generally minimal to moderate severity. Thus, the application of a 10-fold uncertainty factor for use of a LOAEL should provide adequate protection for the observed effects.

Adjustment for Intermittent Exposure: The animals in the study by Hamm et al. (1984) were exposed 6 hours/day, 5 days/week. Therefore, the LOAEL of 5 ppm was adjusted for intermittent exposure as follows:

$$LOAEL_{ADJ} = LOAEL \times \frac{6\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 5ppm \times \frac{6\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 0.89\ ppm$$

Human Equivalent Concentration: Given that all of the effects of nitrobenzene seen at the LOAEL were systemic, the $LOAEL_{ADJ}$ was converted to a human equivalent concentration ($LOAEL_{HEC}$) using guidance from EPA (1994) on dosimetric adjustments for extrarespiratory (systemic) effects. Blood:gas partition coefficients were not identified in the available literature for nitrobenzene. In the absence of a

chemical-specific blood:gas partition coefficient, EPA (1994) recommends using a default value of 1. Therefore, the $LOAEL_{HEC}$ was calculated by the following equation:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times \frac{(HB/g)_A}{(HB/g)_H} = 0.89 * 1 = 0.89\ ppm$$

where:

$\frac{(HB/g)_A}{(HB/g)_H}$ = is the blood: air partition coefficient for animals (a) to humans (h)

Uncertainty Factor: The $LOAEL_{HEC}$ is divided by a total uncertainty factor of 300:

- 10 for human variability
- 10 for use of a LOAEL
- 3 for animal to human extrapolation after dosimetric adjustment

MRL = $LOAEL_{HEC}$ ÷ uncertainty factors
MRL = 0.89 ppm ÷ (3x10x10) = 0.003 ppm

Other Additional Studies or Pertinent Information that Lend Support to this MRL: In human case studies of both inhalation and ingestion exposure to nitrobenzene, methemoglobinemia was the most common finding (Agrawal et al. 2011; Gupta et al. 2012; Ikeda and Kita 1964; Lee et al. 2013; Perera et al. 2009; Saxena and Prakash Saxena 2010). Additionally, experimental animal studies have demonstrated increased methemoglobin levels in mice and rats of both sexes exposed through any exposure route with acute, intermediate, and chronic (≥365 days) exposure durations (Biodynamics 1984; Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983b). A variety of related adverse effects in the hematologic system, such as hemolytic anemia; extramedullary hematopoiesis, hemosiderosis, congestion, and lymphoid depletion of the spleen; and changes in bone marrow in response to anemia have also been observed with inhalation, oral, and dermal exposures in B6C3F1 mice, F344 rats, and Sprague-Dawley rats (Burns et al. 1994; Cattley et al. 1994, 1995; CIIT 193; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b).

Agency Contacts (Chemical Managers): Malcolm Williams

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s):	Nitrobenzene
CAS number(s):	98-95-3
Date:	January 2024
Profile Status:	Final
Route:	Inhalation
Duration:	Chronic
MRL	0.0002 ppm (0.001 mg/m^3)
Critical Effect:	Hyperplasia of the nasal squamous epithelium
Reference:	Cattley et al. 1994, 1995; CIIT 1993
Point of Departure:	LOAEL= 1 ppm ($LOAEL_{HEC}$= 0.054 ppm)
Uncertainty Factor:	300
LSE Graph Key:	12
Species:	Rat

MRL Summary: A chronic-duration inhalation MRL of 0.0002 ppm was derived for nitrobenzene based on nasal hyperplasia of the squamous epithelium in male CD rats following exposure to nitrobenzene via inhalation for 2 years (Cattley et al. 1994, 1995; CIIT 1993). The MRL is based on a LOAEL of 1 ppm, which was adjusted to continuous-duration exposure and converted to a $LOAEL_{HEC}$ of 0.054 ppm. The $LOAEL_{HEC}$ was divided by a total uncertainty factor of 300 (10 for use of a LOAEL, 10 for human variability, and 3 for animal to human extrapolation after applying dosimetric adjustment).

Selection of the Critical Effect: The database of chronic-duration inhalation toxicity studies for nitrobenzene consists of 2-year experiments in male and female F344 rats, male CD rats, and male and female B6C3F1 mice reported in a single publication (Cattley et al. 1994, 1995). The unpublished report (CIIT 1993) provides additional details not reported in the publication. An overview of the lowest NOAEL and LOAEL values from this study are presented in Table A-8. The effects observed at the lowest exposure level in rats (1 ppm) and the lowest exposure level in mice (5 ppm) included effects on the respiratory tract (nasal lesions in rats and mice; pulmonary changes in mice) as well as systemic effects (hematological and hepatic effects). To provide a consistent basis for comparison across species, the LOAELs were adjusted for intermittent exposure and converted to HECs following EPA (1994) methodology. NOAELs were not identified for the most sensitive effects, so $NOAEL_{HECs}$ were not calculated. For systemic (extrarespiratory) effects, the HEC is calculated by multiplying the duration-adjusted animal NOAEL or LOAEL by the ratio of the blood:gas partition coefficients in animals and humans. Partition coefficients were not located for nitrobenzene, so the default value of 1 was used for the ratio. For effects on the respiratory tract, the regional gas dose ratio (RGDR) corresponding to the part of the respiratory tract that is affected was used. Thus, the RGDR for extrathoracic effects was used for nasal lesions and the RGDR for pulmonary effects was used for lung lesions. The $RGDR_{ET}$ (extrathoracic) values for mice (0.2) and rats (0.3) were calculated as follows:

$$RGDR_{ET} = (Mva/Saa) \div (MVh/Sah)$$

where:

Mva = minute volume for mice = 0.06 m^3/day; for rats = 0.47 m^3/day
MVh = minute volume for humans = 20 m^3/day
Saa = extrathoracic surface area for mice = 3 cm^2; for rats = 15 cm^2
Sah = extrathoracic surface area for humans = 200 cm^2

Table A-8. Summary of Relevant NOAEL and LOAEL Values Considered for Derivation of a Chronic Inhalation MRL for Nitrobenzene

Species	Duration	NOAEL (ppm)	LOAEL (ppm)	$NOAEL_{ADJ}$[a] (ppm)	$LOAEL_{ADJ}$[a] (ppm)	$LOAEL_{HEC}$ (ppm)	Effect	Reference
Respiratory effects								
CD rat (male)	2 years, 5 days/week, 6 hours/day		1		0.18	0.054	Increased nasal squamous epithelial hyperplasia/metaplasia and olfactory epithelial pigment deposition	Cattley et al. 1994, 1995; CIIT 1993
F344 rat	2 years, 5 days/week, 6 hours/day		1		0.18	0.054	Increased nasal olfactory epithelial pigment deposition (both sexes)	Cattley et al. 1994, 1995; CIIT 1993
B6C3F1 mouse	2 years, 5 days/week, 6 hours/day		5		0.89	0.18 (nasal) 2.89 (lung)	Increased nasal olfactory degeneration/necrosis in females; nasal olfactory epithelial pigment deposition (both sexes); bronchiolization of alveolar walls (both sexes)	Cattley et al. 1994, 1995; CIIT 1993
Hematologic effects								
CD rat (male)	2 years, 5 days/week, 6 hours/day		1		0.18	0.18	Increased methemoglobin at 15-month sacrifice (4.08 versus 1.18% in controls); spleen congestion at termination	Cattley et al. 1994, 1995; CIIT 1993
F344 rat	2 years, 5 days/week, 6 hours/day		1		0.18	0.18	Spleen congestion (both sexes); pigment deposition in spleen (males)	Cattley et al. 1994, 1995; CIIT 1993
B6C3F1 mouse	2 years, 5 days/week, 6 hours/day	5	24.8	0.89	4.43	4.43	Increased methemoglobin (in males (3.97 versus 1.97% in controls) and females (2.22 versus 1.39%)	Cattley et al. 1994, 1995; CIIT 1993

Table A-8. Summary of Relevant NOAEL and LOAEL Values Considered for Derivation of a Chronic Inhalation MRL for Nitrobenzene

Species	Duration	NOAEL (ppm)	LOAEL (ppm)	$NOAEL_{ADJ}$[a] (ppm)	$LOAEL_{ADJ}$[a] (ppm)	$LOAEL_{HEC}$ (ppm)	Effect	Reference
Hepatic effects								
CD rat (male)	2 years, 5 days/week, 6 hours/day		1		0.18	0.18	Kupffer cell pigmentation	Cattley et al. 1994, 1995; CIIT 1993
B6C3F1 mouse	2 years, 5 days/week, 6 hours/day		5		0.89	0.89	Increased centrilobular hepatocytomegaly and multinucleated hepatocytes in males	Cattley et al. 1994, 1995; CIIT 1993
Endocrine effects								
B6C3F1 mouse	2 years, 5 days/week, 6 hours/day		24.8		4.43	4.43	Thyroid follicular cell hyperplasia in males	Cattley et al. 1994, 1995; CIIT 1993
Renal effects								
B6C3F1 mouse	2 years, 5 days/week, 6 hours/day		49.1		8.77	8.77	Kidney cysts in males	Cattley et al. 1994, 1995; CIIT 1993

[a]Inhalation studies were adjusted from intermittent daily exposures to the equivalent of 24-hour continuous exposure. The duration adjusted values were calculated as:

$$Adjusted\ Daily\ Dose = Intermittent\ dose \times \frac{hours\ per\ day\ exposed}{24\ hours} \times \frac{days\ per\ week\ exposed}{7\ days}$$

LOAEL = lowest-observed-adverse-effect level; $LOAEL_{ADJ}$ = LOAEL adjusted to continuous exposure; $LOAEL_{ADJ(HEC)}$ = LOAEL adjusted to continuous exposure and converted to human equivalent concentration (HEC; see text); NOAEL = no-observed-adverse-effect level; $NOAEL_{ADJ}$ = NOAEL adjusted to continuous exposure

The $RGDR_{PU}$ (pulmonary) was calculated as follows:

$$RGDR_{PU} = (Mva/Saa) \div (MVh/Sah)$$

where:

Mva = minute volume for mice = 0.06 m^3/day
MVh = minute volume for humans = 20 m^3/day
Saa = pulmonary surface area for mice = 0.05 m^2
Sah = pulmonary surface area for humans = 54 m^2

As Table A-8 shows, the lowest $LOAEL_{HEC}$ values were 0.054 ppm for nasal epithelial hyperplasia and olfactory pigment deposition in rats and 0.18 ppm for nasal olfactory degeneration in mice, and increased methemoglobin (male CD rats), spleen congestion (F344 and CD rats), and Kupffer cell pigmentation in the liver (male CD rats).

***Selection of the Principal Study*:** Only one chronic inhalation study of nitrobenzene was located (Cattley et al. 1994, 1995; CIIT 1993). In this study, male and female B6C3F1 mice, male and female F344 rats and male CD rats were exposed by inhalation for 2 years and comprehensive toxicological endpoints were evaluated. This study was selected as the principal study for derivation of the chronic-duration inhalation MRL.

Summary of the Principal Study:

Cattley RC, Everitt JI, Gross EA, et al. 1994. Carcinogenicity and toxicity of inhaled nitrobenzene in B6C3F1 mice and F344 and CD rats. Fundam Appl Toxicol 22(3):328-340.

Cattley RC, Everitt JI, Gross EA, et al. 1995. Erratum: Carcinogenicity and toxicity of inhaled nitrobenzene in B6C3F1 mice and F344 and CD rats. Fundam Appl Toxicol 25:159. https://doi.org/10.1006/faat.1994.1039.

CIIT. 1993. Initial submission: A chronic inhalation toxicity study of nitrobenzene in B6CF1 mice, Fischer 344 rats and Sprague-Dawley (CD) rats. First Chemical Corporation. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0538399. 88930000170. 8EHQ02938723. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0538399.xhtml. November 9, 2022.

Cattley et al. (1994, 1995; CIIT 1993) evaluated the chronic toxicity and carcinogenicity of inhaled nitrobenzene in groups of 70 male and female F344 rats, male CD rats, and male and female B6C3F1 mice. Only selected results were included in the published report (Cattley et al. 1994, 1995); additional details were obtained from the unpublished version (CIIT 1993). All animals were exposed to nitrobenzene for 6 hours/day, 5 days/week for 2 years in an inhalation chamber. Rats were exposed to target concentrations of 0, 1, 5, or 25 ppm nitrobenzene, and mice were exposed to 0, 5, 25, or 50 ppm nitrobenzene (analytically measured concentrations for 0, 1, 5, 25, and 50 ppm exposures were 0, 1, 5, 24.8, and 49.1 ppm, respectively). Animals were examined twice daily for mortality and clinical abnormalities. Body weight was measured weekly for 13 weeks and biweekly thereafter. Groups of 10 rats of each strain and sex were sacrificed after 15 months of exposure; all other animals were sacrificed after the 2-year exposure period. At sacrifice, evaluations included hematology, clinical chemistry, organ weights (spleen, liver, kidney, and brain), gross necropsy, and histopathology (including nose, lung, liver, thyroid, parathyroid, spleen, adrenal glands, femur [bone marrow], sternum, and any tissues with gross lesions). There was no difference in survival between control groups and the

nitrobenzene exposure groups. No exposure-related clinical signs or biologically significant (≥10% less than controls) effects on body weight were observed in the animals. Treatment-related hematology changes were observed in both rats and mice. Male and female F344 rats exhibited decreased erythrocyte counts, hematocrit, and hemoglobin as well as increased methemoglobin at 24.8 ppm; these changes were evident at both the interim and final sacrifices in F344 rats. At the interim sacrifice, male CD rats exhibited increased methemoglobin at all exposure levels (≥1 ppm), while at termination the difference from control was significant only at 24.8 ppm. In male mice the changes consisted of decreased erythrocyte counts and hematocrit, and increased methemoglobin at 49.1 ppm. In female mice, methemoglobin was increased at ≥24.8 ppm.

Organ weight changes in both male and female F344 rats consisted of increased absolute and relative liver and kidney weights at 24.8 ppm. Male CD rats showed no significant changes in spleen, liver, kidney, or brain weights at any exposure level. In female mice, absolute and relative liver and kidney weights were increased at the highest exposure level (49.1 ppm); no biologically relevant organ weight changes were observed in male mice.

Treatment-related increases in the incidences of nonneoplastic lesions were observed in the nose, spleen, liver, kidneys, and pancreas of F344 rats; in the nose, liver, testes, and epididymides of CD rats; and in the nose, lung, bone marrow, liver, kidney, thyroid, pancreas, and thymus of B6C3F1 mice. Nasal lesions occurring at all exposure concentrations (≥1 ppm) in rats included olfactory epithelial pigment deposition (F344 and CD) and squamous epithelial hyperplasia (CD rats). At higher exposures, nasal findings in the rats included inflammation, sometimes with submucosal gland hypertrophy, and suppurative exudate. Mice exhibited more severe lesions in the nasal passages, including the following findings that were seen at significantly increased incidences at all exposure levels (≥5 ppm): glandularization of the respiratory epithelium, olfactory epithelial degeneration, increased secretory product in the respiratory epithelium, olfactory epithelial pigment deposition, and dilatation of the submucosal glands.

No lung effects of nitrobenzene were seen in rats. Male and female mice exhibited increased incidences of alveolar bronchiolization at all exposure levels (≥5 ppm), and males showed increased alveolar/ bronchiolar hyperplasia at ≥24.8 ppm.

Increased incidences of spleen congestion were reported in male and female F344 rats and male CD rats at all exposure levels (≥1 ppm), and increased spleen pigmentation was reported at all exposures in male F344 rats. Splenic lesions were not observed in male mice; females showed an increased incidence of lymphoid hyperplasia at 49.1 ppm. An increased incidence of bone marrow hypercellularity was reported for male mice exposed to 49.1 ppm.

The liver was a target organ for nitrobenzene in both rats and mice. In F344 rats, increased incidences of eosinophilic foci and centrilobular hepatocytomegaly were observed in males exposed to ≥5 ppm, and an increased incidence of spongiosis hepatis was seen at 24.8 ppm. Increased incidences of eosinophilic foci and spongiosis hepatis were noted in females exposed to 24.8 ppm. An increase in the incidence of pigmentation in the Kupffer cells occurred in male CD rats at all exposure levels (≥1 ppm); in addition, increased centrilobular hepatocytomegaly and increased spongiosis hepatis occurred at ≥5 and 24.8 ppm, respectively. In mice, increased incidences of centrilobular hepatocytomegaly and multinucleated hepatocytes were reported for males at all exposure levels (≥5); females exhibited an increased incidence of centrilobular hepatocytomegaly at the highest exposure (49.1 ppm).

Renal effects were seen in both rats and mice. In F344 rats, increases in renal tubular hyperplasia, cysts, (in males) and chronic nephropathy (females) were reported at 24.8 ppm, as well as renal tubular suppurative inflammation in both sexes. The only renal effect observed in male CD rats was

mineralization at 24.8 ppm. Male mice, but not female mice, showed a higher incidence of kidney cysts at 49.1 ppm.

Effects on male reproductive organs were noted at higher concentrations. Male CD rats had increased incidences of bilateral atrophy of the testes, bilateral hypospermia in the epididymis, and atrophy of the seminal vesicles at 24.8 ppm. Male mice exposed to nitrobenzene also exhibited hypospermia of the epididymis at 49.1 ppm.

In addition to the effects noted above, male F344 rats exhibited an increased incidence of focal pancreatic acinar cell hyperplasia at 24.8 ppm. Other effects reported to occur at increased incidence in male CD rats at 24.8 ppm included mineralization of the aorta, myocardium, stomach muscle, and kidney; and fibrous osteodystrophy in the nose and bone. In male mice, increased incidences of thyroid follicular cell hyperplasia were seen at ≥24.8 ppm. Other effects observed in female mice consisted of increased incidences of adrenal gland cortical cell vacuolization (≥24.8 ppm), as well as thymic involution and mononuclear cell infiltrate of the pancreas (at 49.1 ppm).

Table A-9 shows the incidences of treatment-related nonneoplastic histopathology changes that occurred at all exposure levels in rats or mice.

Table A-9. Significant Noncancer Effects[a] Occurring at All Concentrations in Chronic Inhalation Study of Rats and Mice

Effect	0	1 ppm	5 ppm	24.8 ppm	49.1 ppm
F344 rats, male					
Spleen, congestion	43/69 (62)	61/69 (88)*	62/78 (79)*	61/78 (78)*	
Spleen, pigmentation	55/69 (80)	63/69 (91)*	64/70 (91)*	70/70 (100)*	
Nose, olfactory epithelium pigment deposition	40/67(60)	53/67 (79)*	67/70 (96)*	68/69 (99)*	
F344 rats, female					
Spleen, congestion	42/69 (61)	55/66 (83)*	58/66 (88)*	65/69 (94)*	
Nose, olfactory epithelium pigment deposition	37/67 (55)	54/65 (83)*	60/65 (92)*	66/66 (100)*	
CD rats, male					
Methemoglobin (%), 15 months[b]	1.18±0.34	4.08±0.80*	6.22±1.6*	5.85±0.83*	
Spleen, congestion	25/63 (40)	43/67 (64)*	44/69 (64)*	38/65 (58)*	
Nose (L1), squamous epithelial hyperplasia	11/63 (17)	42/65 (65)*	34/66 (52)*	42/62 (68)*	
Liver, Kupffer cell pigmentation	0/63	6/67 (9)*	10/70 (14)*	42/65 (65)*	
B6C3F1 mice, male					
Lung, bronchiolization	0/68		58/67 (87)*	58/65 (89)*	62/66 (94)*
Liver, centrilobular hepatocytomegaly	1/68 (1)		15/65 (23)*	44/65 (68)*	57/64 (89)*
Liver, multinucleated hepatocytes	2/68 (3)		14/65 (22)*	45/65 (69)*	56/64 (88)*
Nose (L1), glandularization of respiratory epithelium	4/67 (6)		36/67(54)*	45/65 (69)*	50/66 (76)*
Nose (L3), olfactory epithelium pigment deposition	0/67		7/66 (11)*	46/65 (71)*	49/66 (74)*
Nose (L3), dilatation of submucosal glands	3/67 (4)		13/66 (20)*	39/65 (50)*	32/66 (48)*

Table A-9. Significant Noncancer Effects[a] Occurring at All Concentrations in Chronic Inhalation Study of Rats and Mice

Effect	0	1 ppm	5 ppm	24.8 ppm	49.1 ppm
	B6C3F1 mice, female				
Lung, bronchiolization	0/53		53/60 (92)*	63/64 (98)*	62/62 (100)*
Nose (L1) glandularization of respiratory epithelium	3/52 (6)		17/60 (28)*	28/63 (44)*	46/62 (74)*
Nose (L1), increased secretory product in respiratory epithelium	0/52		34/60 (57)*	50/63 (79)*	35/62 (56)*
Nose (L2), olfactory degeneration	0/52		9/60 (15)*	28/63 (44)*	32/62 (52)*
Nose (L3), olfactory degeneration	0/52		19/60 (32)*	47/63 (75)*	42/61 (69)*
Nose (L4), olfactory degeneration	0/52		30/59 (51)*	37/63 (59)*	48/61 (79)*
Nose (L3), olfactory epithelium pigment deposition	0/52		6/60 (10)*	37/63 (59)*	29/61 (48)*
Nose (L3), dilatation of submucosal glands	9/52 (17)		22/60 (37)*	40/63 (63)*	46/61 (75)*
Nose (L4), dilatation of submucosal glands	2/52 (4)		11/59 (19)*	20/63 (32)*	39/61 (64)*

[a]Histopathology findings are reported as number affected/number examined (percent incidence). Results marked with an asterisk (*) are significantly different from control ($p<0.05$) as reported by the study authors.
[b]Methemoglobin levels are reported as mean±standard error.

L1, L2, L3, L4 = segments of the nasal cavity; shaded = concentration not tested

Sources: Cattley et al. 1994, 1995; CIIT 1993

Exposure to nitrobenzene was associated with increased incidences of several tumor types. Male F344 rats exhibited increased incidences of hepatocellular adenomas or carcinomas and renal tubular adenomas or carcinomas at 24.8 ppm. An increased incidence of hepatocellular adenomas or carcinomas was also observed in male CD rats exposed to 24.8 ppm. In female F344 rats, the only treatment-related neoplastic change was an increased incidence of endometrial stromal polyps at 24.8 ppm. Male B6C3F1 mice exhibited significantly increased incidences of alveolar/bronchiolar adenomas or carcinomas at all exposure levels (≥5 ppm). Female mice showed a significant increase in the incidence of mammary gland adenocarcinomas at 49.1 ppm; mammary glands were not examined for histopathology at lower concentrations.

Selection of the Point of Departure for the MRL: After conversion to HECs, the lowest LOAEL values (see Table A-8) were for nasal lesions in rats (squamous epithelial hyperplasia in male CD rats and olfactory epithelium pigment deposition in F344 rats). The selection of this endpoint is supported by the fact that rats are generally more sensitive to the toxicity of nitrobenzene than mice are (see Section 3.1.6); thus, rats were tested at a lower exposure concentration (1 ppm) than mice (5 ppm) in the chronic study (Cattley et al. 1994, 1995; CIIT 1993). The nasal lesions in male CD rats included squamous epithelial hyperplasia and olfactory epithelium pigment deposition. The toxicological significance of the pigment deposition is uncertain. Pigment deposition observed in the livers and spleens of exposed rats could have resulted from hemolysis, an established effect of nitrobenzene; however, it seems unlikely that products of hemolysis would be distributed to or preferentially deposited in the nasal passages. Given the uncertainty in the relevance of this effect, pigment

deposition was not considered further for use in deriving the MRL. The data on squamous epithelial hyperplasia in male CD rats were subjected to BMD modeling.

The data were fit to all dichotomous models in EPA's BMDS (version 3.2) using a BMR of 10% relative deviation. Adequate model fit was judged by four criteria: chi-square goodness-of-fit p-value ($p \geq 0.1$), visual inspection of the dose-response curve, BMDL <10 times the lowest non-zero dose, and scaled residual (>-2 and <+2) at the data point (except the control) closest to the predefined BMR. Among models providing adequate fit to the data, the lowest $BMDL_{10}$ was selected as the POD when the difference between the BMDLs estimated from these models was >3 fold; otherwise, the $BMDL_{10}$ from the model with the lowest AIC was chosen. No model fit was achieved with the data.

In the absence of a suitable POD from modeling of the data, the LOAEL of 1 ppm for squamous epithelial hyperplasia in the nose of rats was selected as the POD.

Adjustment for Intermittent Exposure: The animals in the study by Cattley et al. (1994, 1995; CIIT 1993) were exposed 6 hours/day, 5 days/week. Therefore, the LOAEL of 1 ppm was adjusted for intermittent exposure as follows:

$$LOAEL_{ADJ} = LOAEL \times \frac{6\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 1\ ppm \times \frac{6\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 0.18\ ppm$$

Human Equivalent Concentration: The critical effect at the LOAEL was in the respiratory system (nasal epithelial hyperplasia). Therefore, the $LOAEL_{ADJ}$ was converted to a $LOAEL_{HEC}$ using guidance from EPA (1994) on dosimetric adjustments for respiratory effects. The $LOAEL_{ADJ}$ was converted to a $LOAEL_{HEC}$ using the RGDR for extrathoracic effects (EPA 1994) calculated as follows:

$$RGDR_{ET} = \frac{MV_a}{SA_a} \div \frac{MV_h}{SA_h}$$

where:

MV_a = minute volume for rats = 0.47 m^3 per day
SA_a = ET surface area for rats = 15 cm^2
MV_h = minute volume for humans = 20 m^3 per day
SA_h = ET surface area for humans = 200 cm^2

Applying this equation results in an RGDR of 0.3 for extrathoracic effects in rats, and the HEC is calculated as:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 0.18\ ppm \times 0.3 = 0.054\ ppm$$

Uncertainty Factor: The $LOAEL_{HEC}$ was divided by a composite uncertainty factor of 300:

- 10 for use of a LOAEL
- 10 for human variability
- 3 for animal to human extrapolation after dosimetric adjustment.

This results in the following MRL:

$$MRL = \frac{LOAEL_{HEC}}{UFs} = \frac{0.054\ ppm}{300} = 0.0002\ ppm$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: Results from inhalation studies indicate effects of nitrobenzene exposure on the nasal passages of rats and mice and the lungs of mice. In chronic-duration studies, mice had degeneration of the nasal olfactory epithelium and glandularization of respiratory epithelium and rats had squamous epithelial hyperplasia, pigment deposition in the olfactory epithelium, and inflammatory changes (Cattley et al. 1994, 1995; CIIT 1993). In the same study, increases in bronchiolization of the alveoli and alveolar/bronchiolar hyperplasia were observed in mice (Cattley et al. 1994, 1995; CIIT 1993). Acute- and intermediate-duration dermal exposure studies have demonstrated lung congestion after nitrobenzene exposure in F344 rats (NTP 1982).

Agency Contacts (Chemical Managers): Malcolm Williams

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s): Nitrobenzene
CAS number(s): 98-95-3
Date: January 2024
Profile Status: Final
Route: Oral
Duration: Acute
MRL: 0.05 mg/kg/day
Critical Effect: Proliferative changes in the bone marrow
Reference: Burns et al. 1994
Point of Departure: $BMDL_{1SD}$ of 4.7 mg/kg/day
Uncertainty Factor: 100
LSE Graph Key: 10
Species: Mouse

MRL Summary: An acute-duration oral MRL of 0.05 mg/kg/day was derived for nitrobenzene based on a LOAEL of 30 mg/kg/day ($BMDL_{1SD}$ of 4.7 mg/kg/day) for increased DNA synthesis indicating proliferative changes in the bone marrow in a 14-day study in mice (Burns et al. 1994). The $BMDL_{1SD}$ was divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Selection of the Critical Effect: There are nine studies that evaluated toxicity in animals administered oral doses for acute durations (Burns et al. 1994; Iida et al. 1997; Kawaguchi et al. 2004; Kawashima et al. 1995; Levin et al. 1988; Linder et al. 1992; McLaren et al. 1993a; Morgan et al. 1985). In eight of these studies, a single dose, usually 60 mg/kg, was used to evaluate the effect on the male reproductive systems. The only study testing multiple doses and evaluating other health outcomes was the 14-day gavage study in mice by Burns et al. (1994). Burns et al. (1994) administered nitrobenzene to female B6C3F1 mice via gavage in corn oil for 14 days and evaluated immune system endpoints in addition to several other systemic endpoints for acute toxicity. Table A-10 shows the lowest effect levels from the acute-duration oral studies of nitrobenzene.

Table A-10. Summary of Relevant NOAEL and LOAEL Values Considered for Derivation of an Acute Oral MRL for Nitrobenzene

Species	Duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
Hematological effects					
B6C3F1 mouse	Once/day for 14 days (GO)		30	Increased number of cells, DNA synthesis, and number of granulocyte-monocyte progenitor cells in bone marrow	Burns et al. 1994
Reproductive effects					
Sprague-Dawley rat	Once/day for 14 days (GO)		60	Decreased testes weight and epididymal weights, 34% decrease in sperm count, and significant decrease in sperm motility with no effect on copulation or fertility rate	Iida et al. 1997; Kawashima et al. 1995

Table A-10. Summary of Relevant NOAEL and LOAEL Values Considered for Derivation of an Acute Oral MRL for Nitrobenzene

Species	Duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
Rat (Wistar)	Once/day for 14 days (GO)		100	Decreased testicular and epididymal weights; decreased serum prolactin, luteinizing hormone, follicle stimulating hormone, and testosterone; atrophic and degenerated seminiferous tubules	Oladele et al. 2020c
Other effects					
B6C3F1 mouse	Once/day for 14 days (GO)	30	100	Hepatic: Increased liver weight, hepatomegaly Immune: Decreased IgM AFC in spleen cells; decreased natural killer cell activity	Burns et al. 1994
Rat (Wistar)	Once/day for 14 days (GO)		100	Renal: Increased serum urea and creatinine; renal lesions including mild fibrosis and hemorrhage and marked glomerular shrinkage Neurological (SLOAEL): Neurobehavioral changes (decreased exploratory behavior and increased defecation); increased acetylcholinesterase activity in brain; degenerative lesions in the cerebellum, cerebrum, and hippocampus Endocrine: Decreased serum TSH Reproductive: Decreased testicular and epididymal weights; decreased serum prolactin, luteinizing hormone, follicle stimulating hormone, and testosterone; atrophic and degenerated seminiferous tubules	Oladele et al. 2020a, 2020b, 2021

AFC = antibody-forming cell; DNA = deoxyribonucleic acid; GD = gestation day; (GO) = gavage in oil vehicle; IgM = immunoglobulin M; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; SLOAEL = serious LOAEL; TSH = thyroid-stimulating hormone

The lowest LOAEL was 30 mg/kg/day in the study by Burns et al. (1994). Effects seen at the LOAEL included increased DNA synthesis, cell number, and numbers of granulocyte-monocyte progenitor cells in the bone marrow, indicating proliferative effects on this tissue. A NOAEL was not determined. These effects were considered for use in deriving the acute-duration MRL.

Selection of the Point of Departure: Burns et al. (1994) is the only oral exposure 14-day study that had reliable, dose-related data to inform an MRL derivation for the acute exposure duration. There was a clear and significant impact on bone marrow at 30 mg/kg/day that was linked to hematological effects

observed at ≥100 mg/kg/day (significant increase in reticulocytes) and consistent with effects seen in other studies of nitrobenzene (Burns et al. 1994).

Summary of the Principal Study:

Burns LA, Bradley SG, White KL, et al. 1994. Immunotoxicity of nitrobenzene in female B6C3F1 mice. Drug Chem Toxicol 17(3):271-315.

Groups of female B6C3F1 mice (eight/group) were administered nitrobenzene via gavage in corn oil at doses of 0 (vehicle control), 30, 100, or 300 mg/kg/day for 14 days. Animals were weighed on study days 1, 8, and 15. Animals were sacrificed on day 15 and blood was collected for hematology (erythrocyte and leukocyte number, hemoglobin, hematocrit, MCV, MCH, mean corpuscular hemoglobin concentration [MCHC], and total and differential leukocyte counts) and serum chemistry (aspartate aminotransferase [AST], ALT, urea nitrogen, glucose, albumin, and total protein). Animals underwent necropsy and select organs (liver, thymus, spleen, lungs, lymph nodes, kidneys, and brain) were removed, weighed, and prepared for histologic examination. Bone marrow was harvested for examination of immune cell (macrophage and granulocyte/monocyte) progenitors and DNA synthesis. Immune function assays included spleen IgM and IgG antibody response following stimulation with sheep erythrocytes (sRBC), spleen cell proliferation following stimulation with various mitogens, mixed leukocyte response, delayed hypersensitivity response, serum complement proteins, reticuloendothelial system sRBC clearance, peritoneal cell number, macrophage phagocytic activity, natural killer cell activity, and host resistance assay.

Death occurred in 8.5% of mice across several experiments at 300 mg/kg/day. Animals in the 300 mg/kg/day group showed treatment-related clinical signs, including ataxia, lethargy, circling, and head bobbing. No biologically significant (≥10% less than controls) effects on body weight were observed. Treatment-related hematology changes were observed and include increased MCV, MCH, and percent reticulocytes at ≥100 mg/kg/day. At 300 mg/kg/day, erythrocytes were decreased by 9%. Serum chemistry changes included increased ALT and bilirubin at 300 mg/kg/day. Total protein was increased at all doses.

Terminal body weights at 300 mg/kg/day were increased by 11%. Organ weight changes included increased absolute and relative liver weights and spleen weights at ≥100 mg/kg/day. Absolute lung weights were increased at all doses and relative lung to brain weight was increased at 300 mg/kg/day.

Mice exhibited hepatomegaly and splenomegaly and the spleen was dark and congested at ≥100 mg/kg/day. In mice in the 300 mg/kg group, there was mild hydropic degeneration foci around central veins in the liver. Mice in the 100 mg/kg group had mild congestion in the red pulp areas of the spleen and at 300 mg/kg/day, the spleen was dark red, with enlarged red pulp areas exhibiting severe congestion, hemosiderin, and extramedullary hematopoiesis (indicated by nucleated erythrocytes and immature granulocytes present). Incidence of these lesions was not reported.

Analysis of bone marrow showed increased DNA synthesis, cells per femur, and increased CFUs (granulocyte monocyte) per femur at all doses.

In the sRBC assay on day 4, there was a significant increase in spleen weight and spleen cell number at 300 mg/kg/day and reduced IgM AFC/10^6 spleen cells at ≥100 mg/kg/day. After 5 days, there was a significant increase in spleen weight and spleen cell number at ≥100 mg/kg/day. These effects did not persist after a 20-day recovery period.

In a spleen cell mitogenic response assay, 3H-thymidine incorporation in response to T cell mitogens (phytohemagglutinin and concanavalin A) was suppressed at ≥100 mg/kg/day. No effect was observed on B-cell mitogen (LPS) response. Decreased stimulation index was observed at ≥100 mg/kg/day, indicating reduced ability of splenic T cells to recognize and respond to alloantigens. At 300 mg/kg/day, there was an increase in the number of peritoneal cells and an increase in phagocytic activity. Natural killer cell activity in peritoneal cells was decreased at ≥100 mg/kg/day.

In the host resistance assay, mice treated with 300 mg/kg/day of nitrobenzene were more susceptible to *L. monocytogenes* and had a 338% higher mortality rate following a challenge of $6x10^3$ CFUs. Susceptibility to *Streptococcus pneumoniae* or *Plasmodium berghei* or herpes simplex virus were comparable to controls.

Table A-11 summarizes the statistically significant treatment-related effects that occurred at all dose levels, as well as the related data on reticulocyte count increases at ≥100 mg/kg/day.

Table A-11. Significant Noncancer Effects[a] Female B6C3F1 Mice Orally Exposed to Nitrobenzene for 14 Days

Effect	0	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day
Hematological effects				
Reticulocyte percent[b]	1.03±0.09	1.09±0.17	3.51±0.43*	4.57±0.48*
Cells/femur ($x10^6$)	6.3±0.6	9.5±0.6*	9.4±0.7*	10.2±0.7*
DNA synthesis (cpm $x10^3$)	71.0±6.5	117.3±8.8*	126.6±4.7*	127.6±7.2*
CFU-GM/femur ($x10^4$)	0.58±0.06	0.88±0.06*	0.82±0.05*	1.01±0.06*

[a]Hematology changes are reported as mean ± standard error. Results marked with an asterisk (*) are significantly different from control ($p<0.05$) as reported by the study authors. Number of animals n=7–8 CFU-GM.
[b]Reticulocyte count is reported as the percentage of red blood cells.

CFU = colony-forming unit; cpm = counts per minute; DNA = deoxyribonucleic acid; GM = granulocyte monocyte

Source: Burns et al. 1994

Selection of the Point of Departure for the MRL: BMD modeling was conducted on the bone marrow effects occurring at all doses as reported in Table A-11 after converting the standard errors to SDs. The data were fit to all available continuous models in EPA's BMDS (version 3.2) using a BMR of 1 SD change from control. Adequate model fit was judged by four criteria: goodness-of-fit statistics (p-value >0.1), visual inspection of the dose-response curve, a BMD) that is not 10 times lower than the lowest non-zero dose, and scaled residual within ±2 units at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest BMDL was selected as the POD when the difference between the BMDLs estimated from these models was >3-fold; otherwise, the BMDL from the model with the lowest AIC was chosen. No suitable models were identified for CFU-granulocyte monocyte/femur.

In accordance with the selection criteria mentioned above, the exponential 4 model (constant variance) was selected as the best fit for the number of cells/femur data and the exponential model 5 (constant variance) was selected as the best fit for the DNA synthesis data. The BMD_{1SD} and $BMDL_{1SD}$ for increased number of cells/femur were 10.6 and 6.8 mg/kg/day, respectively. The BMD_{1SD} and $BMDL_{1SD}$ for increased DNA synthesis were 6.7 and 4.7 mg/kg/day, respectively. Therefore, the $BMDL_{1SD}$ of 4.7 mg/kg/day for increased DNA synthesis (as a marker for cell proliferation in the bone

marrow) was selected as the POD for the acute-duration oral MRL. The output from the BMDS modeling for increased DNA synthesis is presented in Table A-12 and the fit of the data to the selected model is presented in Figure A-2.

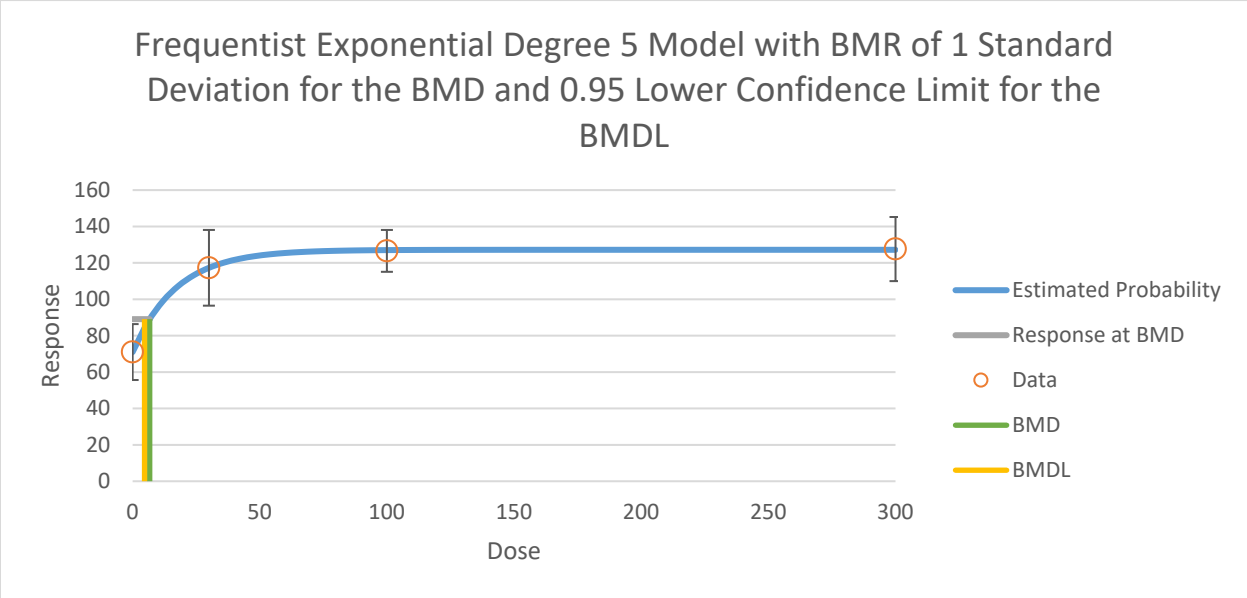
Table A-12. Results from BMD Analysis (Constant Variance) for DNA Synthesis in Femur Bone Marrow of Female B6C3F1 Mice after 14 Days of Oral Exposure to Nitrobenzene (Burns et al. 1994)

					Scaled residuals[c]	
Model	BMD_{1SD}[a] (mg/kg/day)	$BMDL_{1SD}$[a] (mg/kg/day)	p-Value[b]	AIC	Dose below BMD	Dose above BMD
Exponential (model 2)[d]			<0.0001	286.57	1.83	-0.64
Exponential (model 3)[d]			<0.0001	286.57	1.83	-0.64
Exponential (model 4)[d]	6.72	4.69	0.93	266.83	-0.0006	0.005
Exponential (model 5)[d,e]	**6.72**	**4.69**	**0.93**	**266.83**	**-0.0004**	**0.003**
Hill[f]			NA	268.83	-5.7×10^{-8}	3.96×10^{-8}
Polynomial (3-degree)[f]			<0.0001	285.87	1.78	-0.79
Polynomial (2-degree)[f]			<0.0001	285.87	1.78	-0.79
Power			<0.0001	285.87	1.78	-0.79
Linear			<0.0001	285.87	1.78	-0.79

[a]BMD and BMDL values for models that do not provide adequate fit are not included in this table.
[b]Values <0.1 fail to meet conventional χ^2 goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD.
[d]Power restricted to ≥1.
[e]Selected model. The constant variance model provided an adequate fit to the data. Only the Exponential 4 and 5 models provided adequate fits to the means. The BMDLs were similar and sufficiently close (<3-fold); therefore, the model with the (slightly) lowest AIC was selected (Exponential 5 model).
[f]Coefficients restricted to be positive.

AIC = Akaike Information Criterion; BMD = benchmark dose (maximum likelihood estimate of the concentration associated with the selected benchmark response); BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response); SD = standard deviation

Figure A-2. Fit of the Exponential 5 Model (Constant Variance) to Data for DNA Synthesis in Female B6C3F1 Mice with 14 Days of Oral Exposure to Nitrobenzene (Burns et al. 1994)



Using this POD an MRL was derived as follows.

Uncertainty Factor: The $BMDL_{1SD}$ of 4.7 mg/kg/day was divided by a total uncertainty factor of 100:

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

This results in the following MRL:

$$MRL = \frac{BMDL}{UF} = \frac{4.7}{100} = 0.05 \text{ mg/kg/day}$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: In human case studies of both inhalation and ingestion exposure to nitrobenzene, methemoglobinemia was the most common finding (Agrawal et al. 2011; Gupta et al. 2012; Ikeda and Kita 1964; Lee et al. 2013; Perera et al. 2009; Saxena and Prakash Saxena 2010). Additionally, experimental animal studies have demonstrated increased methemoglobin levels in mice and rats of both sexes exposed through any exposure route with acute, intermediate, and chronic (≥365 days) exposure durations (Biodynamics 1984; Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983b). A variety of related adverse effects in the hematologic system, such as hemolytic anemia; extramedullary hematopoiesis, hemosiderosis, congestion, and lymphoid depletion of the spleen; and changes in bone marrow in response to anemia have also been observed with inhalation, oral, and dermal exposures in B6C3F1 mice, F344 rats, and Sprague-Dawley rats (Burns et al. 1994; Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b).

Agency Contacts (Chemical Managers): Malcolm Williams

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s): Nitrobenzene
CAS number(s): 98-95-3
Date: January 2024
Profile Status: Final
Route: Oral
Duration: Intermediate
MRL 0.02 mg/kg/day
Critical Effect: Increased methemoglobin
Reference: NTP 1983a
Point of Departure: $BMDL_{1SD}$ of 1.8 mg/kg/day
Uncertainty Factor: 100
LSE Graph Key: 16
Species: Rat

MRL Summary: An intermediate-duration oral MRL of 0.02 mg/kg/day was derived for nitrobenzene based on evidence of increased methemoglobin levels in male F344 rats administered nitrobenzene via gavage at 9.375 mg/kg/day for 90 days (NTP 1983a). The MRL is based on a $BMDL_{1SD}$ of 1.8 mg/kg/day for increased methemoglobin. This was divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Selection of the Critical Effect: There are three intermediate-duration studies that evaluated the adverse effects of nitrobenzene on the male reproductive system at the same doses (0 or 60 mg/kg/day) (Iida et al. 1997; Kawaguchi et al. 2004; Kawashima et al. 1995). In all of these studies, adverse effects on the testes and epididymis were observed. In addition, Kawaguchi et al. (2004) and Kawashima et al. (1995) reported adverse effects on sperm, including reduced count or concentration, motility, and viability.

There are two multi-dose oral intermediate-duration studies (Mitsumori et al. 1994; NTP 1983a) that also tested lower doses and evaluated comprehensive endpoints. Table A-13 shows the lowest effect levels from these two studies. Mitsumori et al. (1994) was a combined repeated-dose and reproduction/ developmental toxicity study with Sprague-Dawley rats in which a LOAEL of 20 mg/kg/day was identified for hematological, hepatic, renal, and reproductive effects. No NOAEL was identified. NTP (1983a) administered nitrobenzene doses via gavage to B6C3F1 mice and F344 rats for 90 days and evaluated a comprehensive list of endpoints. Effects observed at the lowest dose (9.375 mg/kg/day) included hematological, hepatic, and renal effects. All of these effects were considered for use in deriving the intermediate-duration oral MRL.

Table A-13. Summary of Relevant NOAEL and LOAEL Values Evaluated for Derivation of an Intermediate Oral MRL for Nitrobenzene

Species	Duration/route	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
Hematological effects					
F344 rat	Once/day for 90 days (GO)		9.375	Increased absolute reticulocyte count and decreased hemoglobin; increased methemoglobin in males (2.752 versus 1.131% in controls) and females (2.059 versus 0.941% in controls)	NTP 1983a
B6C3F1 mouse	Once/day for 90 days (GO)		18.75	Increased methemoglobin in males (2.162 versus 1.074% in controls) and females (1.198 versus 0.871% in controls), increased reticulocytes in females	NTP 1983a
F344 rat	Once/day for 54 days (GO)		20	Decreased erythrocytes, hemoglobin, and packed cell volume; increased hematopoiesis in bone marrow; increased spleen weight; extramedullary hematopoiesis and hemosiderin deposition in the spleen in males	Mitsumori et al. 1994
Hepatic effects					
F344 rat	Once/day for 90 days (GO)		9.375	Increased liver weights	NTP 1983a
F344 rat	Once/day for 54 days (GO)		20	Increased liver weights; centrilobular swelling of hepatocytes, hemosiderin deposition in Kupffer cells, extramedullary hematopoiesis in males	Mitsumori et al. 1994
Renal effects					
F344 rat	Once/day for 90 days (GO)		9.375 M	Increased kidney weights in males	NTP 1983a

Table A-13. Summary of Relevant NOAEL and LOAEL Values Evaluated for Derivation of an Intermediate Oral MRL for Nitrobenzene

Species	Duration/route	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
Reproductive effects					
F344 rat	Once/day for 90 days (GO)	18.75 M	37.5 M	Atrophic seminiferous tubules in one male; atrophied uteri in two females	NTP 1983a
Sprague-Dawley rat	Once/day for 54 days (GO)	20 M	60 M (SLOAEL)	~60% decrease in testes weight and ~20% decrease in epididymides weight; atrophy of seminiferous tubules (all males), Leydig cell hyperplasia, loss of intraluminal sperm or cell debris in epididymis	Mitsumori et al. 1994
B6C3F1 mouse	Once/day for 90 days (GO)	75M	150 M	Testicular atrophy	NTP 1983a

(GO) = gavage in oil vehicle; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; SLOAEL = serious LOAEL

Selection of the Principal Study and Point of Departure: While both Mitsumori et al. (1994) and NTP (1983a) are well-conducted studies demonstrating an array of toxicological effects of nitrobenzene exposure, the LOAEL of 9.375 mg/kg/day in the study by NTP (1983a) is lower than the LOAEL of 20 mg/kg/day (lowest dose tested) in Mitsumori et al. (1994). Neither study identified a NOAEL. Therefore, NTP (1983a) was selected as the principal study for derivation of the intermediate-duration oral MRL.

Summary of the Principal Study:

NTP. 1983a. Report on subchronic toxicity via gavage of nitrobenzene (C60082) in Fischer 344 rats and B6C3F1 mice. Worcester, MA: National Toxicology Program. MRI-NTP 09-83-19.

Groups of male and female F344 rats and B6C3F1 mice were administered nitrobenzene via gavage in corn oil at doses of 0, 9.345, 18.75, 37.5, 75, and 150 mg/kg/day and 0, 18.75, 37.5, 75, 150, and 300 mg/kg/day, respectively, for 90 days. Experimental groups consisted of 10 male and 10 female animals of each strain and species. Animals were examined for clinical abnormalities twice daily and weighed weekly. Animals were sacrificed at the end of the exposure period. Blood was drawn from the external jugular for hematology and serum chemistry analysis. Animals underwent gross necropsy and select tissues (liver, brain, right kidney, thymus, heart, lung, and right testis) were weighed. Other tissues were prepared for histological analysis (cerebrum, cerebellum, pituitary gland, larynx, tongue, jejunum, pancreas, gall bladder, thymus, kidney, prostate, sternum, nose/turbinates, thyroid glands, esophagus, ileum, salivary gland, spleen, adrenal glands, testes, uterus, mammary glands, bone marrow, gross lesion, parathyroid glands, stomach, cecum, lymph nodes, heart, lungs, urinary bladder, skeletal muscle, rib bone, trachea, duodenum, colon, rectum, liver, seminal vesicles, epididymides, ovaries, eyes, sciatic nerve, skin, and zymbal's gland).

Mortality occurred in nine male and three female rats in the 150 mg/kg/day group. Mortality in mice included three males at 300 mg/kg/day and one male at 75 mg/kg/day. One female in the control and one in the 18.75 mg/kg/day died accidentally. Clinical signs of ataxia, head tilt, lethargy, and trembling were observed at 150 and 300 mg/kg/day in rats and mice, respectively. Cyanosis was observed in all rats at doses ≥75 mg/kg/day. Due to high mortality in male rats at the high dose, study data from these animals were not included.

Regarding hematologic effects, mice and rats of both sexes had increased methemoglobin levels at all doses. Rats of both sexes had increased reticulocytes at ≥9.375 mg/kg/day as well as decreased hemoglobin. In addition, male rats had decreased MCV and MCH, while female rats had decreased hematocrit at all doses. Increased reticulocytes were observed in female mice at ≥18.75 mg/kg/day and in males at ≥37.5 mg/kg/day; decreases in red blood cells, hematocrit, and hemoglobin were seen in both sexes at ≥150 mg/kg/day. Male mice exhibited anisocytosis and polychromasia at 300 mg/kg/day.

Liver weights and liver to body and brain weight ratios were increased in rats of both sexes at all doses. Right kidney and kidney to body weights were increased in a dose related manner in rats of both sexes at ≥9.375 in males and ≥18.75 in females and right kidney to brain weight ratios were increased ≥18.75 mg/kg/day in rats of both sexes. In male rats, weights of the right testis and right testis to body and brain weight ratios were reduced at 75 mg/kg/day. In female rats, heart weights and heart to brain weight ratios were increased at all doses and heart to body weight ratios were increased at ≥75 mg/kg/day and lung weights and lung to brain and body weight ratios were increased at ≥18.75 mg/kg/day. In male mice, liver weights and liver to brain and body weight ratios were increased ≥150 mg/kg/day. Right testis weight and testis to body and brain weight ratios were decreased in male mice at 300 mg/kg/day. In female mice, liver weights were increased at all doses and liver to body and brain weight ratios were increased at ≥37.5 mg/kg/day. Thymus weights and thymus to body and brain weight ratios were increased at all doses in female mice.

Histological effects include congestion of the spleen in most of the treated rats of both sexes. Hemosiderin pigment was observed in the red pulp of the spleen, while lymphoid depletion was noted in the white pulp. In mice lymphoid depletion of the spleen was noted at ≥150 mg/kg/day. Hepatocellular hypertrophy in the centrilobular zone were observed in mice of both sexes. Female rats had increased incidence of renal tubular cell pigmentation. There was increased incidence of brainstem hemorrhage in 5/10 male rats at 75 mg/kg/day and 7/10 female rats at 150 mg/g/day. In male rats, seminiferous tubules were atrophied at ≥37.5 mg/kg/day; atrophied testes and hypospermatogenesis were noted in male rats (10/10) at 75 mg/kg/day and in male mice (4/10) at 300 mg/kg/day. In mice lymphoid depletion of the spleen was noted at ≥150 mg/kg/day. Hepatocellular hypertrophy in the centrilobular zone was observed in mice of both sexes.

In summary, effects of nitrobenzene were seen at all doses in both rats and mice. Because rats were tested at a lower dose (9.375 versus 18.75 mg/kg/day in mice) and are more sensitive to the effects of nitrobenzene, data for rats were considered for MRL derivation. The main effects (hematologic, hepatic, and renal) observed at ≥9.375 mg/kg/day in rats in this study are shown in Table A-14.

Table A-14. Significant Noncancer Effects[a] F344 Rats Orally Exposed to Nitrobenzene for 90 Days

	Dose (mg/kg/day)					
Effect	0	9.375	18.75	37.5	75	150
Male						
Methemoglobin percent	1.131±0.579	2.752±0.583*	4.22±1.145*	5.624±0.85*	7.307±1.438*	–
Relative (to body weight) liver weight	3.51847± 0.21612	4.03612± 0.20049*	4.37246± 0.14415*	4.76839± 0.21788*	5.1545± 0.14804*	–
Relative (to body weight) kidney weight	3.09542± 0.20207	3.29664± 0.20499*	3.36071± 0.11055*	3.29808± 0.37913	3.44410± 0.23201*	–
Female						
Methemoglobin percent	0.941±0.031	2.059±0.449*	3.623±1.088*	5.269±0.757*	6.851±2.250*	12.771± 1.825*
Relative (to body weight) liver weight	3.42905± 0.15529	3.76085± 0.08417*	3.95375± 0.17757*	4.22849± 0.17951*	4.87532± 0.21802*	5.20999± 0.28071*
Absolute reticulocyte count	0.218±0.034	0.297±0.029*	0.38±0.053*	0.491±0.071*	0.637±0.108*	1.871±0.173*
Relative reticulocyte count[b]	2.6±0.37	3.69±0.32*	4.75±0.68*	6.28±0.9*	8.72±1.49*	32.07±3.56*

[a]Hematology changes are reported as mean±standard deviation. Results marked with an asterisk (*) are significantly different from control ($p<0.05$) as reported by the study authors. Number of animals = 10 except in the 150 mg/kg/day group (n=7).
Greyed cells: data not included for males at the high dose due to high mortality (only one male survived at this dose)
[b]Reticulocyte count is reported as the percentage of red blood cells that are reticulocytes (number of reticulocytes divided by the total number of red blood cells, multiplied by 100). For adults, a normal range is considered to be 0.5–1.5%.

Source: NTP 1983a

Selection of the Point of Departure for the MRL: As shown in Table A-14, the endpoint with the largest change at the LOAEL was methemoglobin (which was more than doubled at the LOAEL, compared with smaller changes in other endpoints), suggesting that methemoglobin is the most sensitive endpoint at the LOAEL. In addition, many of the hematological, hepatic, and renal effects of nitrobenzene are related to the induction of methemoglobin (see *Metabolic Mechanisms in* Section 3.1.3). Therefore, the data on methemoglobin in blood of rats were selected for use in deriving the MRL. Table A-14 shows the methemoglobin data in male and female F344 rats in the study by NTP (1983a).

BMD modeling was conducted on the methemoglobin data for both sexes of rat. The highest dose group was dropped for males as a result of a high mortality rate. The data were fit to all available continuous models in EPA's BMDS (version 3.2) using a BMR of 1 SD. Adequate model fit was judged by four criteria: goodness-of-fit statistics (p-value >0.1), visual inspection of the dose-response curve, a BMDL that is not 10 times lower than the lowest non-zero dose and scaled residual within ±2 units at the data point (except the control) closest to the predefined BMR. Among the models providing adequate fit to the data, the lowest BMDL was selected as the POD when the difference between the BMDLs estimated from these models was >3-fold; otherwise, the BMDL from the model with the lowest AIC was chosen. No suitable models were identified for the female rats. The constant variance model did not provide an adequate fit to the male rat variance data. With a nonconstant variance (NCV) model applied, an adequate fit to the variance was provided. The Exponential 4 and 5 models and the Hill model provided adequate fit to the means.

The results of the modeling are presented in Table A-15. In accordance with the above guidance, the model with the lowest AIC among the adequately fitting models was selected; this was the Exponential 5-NCV model. The exposure-response curve for this model is displayed in Figure A-3. The $BMDL_{1SD}$ from this model is 1.8 mg/kg/day.

Table A-15. Results from BMD Analysis (Non-Constant Variance) for Increased Methemoglobin in Male F344 Rats Exposed to Nitrobenzene via Gavage for 90 Days (NTP 1983a)

					Scaled residuals[c]	
Model	BMD_{1SD}[a] (mg/kg/day)	$BMDL_{1SD}$[a] (mg/kg/day)	p-Value[b]	AIC	Dose below BMD	Dose above BMD
Exponential (model 2)[d]			<0.0001	177.02	2.04	2.72
Exponential (model 3)[d]			<0.0001	177.02	2.04	2.72
Exponential (model 4)[d]	2.62	1.81	0.65	136.42	-0.07	-0.20
Exponential (model 5)[d,e]	**2.62**	**1.81**	**0.65**	**136.42**	**-0.06**	**-0.20**
Hill[f]	3.03	1.65	0.42	138.21	-0.08	-0.09
Polynomial (4-degree)[f]			<0.0001	157.95	-2.33	0.84
Polynomial (3-degree)[f]			<0.0001	157.95	-2.33	0.84
Polynomial (2-degree)[f]			<0.0001	157.95	-2.33	0.84

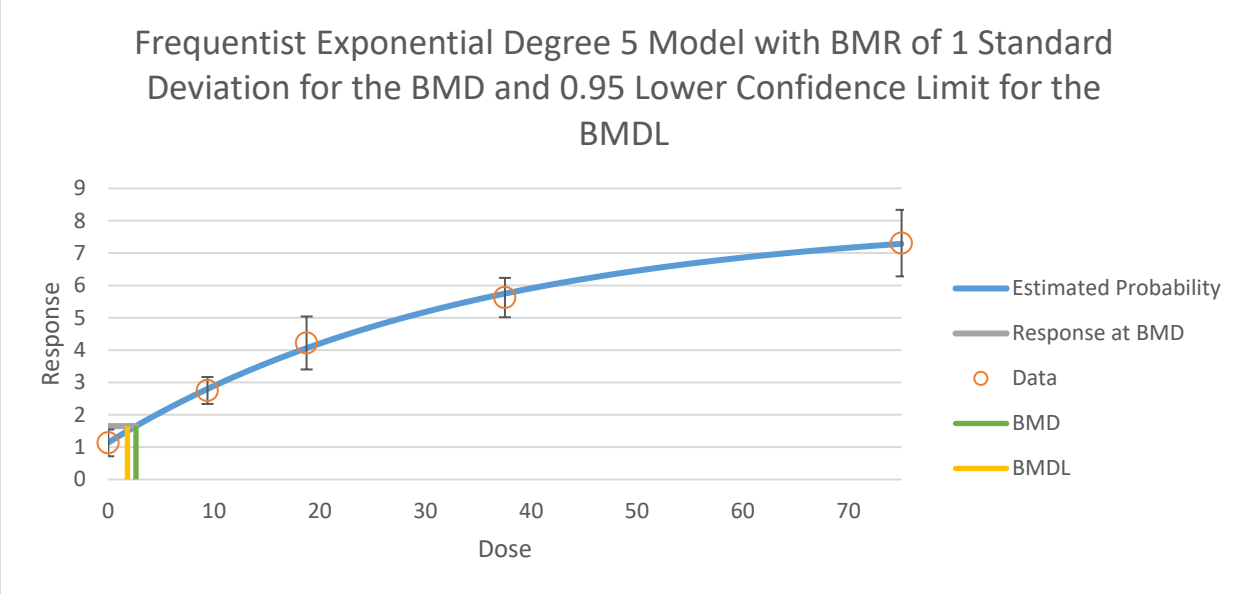
Table A-15. Results from BMD Analysis (Non-Constant Variance) for Increased Methemoglobin in Male F344 Rats Exposed to Nitrobenzene via Gavage for 90 Days (NTP 1983a)

Model	BMD_{1SD}[a] (mg/kg/day)	$BMDL_{1SD}$[a] (mg/kg/day)	p-Value[b]	AIC	Scaled residuals[c]	
					Dose below BMD	Dose above BMD
Power			<0.0001	157.95	-2.33	0.84
Linear			<0.0001	157.95	-2.33	0.84

[a]BMD and BMDL values for models that do not provide adequate fit are not included in this table.
[b]Values <0.1 fail to meet conventional χ^2 goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the BMD.
[d]Power restricted to ≥1.
[e]Selected model. The constant variance model did not provide an adequate fit to the data. With nonconstant variance applied, only the Exponential 4 and 5 models and the Hill model provided adequate fits to the means. The BMDLs of the fit models were sufficiently close (<3-fold); therefore, the model with the lowest AIC was selected (Exponential 5 model).
[f]Coefficients restricted to be positive.

AIC = Akaike Information Criterion; BMD = benchmark dose (maximum likelihood estimate of the concentration associated with the selected benchmark response); BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response); SD = standard deviation

Figure A-3. Fit of Exponential 5 Model (Nonconstant Variance) to Data on Increased Methemoglobin in Male F344 Rats Exposed to Nitrobenzene via Gavage for 90 Days (NTP 1983a)



Uncertainty Factor: The $BMDL_{1SD}$ of 1.8 mg/kg/day was divided by a total uncertainty factor of 100:

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

This results in the following MRL:

$$MRL = \frac{BMDL}{UF} = \frac{1.8}{100} = 0.02\frac{mg}{kg}/day$$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: In human case studies of both inhalation and ingestion exposure to nitrobenzene, methemoglobinemia was the most common finding (Agrawal et al. 2011; Gupta et al. 2012; Ikeda and Kita 1964; Lee et al. 2013; Perera et al. 2009; Saxena and Prakash Saxena 2010). Additionally, experimental animal studies have demonstrated increased methemoglobin levels in mice and rats of both sexes exposed through any exposure route with acute, intermediate, and chronic (≥365 days) exposure durations (Biodynamics 1984; Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983b). A variety of related adverse effects in the hematologic system, such as hemolytic anemia; extramedullary hematopoiesis, hemosiderosis, congestion, and lymphoid depletion of the spleen; and changes in bone marrow in response to anemia have also been observed with inhalation, oral, and dermal exposures in B6C3F1 mice, F344 rats, and Sprague-Dawley rats (Burns et al. 1994; Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b).

Agency Contacts (Chemical Managers): Malcolm Williams

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical name(s): Nitrobenzene
CAS number(s): 98-95-3
Date: January 2024
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL.

Rationale for Not Deriving an MRL: No studies that evaluated chronic-duration oral exposure to nitrobenzene were located; therefore, no MRL can be derived.

Agency Contacts (Chemical Managers): Malcolm Williams

APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR NITROBENZENE

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 nitrobenzene.

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 nitrobenzene. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of nitrobenzene 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 nitrobenzene 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
Developmental effects
Other noncancer effects

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

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 Draft Toxicological Profile for Nitrobenzene released for public comment in 2022; thus, the literature search was restricted to studies published between January 2019 and July 2022. The following main databases were searched in July 2022:

- 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 nitrobenzene. 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 nitrobenzene 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	
07/2022	(98-95-3[rn] OR "1-Nitrobenzene"[tw] OR "mononitrobenzene"[tw] OR "Nitrobenzene"[tw] OR "Nitrobenzol"[tw] OR "Oil of Mirbane"[tw] OR "p-Nitrobenzene"[tw] OR "benzene, nitro"[tw] OR "Benzene, nitro-"[tw] OR "Essence of mirbane"[tw] OR "Essence of Myrbane"[tw] OR "Mirbane oil"[tw] OR "Oil of Myrbane"[tw]) AND (2019:2022[mhda] OR 2019:2022[edat] OR 2019:2022[crdat] OR 2019:2022[dp])
NTRL	
07/2022	2019-present "Nitrobenzene" OR "Oil of Mirbane" OR "1-Nitrobenzene" OR "Mononitrobenzene" OR "p-Nitrobenzene" OR "Nitrobenzol" OR "Essence of mirbane" OR "Essence of Myrbane" OR "Mirbane oil" OR "Oil of Myrbane" OR "Benzene, nitro-" OR "benzene, nitro"
Toxcenter	
07/2022	FILE 'TOXCENTER' ENTERED AT 13:29:49 ON 26 JUL 2022 CHARGED TO COST=EH038.13.07.LB.04 L1 7736 SEA FILE=TOXCENTER 98-95-3 L2 7673 SEA FILE=TOXCENTER L1 NOT TSCATS/FS L3 6570 SEA FILE=TOXCENTER L2 NOT PATENT/DT L4 4498 SEA FILE=TOXCENTER L3 AND PY>=1997 ACTIVATE TOXQUERY/Q --------- 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?)

Table B-2. Database Query Strings

Database search date	Query string
	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 L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29
	L31 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L32 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L33 QUE L30 OR L31 OR L32
	L34 QUE (NONHUMAN MAMMALS)/ORGN
	L35 QUE L33 OR L34
	L36 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L37 QUE L35 OR L36

	L38 177 SEA FILE=TOXCENTER L4 AND L31
	D SCAN L38

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS via ChemView	
07/2022	98-95-3
NTP	
07/2022	"Nitrobenzene" "Oil of Mirbane" "1-Nitrobenzene" "Mononitrobenzene" "p-Nitrobenzene" "Nitrobenzol" "Essence of mirbane" "Essence of Myrbane" "Mirbane oil" "Oil of Myrbane" "Benzene, nitro-" "benzene, nitro"
Regulations.gov	
07/2022	Nitrobenzene 1-Nitrobenzene p-Nitrobenzene mononitrobenzene Nitrobenzol Oil of Mirbane benzene, nitro Benzene, nitro- Essence of mirbane Essence of Myrbane Mirbane oil Oil of Myrbane
NPIRS	
07/2022	98-95-3
NIH RePORTER	
10/2022	Search Criteria-- Fiscal Year: Active Projects; Text Search: "1-Nitrobenzene" OR "mononitrobenzene" OR "Nitrobenzene" OR "Nitrobenzol" OR "Oil of Mirbane" OR "p-Nitrobenzene" OR "benzene, nitro" OR "Benzene, nitro-" OR "Essence of mirbane" OR "Essence of Myrbane" OR "Mirbane oil" OR "Oil of Myrbane" (advanced) Limit to: Project Title, Project Terms, Project Abstracts
Other	Identified throughout the assessment process

The 2022 results were:

- Number of records identified from PubMed, NTRL, and TOXCENTER (after duplicate removal): 677
- Number of records identified from other strategies: 55
- Total number of records to undergo literature screening: 732

B.1.2 Literature Screening

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

- 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.

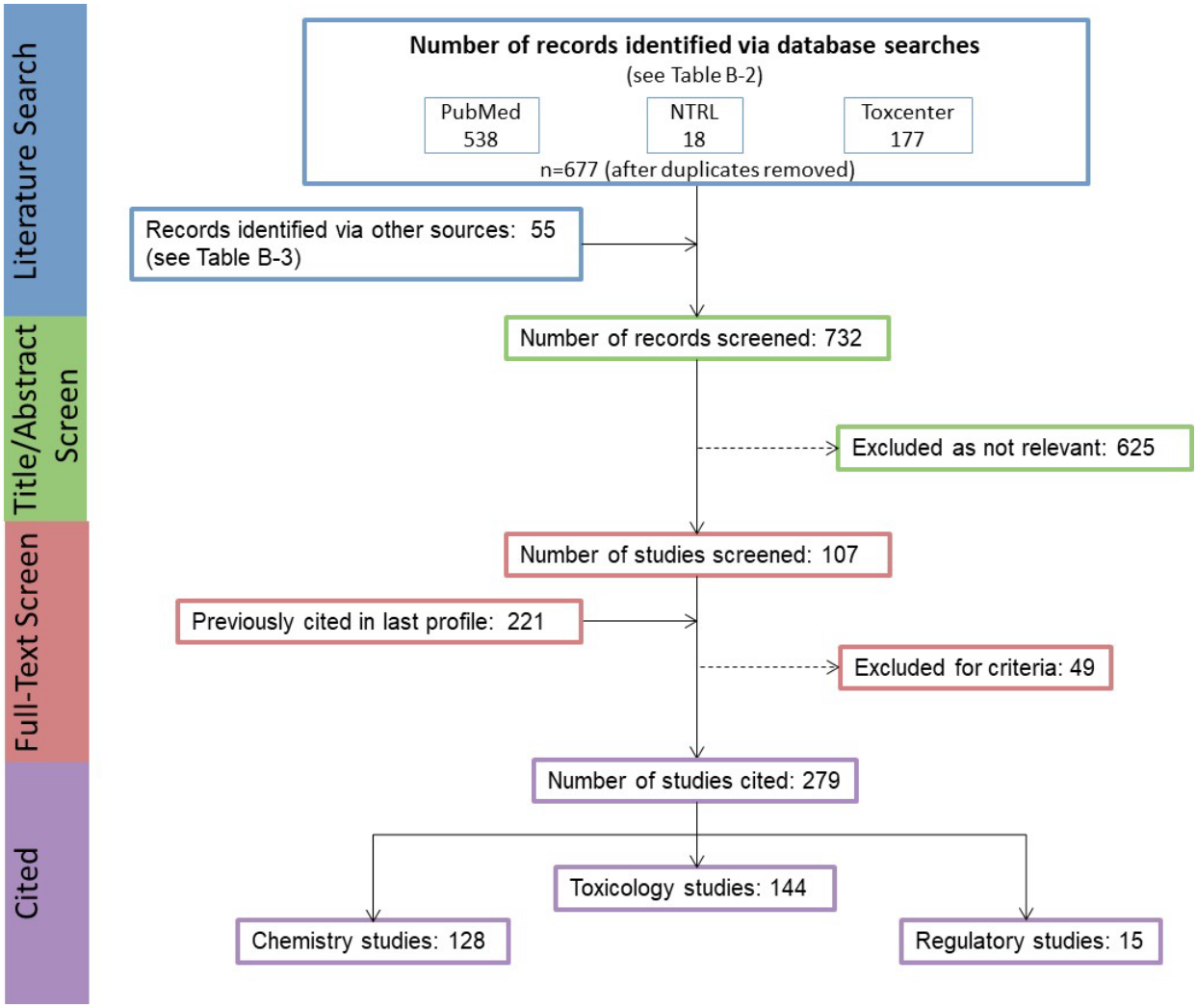
- Number of titles and abstracts screened: 732
- Number of studies considered relevant and moved to the next step: 107

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: 107
- Number of studies cited in the pre-public draft of the toxicological profile: 221
- Total number of studies cited in the profile: 279

A summary of the results of the literature search and screening is presented in Figure B-1.

Figure B-1. July 2022 Literature Search Results and Screen for Nitrobenzene



APPENDIX C. 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 C-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 C-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.

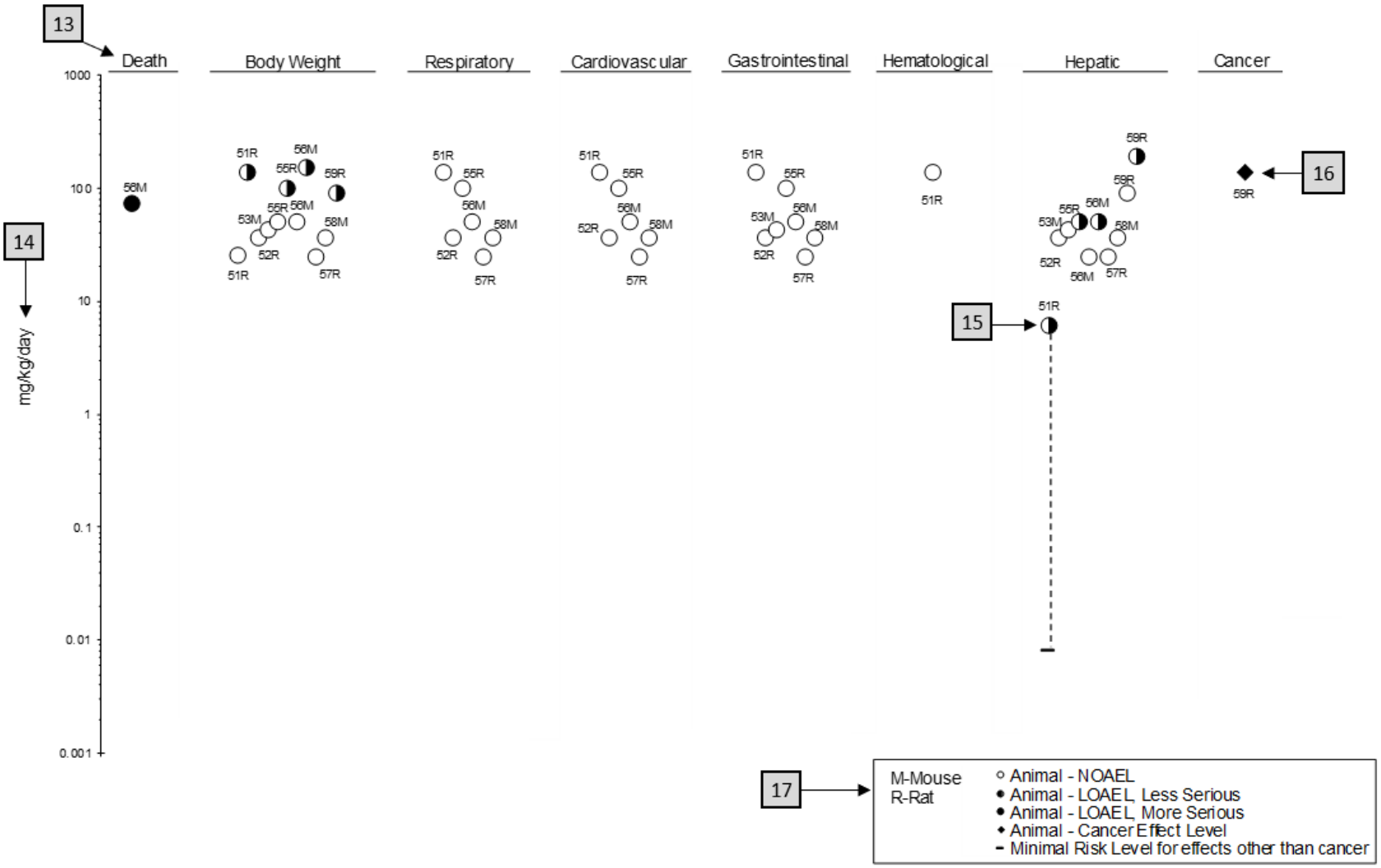
Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
CHRONIC EXPOSURE									
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									
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.
[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).

APPENDIX C

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral



APPENDIX D. 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 discuss health effects and approaches to patient management in a brief/factsheet style. *Clinician Overviews* 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, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • 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 E. 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 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 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 dose 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 dose, 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.

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 F. 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 Substance 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